Survival of *Escherichia coli* O157:H7 in Cucumber Fermentation Brines

Frederick Breidt, Jr. and Jane M. Caldwell

Abstract: Bacterial pathogens have been reported on fresh cucumbers and other vegetables used for commercial fermentation. The Food and Drug Administration currently has a 5-log reduction standard for *E. coli* O157:H7 and other vegetative pathogens in acidified pickle products. For fermented vegetables, which are acid foods, there is little data documenting the conditions needed to kill acid resistant pathogens. To address this knowledge gap, we obtained 10 different cucumber fermentation brines at different stages of fermentation from 5 domestic commercial plants. Cucumber brines were used to represent vegetable fermentations because cabbage and other vegetables may have inhibitory compounds that may affect survival. The 5-log reduction times for *E. coli* O157:H7 strains in the commercial brines were found to be positively correlated with brine pH, and ranged from 3 to 24 d for pH values of 3.2 to 4.6, respectively. In a laboratory cucumber juice medium that had been previously fermented with *Lactobacillus plantarum* or *Leuconostoc mesenteroides* (pH 3.9), a 5-log reduction was achieved within 1 to 16 d depending on pH, acid concentration, and temperature. During competitive growth at 30 °C in the presence of *L. plantarum* or *L. mesenteroides* in cucumber juice, *E. coli* O157:H7 cell numbers were reduced to below the level of detection within 2 to 3 d. These data may be used to aid manufacturers of fermented vegetable products determine safe production practices based on fermentation pH and temperature.

Keywords: cucumbers, *E. coli* O157:H7, fermentation brine, fermented vegetables, survival times

Practical Application: Disease causing strains of the bacterium *E. coli* may be present on fresh vegetables. Our investigation determined the time needed to kill *E. coli* in cucumber fermentation brines and how *E. coli* strains are killed in competition with naturally present lactic acid bacteria. Our results showed how brine pH and other brine conditions affected the killing of *E. coli* strains. These data can be used by producers of fermented vegetable products to help assure consumer safety.

Introduction

Acid-resistant, vegetative bacterial pathogens can be present on fresh produce and ingredients that are used in the manufacture of fermented and acidified foods. The microflora on fresh fruits, grains, and vegetables can range from 10^2 to 10^9 colony forming units (CFU) per gram (Lund 1992; Nguyen-the and Carlin 1994). On pickling cucumbers, the aerobic microflora are usually between 10^4 and 10^6 CFU/g with lactic acid bacteria (LAB) around 10 to 10^4 CFU/g (Fleming and others 1995). A variety of pathogens, including *Salmonella* and *Shigella* species, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Campylobacter*, *Listeria monocytogenes*, *E. coli*, including acid-resistant enterohemorrhagic O157:H7, O145, and other pathogenic serotype strains, may be present on vegetables that are used in ready-to-eat and processed foods (Beuchat 1996, 2002; Brackett 1999; Taormina and Beuchat 1999). Removal of pathogenic and spoilage bacteria from fresh fruits and vegetables prior to processing is difficult. Biofilms containing bacteria on fruits and vegetables may be more resistant to sanitizing agents and organic acid treatments than free or planktonic cells (Kumar and Anand 1998; Bower and Daeschel 1999; Riordan and others 2001; Sapers 2001). Washing procedures with water or chemical sanitizers typically result in only a 1- to 2-log decrease in bacterial cell numbers, and bacteria may be protected in inaccessible locations on fruits and vegetables, such as the core and calyx of apples (Riordan and others 2001). Attachment of bacteria to wounded or cut surfaces, or entry of bacteria into the interior of fruits and vegetables through stomata may occur (Daeschel and Fleming 1981; Seo and Frank 1999; Takeuchi and Frank 2000; Reina and others 2002). As a result of large scale commercial processing of vegetables, the spread of pathogens may occur. Disease outbreaks have occurred in some acid foods such as apple cider and apple juice (Besser and others, 1993; CDC, 1996), which have pH values around 3.7, similar to many acidified vegetable products.

Acid and acidified foods are defined in the United States Code of Federal Regulations (21 CFR part 114) as having a pH value of $\leq$ 4.6. This is the pH upper limit that prevents *Clostridium botulinum* spore outgrowth and neurotoxin production (Ito and

MS 20100970 Submitted 8/27/2010, Accepted 12/21/2010. *Authors are with USDA-ARS, SAA Food Science Research Unit, 322 Schaub Hall, Box 7624, North Carolina State Univ., Raleigh, NC 27695-7624, U.S.A. Direct inquiries to author Breidt (E-mail: Fred.Breidt@ars.usda.gov).*

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doi: 10.1111/j.1750-3841.2011.02045.x

Journal of Food Science A Publication of the Institute of Food Technologists

others 1976). Acidified foods, to which acid or acid food ingredients are added to lower the pH below 4.6, include pickled vegetables sold in hermetically sealed (anaerobic) containers. While the focus of the regulation is to prevent the growth of *C. botulinum* and toxin production, 21 CFR part 114 specifies processing of acidified foods "to the extent that is sufficient" to destroy vegetative cells of microorganisms of public health significance. Acidified vegetable products can be heat processed to assure shelf stability and safety; and the times and temperatures needed for that purpose have been reported (Breidt and others 2005; Breidt 2006), and a 5-log reduction standard has been adapted by FDA (Breidt and others 2010). However, up to half of the $1.5 billion per year pickled vegetable market consists of products that are preserved without thermal processing, including fermented products, which are naturally acidified and defined as acid foods.

Low pH (3 to 4) and organic acids in acidified and fermented vegetable products prevents the growth of bacterial pathogens and results in pathogen death. These products have an excellent history of consumer safety, but definitive data are lacking to show that *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica* will die off over the range of conditions under which commercial fermentations occur. Previous research has shown that *E. coli* O157:H7 is the most acid resistant pathogen of concern in acidified vegetables, and for nonheat processed acidified vegetables containing acetic acid (pH 3.3 or below), the holding times needed to assure destruction of this pathogen are 6 d at 10 °C and 2 d at 25 °C (Breidt and others 2007). No research has been done to determine the survival of acid-resistant vegetative pathogens in vegetable fermentations. Of particular concern are fermentations conducted under colder temperatures such as 10 °C, which increases the survival of *E. coli* O157:H7 in acid solutions (Breidt and others 2007).

Our research objectives were to determine the survival of *E. coli* O157:H7 in sterile, filtered commercial fermentation brines, in competitive growth with *L. plantarum* and *L. mesenteroides* in cucumber juice, and in cucumber juice that has previously been fermented by these LAB. We examined the effect of temperature, pH, protonated acid concentrations, NaCl concentrations, and other factors on the survival of *E. coli* O157:H7 in these brines. From these data we determined the times and temperatures needed to assure safety of vegetable fermentations.

Materials and Methods

Preparation of cucumber brines

Size 2B pickling cucumbers (approximately 3.5 cm diameter) were obtained from a local supplier. Cucumbers were rinsed and scrubbed in tap water to remove dirt and debris then processed to slurry in a Waring blender after 1 : 1 dilution in distilled water (wt/vol). Slurry was frozen at −20 °C for several days or weeks. To create cucumber juice medium (CJ), slurry was thawed at 4 °C overnight and centrifuged in a Sorval RC-5B plus superspeed centrifuge (Thermo Electron Corp., Asheville, N.C., U.S.A.) at 7.5k (8539 × *g*) for 20 min. Supernatant was aspirated from the solids and the medium was sterilized by filtration using a 0.45 micron bottle filter (430514, Fisher Scientific, Suwanee, Ga., U.S.A.). Sodium chloride was added to a final concentration of 2%. Fermented cucumber juice (FCJ) was obtained by inoculating CJ with a single colony of *L. plantarum* or *L. mesenteroides* (Table 1) and incubating at 30 °C for 96 h, followed by centrifugation and filtration as described previously. Both CJ and FCJ were stored at 4 °C until use. Fermented cucumber brines (approximately 250 mL) at different stages of fermentation were obtained from commercial sources, sterilized by filtration (0.45 um) and frozen at −20 °C until use.

Preparation and handling of bacterial cells

A total of 5 enterotoxigenic *E. coli* O157:H7 strains (B200 to B204) were grown independently and combined to form an inoculation cocktail (Table 1). *E. coli* strains were grown statically at 37 °C for 16 h in LB broth (Difco, Becton Dickinson, Sparks, Md., U.S.A.) supplemented with 1% glucose to induce acid resistance. Overnight cultures were centrifuged at 5k (3000 × *g*) for 10 min, supernatant was removed, then combined and concentrated 10-fold in sterile saline with approximately equal concentrations of each bacterial culture. The *E. coli* cocktail was inoculated at 10^6, 10^8, and 10^8 CFU/mL for CJ, FCJ, and commercial brines, respectively. Brines were incubated for the indicated times and temperatures, and samples (0.5 mL) were removed and diluted prior to plating with a spiral plater (Spiral Biotech Inc. Norwood, Mass., U.S.A.) on nonselective LB agar (Difco). After 24 h incubation at 37 °C, colonies were counted with an automated spiral plate counter (Q-Count, Spiral Biotech Inc.). The lower detection limit was between 10^2 and 10^3 CFU/mL by this method.

For bacterial competition assays in CJ, *L. mesenteroides* (LA430) and *L. plantarum* (LA445) were grown statically at 30 °C for 16 h in MRS broth (Difco). Cultures were centrifuged and concentrated as described for *E. coli* previously and inoculated at 10^3 CFU/mL into CJ containing 10^6 CFU/mL of the *E. coli* cocktail. Mixed culture fermentation samples were serially diluted and plated on MRS agar (Difco), and LB agar followed by incubation at 30 and 37 °C, respectively. We discovered that *E. coli* does not form colonies on MRS agar at 30 °C within 48 h, and *L. mesenteroides* and *L. plantarum* will not grow on LB at 37 °C in 24 h, allowing independent enumeration of the LAB and the *E. coli* strains.

Biochemical analysis

Samples (2 mL) were withdrawn aseptically at indicated times for high-performance liquid chromatography (HPLC), pH, and NaCl concentrations. The pH was determined with an Accumet AR25 pH meter (Fisher, Atlanta, Ga., U.S.A.). Sodium chloride concentration was determined by chloride ion titration using silver nitrate and fluorescein dye indicator (Collier 1936). Organic acids, sugars, and ethanol concentrations were measured with a Thermo Separation Products HPLC (ThermoQuest Inc., San Jose, Calif., U.S.A.) system consisting of a P1000 pump, an SCM100 solvent degasser, an AS3000 autosampler, and a UV6000 diode array detector (ThermoQuest) (Breidt and others 2004). A Bio-Rad HPX-87H column, 300 by 7.8 mm (Bio-Rad, Hercules, Calif., U.S.A.) was used with a differential refractometer (Waters model 410 Millipore, Milford, Mass., U.S.A.) and a UV detector (UV6000LP, Thermo Separation Products, San Jose, Calif., U.S.A.) for detection of the analytes. Operating conditions of the system included a sample tray at 6 °C, a column at 65 °C and

Table 1–Bacterial strains.

Strain ID	Strain name	Previous ID	Origin
B0200	*E. coli* O157:H7	ATCC 43888	Human feces
B0201	*E. coli* O157:H7	SRCC 1675	Apple cider outbreak
B0202	*E. coli* O157:H7	SRCC 1486	Salami outbreak
B0203	*E. coli* O157:H7	SRCC 2061	Ground beef
B0204	*E. coli* O157:H7	SRCC 1941	Pork
LA430	*L. mesenteroides*	ATCC 8293	Fermenting olives
LA445	*L. plantarum*	MOP3	Fermenting cucumbers

0.03 N H_2SO_4 eluent at a flow rate of 0.9 mL/min. The UV6000 detector was set to 210 nm at a rate of 1 Hz for data collection and 2 Hz was used for refractive index data. ChromQuest version 4.1 chromatography software was used to control the system and analyze the data, utilizing the peak heights for quantitative integration based on standard solutions with 4 different concentrations.

Regression analysis

The estimated 5-log reduction times and standard errors for the estimate were determined from the killing curves using a linear regression algorithm in Matlab™ (Matlab Central http://www.mathworks.com/matlabcentral/, F. Breidt and J. A. Osborne). Regression analysis to determine correlations between pH, protonated acid, and the predicted 5-log reduction time was carried out using SigmaPlot software (version 10, Systat Software Inc., Chicago, Ill., U.S.A.).

Results

A total of 10 commercial brines obtained during the first week of fermentation were found to have pH values ranging from 3.2 to 4.5, and lactic acid concentrations of 150 mM to nondetectable levels (less than 1 mM) (Table 2). The average NaCl concentration for these brines was approximately 6.8%, ranging from 5.5% to 8.7%. All brines contained acetic acid, an occasional additive to commercial brines to help lower the initial pH, encourage the growth of LAB, and buffer the fermentation. The survival of the *E. coli* O157:H7 cocktail in the filtered commercial brines is shown in Figure 1. The survival curves were approximated by linear models, with an average *R*-squared value of 0.876 (data not shown). From these data, 5-log reduction times were estimated and the standard error of the estimate determined (Table 2). Sample A1, which had the highest pH value (4.53), no detectable lactic acid, and a NaCl concentration of 7.9%, had the highest 5-log reduction time, requiring 22.6 ± 1.3 d at 23 °C.

The 5-log reduction times were used to identify correlations with brine components. We found a correlation between the pH of the brine and the 5-log reduction time, with a *R*-squared value of 0.71 (Figure 2). A similar trend was found for the correlation between the log of the protonated acid concentration (calculated from the Henderson–Hasselbalch equation) and pH, which had a *R*-squared value of 0.704 (data not shown). One outlying data point, brine sample E5, had a relatively short 5-log reduction time (2 d) but a relatively high pH (3.78) compared to the other samples. The NaCl concentration for this sample was higher than the mean for the other samples (7.82% against 6.5%). Although 2 other samples had higher salt concentrations, they also had the highest pH values for 10 brine samples.

To determine how competition with LAB would affect survival of the *E. coli* O157:H7 strains, CJ fermentations were carried at 30 °C with 2% NaCl. In fermentations with *L. mesenteroides*, the cell numbers for the *E. coli* O157:H7 strains initially increased to over 10^8 CFU/mL but then was reduced to below

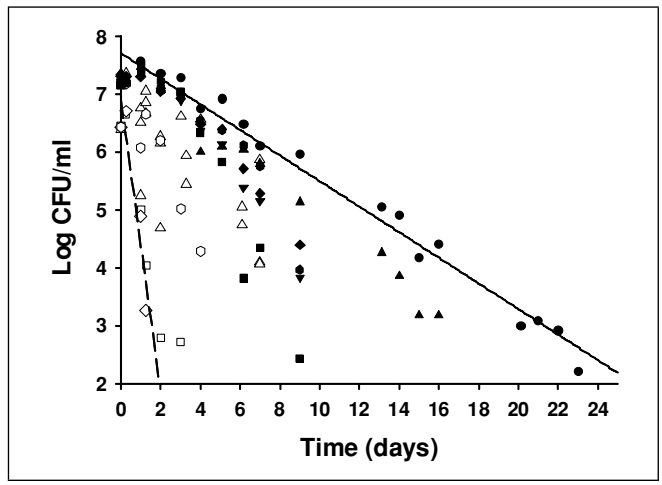

Figure 1–Survival of *E. coli* O157:H7 in brine from commercial fermentations at 23 °C under anaerobic conditions. The survival of *E. coli* O157:H7 strains in brines labeled as shown in Table 1: A1, (filled circle); A2, (filled triangle up); B1, (filled triangle down); B2, (filled square); C1, (filled diamond); D1, (filled hex); E1, (open triangle up); E2, (open square); E5, (open diamond); E6, (open hex). The regression lines for A1 data (solid line, R^2 = 0.977) and E5 data (dashed line, R^2 = 0.881) are shown.

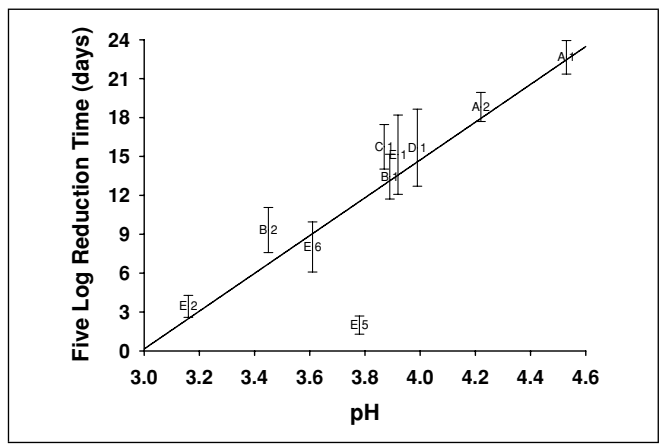

Figure 2–Five log reduction times and pH of commercial brines at 23 °C under anaerobic conditions. The alpha-numeric code for each data point corresponds to the code in Table 1. The error bars represent the standard error for the calculated 5 log reduction times. The R^2 value for the regression line was 0.71.

Table 2–Biochemistry and 5 log reduction times for commercial brines.

Company ID[a]	pH	NaCl (%)	Lactic acid (mM)	Acetic acid (mM)	5 log reduction (d)	SE[b]
A1	4.53	7.90	0.00	11.76	22.6	1.3
A2	4.22	8.70	0.00	14.98	18.8	1.1
B1	3.89	5.50	35.81	4.39	13.4	1.7
B2	3.45	6.30	42.41	4.19	9.3	1.7
C1	3.87	6.90	24.86	22.83	15.7	1.7
D1	3.99	6.00	0.36	27.31	15.7	3.0
E1	3.92	5.64	31.89	20.06	15.1	3.1
E2	3.16	6.95	150.76	34.00	3.4	0.8
E5	3.78	7.82	50.60	14.20	2.0	0.7
E6	3.61	6.23	50.17	19.30	8.0	1.9

[a]Company ID = company identification code.
[b]SE represents the standard error of the estimated 5 log reduction time.

detectable levels (less than 10^2 CFU/mL) in less than 2.3 d (55 h) (Figure 3A). During this time, the *L. mesenteroides* culture grew from approximately 10^3 to 10^7 CFU/mL. In control fermentations with the *E. coli* O157:H7 cocktail or the *L. mesenteroides* culture grown independently of each other, the cultures grew similarly within the first 10 to 20 h (to > 10^8 CFU/mL), but *E. coli* strains maintained viability (approximately 10^7 CFU/mL) during the 55 h of the experiment (Figure 3B). Similar results were seen with the *L. plantarum* culture in competition with the *E. coli* O157:H7 cocktail of strains (Figure 4A and 4B), except the *E. coli* culture was reduced to below the limit of detection in mixed culture in less than 48 h. Interestingly, the final pH values for the mixed culture fermentations with *L. plantarum* and the *E. coli* cocktail were similar to the *L. mesenteriodes*—*E. coli* mixed fermentation (pH 4, data not shown).

The survival of *E. coli* in brines of CJ previously fermented with *L. plantarum* or *L. mesenteroides* was determined under the same conditions as the competitive growth experiment (2% NaCl at 30 °C) (Figure 5). The 5-log reduction for the *E. coli* O157:H7 strain cocktail, as calculated from the slope of the regression line, was achieved within 4.3 h ± 0.8 h for CJ previously fermented with *L. plantarum*, but for CJ previously fermented by *L. mesenteroides* 15 ± 1.3 h was needed for a 5-log reduction. At 10 °C, the times needed for a 5-log reduction were found to be 3.2 ± 0.5 d or 16 ± 1.4 d for brine fermented with *L. plantarum* or *L. mesenteroides*, respectively.

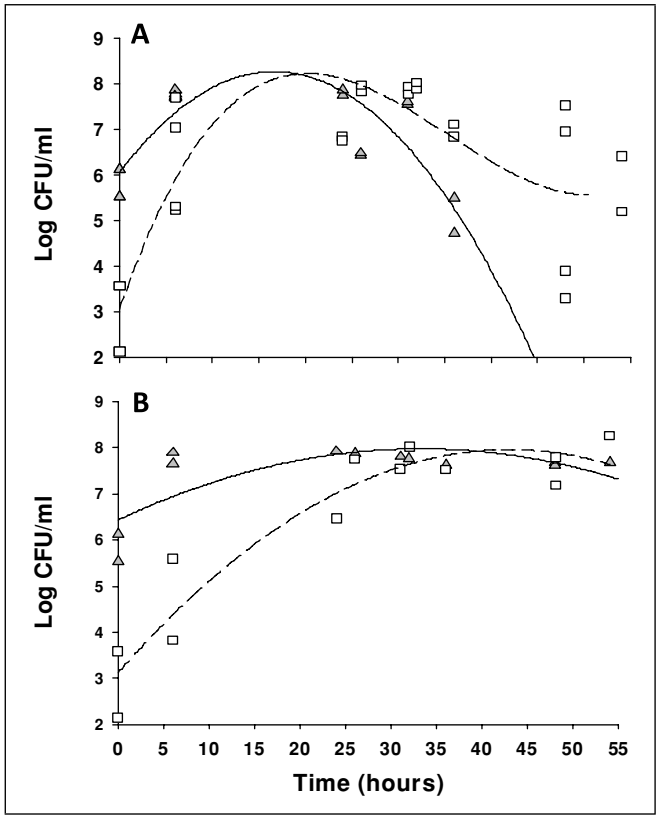

Figure 4–Survival of *E. coli* O157:H7 in competition with *L. plantarum* at 30 °C under anaerobic conditions. *E. coli* O157:H7 (triangles) and *L. plantarum* (squares) cell counts for competitive growth of cells (A) or cells grown separately (B) in CJ medium. The lines indicating the trends of growth and death for *E. coli* O157:H7 strains (solid line) or *L. plantarum* (dashed line) were 2nd or 3rd order polynomials.

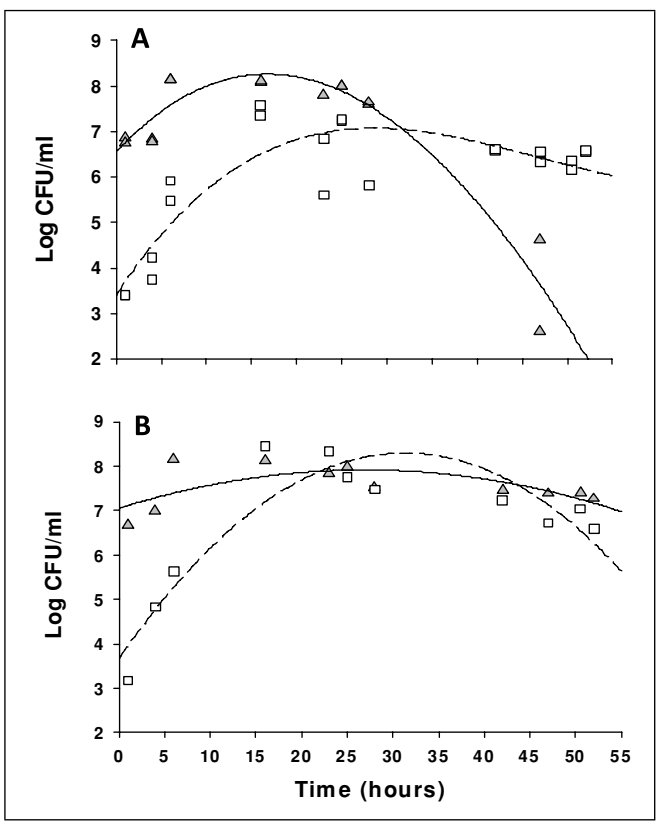

Figure 3–Survival of *E. coli* O157:H7 in competition with *L. mesenteroides* at 30 °C under anaerobic conditions. *E. coli* O157:H7 (triangles) and *L. mesenteroides* (squares) cell counts for competitive growth of cells (A) or cells grown separately (B) in CJ medium. The lines indicating the trends of growth and death for *E. coli* O157:H7 strains (solid line) or *L. mesenteroides* (dashed line) were 2nd or 3rd order polynomials.

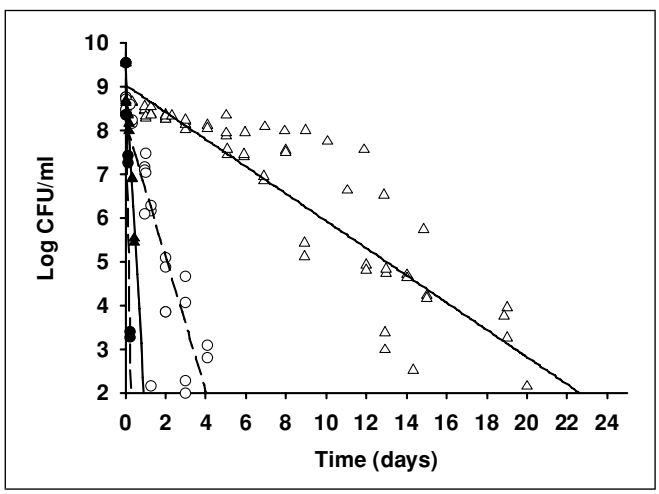

Figure 5–Survival of *E. coli* O157:H7 at 10 °C and 30 °C in brines fermented with *L. Plantarum* or *L. mesenteroides* under anaerobic conditions. Survival of *E. coli* O157:H7 in fermented CJ. *E. coli* O157:H7 strains (5-strain cocktail) were inoculated CJ that had been fermented by *L. mesenteroides* (triangles and solid line) or *L. plantarum* (circles and dashed line). Incubations were carried out at 10 °C (open symbols) or 30 °C (filled symbols) The lines depict linear regression for survival in CJ fermented by *L. mesenteroides* (solid line, $R^2 = 0.82$ or 0.96 for 10 °C or 30 °C, respectively) or *L. plantarum* (dashed line, $R^2 = 0.80$ or 0.92 for 10 °C or 30 °C, respectively).

Discussion

Process filings are required for commercial production of shelf-stable acidified foods in the United States. In addition to maintaining a pH of 4.6 or below, as required by 21 CFR part 114 to prevent botulism, manufacturers also achieve a 5-log reduction in the cell numbers of vegetative pathogens (Breidt and others 2010, 2007, 2005). *E. coli* O157:H7 has been found to be the most acid-resistant vegetative pathogen of concern in acidified foods, and a heat process is typically used to assure safety (Breidt and others 2005, 2007). While fermented foods are exempt from acidified food regulations, little is known about the rate of killing for acid resistant vegetative pathogens during fermentation. For cucumber and sauerkraut fermentations, both Gram-positive and Gram-negative bacteria have been shown to grow during the early stages (first few days) of fermentation, depending on the initial brine conditions and temperature (Fleming and others 1995; Plengvidhya and others 2007). Currently, most manufacturers of fermented cucumbers use recycled brine or add acetic acid to the brine, this can selectively encourage the growth of LAB and accelerate the die off of enteric organisms. Starter cultures are not typically used in vegetable fermentations, so the rate of fermentation and competitive growth inhibition of vegetative pathogens can vary depending on the initial microbiota, temperature, salt and acid concentration, and brine pH.

This study focused on cucumber fermentations and laboratory CJ studies because cabbage contains antimicrobial compounds, which may reduce survival of enteric bacteria in brines (Kyung and Fleming 1997). In cucumber brine that had been fermented by *L. mesenteroides*, *E. coli* O157:H7 strains required more than 2 wk before a 5-log reduction in cell numbers was achieved. Interestingly, the *E. coli* O157:H7 strains did not use significant amounts of the remaining sugars (10 and 12 mM for glucose and fructose, respectively) for up to 22 d incubation at 10 °C (Table 3). This indicates the low temperature, external pH, and organic acids inhibited glycolysis. In competition with *L. mesenteroides* or *L. plantarum*, the *E. coli* O157:H7 strains initially grew at 30 °C from 10^6 CFU/mL to greater than 10^8 CFU/mL; however, the LAB strains, which were inoculated in co-culture at approximately 10^3 CFU/mL were able to predominate in the fermentations, overcoming the 3-log cycle deficit in initial cell numbers within approximately 24 h (Figure 3). The final pH values obtained in the mixed culture fermentations with both *L. mesenteroides* and *L. plantarum* were approximately 4 (data not shown). The growth of the *E. coli* strains interfered with the metabolic activity and growth of the LAB, even though the *E. coli* strains were killed within 2 to 3 d. The factors influencing competitive growth include the production of organic acids by both competitive species during fermentative growth, the reduction in pH, and the utilization of the main carbon sources as well as other nutrients. Because of the complexity of these interactions, we used polynomial functions to approximate the growth and death curves (Figure 3 and 4). Further study of mixed culture growth of bacteria and improved competitive growth models (Breidt and Fleming 1998) may be used to help determine outcomes of competitive growth.

Table 3—Biochemistry of fermented brines inoculated with *E. coli* O157:H7.

ID[a]	Time (h)	pH	Glucose (mM)	Fructose (mM)	Lactic (mM)	Acetic (mM)
FCJ-LM	0	3.70	10.3	n.d.	30.8	24.0
FCJ-LM	24	3.9	10.0	0.0	28.4	23.0
FCJ-LP	0	2.89	10.3	12.8	105.2	0.0
FCJ-LP	24	3.0	9.9	12.0	98.1	0.0

[a]ID: FCJ-LM = CJ fermented by *L. mesenteroides*; FCJ-LP = CJ fermented by *L. plantarum*.

Brine samples were obtained from 9 different commercial fermentations at 5 different fermentation facilities around the United States, sterilized by filtration, and used to determine survival of *E. coli* O157:H7. The results were similar to the observations with brines prepared in the laboratory by fermentation of CJ with *L. plantarum* or *L. mesenteroides* (although with lower NaCl concentration, approximately 2%). The data show a striking correlation between pH and 5-log reduction time. As acid accumulates and pH is reduced in the brine, the 5-log reduction time is correspondingly reduced. Protonated acid concentrations also correlated well with 5-log reduction time (not shown). If buffering capacity of brines and fermentation acid production were similar for the different commercial brines obtained, this result would be expected. Because pH is much easier to measure then protonated acid concentrations, we reported the correlation with pH and 5-log reduction times only. Only brine samples were used for this study. Brine pH and organic acids will rapidly equilibrate between the brine and the vegetable material in the fermentation (Fleming and others, 1995). Although the log reduction observed for bacterial pathogens in the brine was not directly measured using cucumber fruit, the similar pH and acid concentrations should result in similar log reduction for cells embedded in and on the vegetable material. Further research would be necessary to confirm this assumption.

A commercial brine having little or no lactic acid at the start of fermentation (brine sample A1) had a 5-log reduction time of approximately 3 wk, compared to 3 d for a fermented brine with a pH of 3.1 and 150 mM lactic acid (brine sample E2) (Table 2). Under more favorable conditions (lower temperature) for the survival of *E. coli* O157:H7 (2% NaCl, 10 °C with CJ fermentation by *L. mesenteroides*, Figure 5), a 5-log reduction at pH 3.9 required 23 d compared to 16 d for commercial brine samples at 25 °C with similar pH values (brines C1 and D1, Table 2). While the trend of increasing survival as temperature is reduced is similar to that reported by Breidt and others (2007), further study will be needed to clarify the relationship between temperature and brine survival of *E. coli*.

Interestingly, one commercial brine had a 5-log reduction time that was lower than expected based on pH (brine E5) (Table 2 and Figure 2). This sample had higher salt (7.82% NaCl) compared to similar samples (brines E6, C1 with 6.23% and 6.9% NaCl, respectively), but had a 5-log reduction time at least 6 d shorter (2 d compared with 8 or 15.7 d). The apparent effect of higher salt concentration was to increase acid killing, but the there may be other unidentified factors influencing acid killing, including the presence of preservatives. Postassium sorbate is sometimes used in commercial fermentations to prevent the growth of spoilage microflora, including yeasts (Costilow and Uebersax 1982). But, in the commercial brines used in this study we found no evidence of sorbate (<1 mM) by HPLC.

Conclusions

Process filings submitted to FDA for commercial production of acidified foods have a 5-log reduction standard to assure safety. While fermented foods are considered acid foods, and are therefore exempt from acidified food regulations, no published research is available to determine the survival of *E. coli* O157:H7 or other pathogens in these products. Given the most permissive conditions tested for the survival of *E. coli* O157:H7 (pH 4.5 for commercial

brine samples at 23 °C or pH 3.9 with brine fermented by *L. mesenteroides* at 10 °C), a 5-log reduction was achieved within 23 d. We also found that pH and 5-log reduction time were closely correlated for a variety of commercial fermentation brines. Brine pH values below 3.3 required less than 4 d to achieve a 5-log reduction regardless of temperature (10 °C or higher) with commercial brines or in active competition with LAB. These data will help commercial manufacturers assure the safety of fermented vegetable products.

Acknowledgments

The authors acknowledge Drs. Roger F. McFeeters and Jason A. Osborne for helpful discussions. We also thank Sandra Parker for excellent editorial assistance. This study was supported in part by a grant from Pickle Packers Intl. Inc.

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