Journal of Food Protection, Vol. 72, No. 3, 2009, Pages 503–509

Escherichia coli O157:H7 Strains Isolated from Environmental Sources Differ Significantly in Acetic Acid Resistance Compared with Human Outbreak Strains†‡

DEOG-HWAN OH,[1] YOUWEN PAN,[2] ELAINE BERRY,[3] MICHAEL COOLEY,[4] ROBERT MANDRELL,[4] AND FREDERICK BREIDT, JR.[5]*

[1]Department of Food Science and Biotechnology, College of Bioscience and Biotechnology, Kangwon National University, Hyoja 2-dong, Chuncheon 200-701, Republic of Korea; [2]Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27695-7615; [3]U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Spur 18D, Clay Center, Nebraska 68933-0166; [4]U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, California 94710; and [5]U.S. Department of Agriculture, Agricultural Research Service, Department of Food, Bioprocessing, and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina 27695-7624, USA

MS 08-255: Received 3 June 2008/Accepted 7 November 2008

ABSTRACT

A number of studies on the influence of acid on *Escherichia coli* O157:H7 have shown considerable strain differences, but limited information has been reported to compare the acid resistance based on the different sources of *E. coli* O157:H7 isolates. The purpose of this study was to determine the survival of *E. coli* O157:H7 strains isolated from five sources (foods, bovine carcasses, bovine feces, water, and human) in 400 mM acetic acid solutions under conditions that are typical of acidified foods. The isolates from bovine carcasses, feces, and water survived acetic acid treatment at pH 3.3 and 30°C significantly ($P \leq 0.05$) better than did any food or human isolates. However, resistance to acetic acid significantly increased as temperature decreased to 15°C for a given pH, with little ($P \geq 0.05$) difference among the different isolation sources. All groups of *E. coli* O157:H7 strains showed more than 1.8- to 4.5-log reduction at pH 3.3 and 30°C after 25 min. Significantly reduced (less than 1-log reduction) lethality for all *E. coli* O157:H7 strain mixtures was observed when pH increased to 3.7 or 4.3, with little difference in acetic acid resistance among the groups. The addition of glutamate to the acetic acid solution or anaerobic incubation provided the best protection compared with the above conditions for all groups of isolates. These results suggest that temperature, pH, and atmospheric conditions are key factors in establishing strategies for improving the safety of acidified foods.

Enterohemorrhagic *Escherichia coli* O157:H7 has developed multiple mechanisms to survive under low-pH conditions. Because of its low infectious dose, an important component of *E. coli* O157:H7 pathogenesis is thought to be the ability to survive in extremely acidic environments, such as in stomach acid or in areas of the intestine that contain organic acids *(14, 21, 23, 35).* Disease outbreaks caused by *E. coli* O157:H7 in acid foods *(3, 8, 28, 38)* have led to increased research on acid resistance mechanisms of this organism *(2, 17, 36, 40, 41).* Acidified foods such as pickled cucumbers and peppers are typically prepared in hermetically sealed containers that hold acetic acid as the primary acidulant, lack dissolved oxygen, contain NaCl at 2 to 3%, and have pH values between 3.0 and 4.6. Several studies have shown that adaptation to acidic conditions can further enhance the survival of *E. coli* O157:H7 and other pathogens in foods that are preserved by low pH and acids, as well as the cross-protection against heat, salt, and organic acid preservation of foods *(19, 20, 27, 29, 34).* Concern about the survival of *E. coli* O157:H7 in acidified foods has led to recent research to define the heat treatments *(13)* required to assure a 5-log reduction in the numbers of this and other acid-resistant pathogens. Similarly, acidified foods with a pH of 3.3 or below need not be heat processed to assure safety, but do require a holding time after the addition of acetic acid *(12).*

Waterborne outbreaks of *E. coli* O157:H7 have been reported from irrigation or drinking water contaminated with infected feces, although the specific source of the fecal contamination is sometimes unclear *(18).* The major reservoir of *E. coli* O157:H7 is the bovine gastrointestinal tract, and human illness has been associated with the consumption of undercooked ground beef and unpasteurized milk. Human infections have also been associated with a variety of ready-to-eat foods, including fresh vegetable products, as well as acid foods, such as apple cider and fermented meats *(3, 8, 28, 38).*

Organic acids have been used in foods as acidulants, flavor enhancers, and to enhance microbial safety. Organic acids are often used in foods with other preservatives or preservation systems, such as acidic pH, drying, heat, anaerobiosis, chemical preservatives, or refrigeration. Weak acids often have antimicrobial activity associated with their

* Author for correspondence. Tel: 919-513-0186; Fax: 919-513-0810; E-mail: fred.breidt@ars.usda.gov.

† Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable.

‡ Paper no. FSR08-09 of the Journal Series of the Department of Food Science, North Carolina State University, Raleigh, NC 27695-7624.

undissociated form, which can cross cell membranes freely *(10, 15).* Factors affecting the antimicrobial activity of organic acids include pH, acid concentration, and ionic strength, as well as the physiology of the target microorganisms and their environment (growth phase, aerobic or anaerobic atmosphere, acid adaptation, and so forth) *(11, 14, 30, 31).* Temperature is a primary factor influencing acid activity, with rising temperature typically resulting in increasing effectiveness of organic acids *(1, 12, 26).*

Previous studies on the influence of organic acids on *E. coli* O157:H7 have shown strain differences *(9, 26, 33, 37, 42),* but limited information has been reported to compare the acid resistance based on the different sources of *E. coli* O157:H7 isolates under the exposure to various environmental conditions that simulate those found in foods. The purpose of this study was to determine the survival of *E. coli* O157:H7 strains isolated from five different sources (food, bovine carcasses, bovine feces, water, and humans) in acetic acid solution exposed under different environmental conditions.

MATERIALS AND METHODS

***E. coli* isolates and culture conditions.** A total of 52 *E. coli* O157:H7 strains, two *E. coli* O157 strains, and five *E. coli* non-O157:H7 strains from various sources including foods, bovine carcasses, bovine feces, water, and humans were used in this study (Table 1). The non-O157:H7 strains were all commensal nonpathogenic *E. coli* strains of bovine feces origin and included in the study for comparative purposes. Additional details regarding some of the outbreak strains and strains isolated from watersheds have been reported previously *(22).* All strains were stored at −80°C in Bacto tryptic soy broth (TSB, pH 7.2; BD Biosciences, San Jose, CA) supplemented with 16% glycerol. Each culture was streaked from frozen stocks onto plates of Bacto tryptic soy agar (TSA; BD Biosciences) and incubated at 37°C for 24 h. To prepare cells for the acid challenge, an overnight culture of each *E. coli* strain was inoculated into 10.0 ml of TSB supplemented with 1% glucose and incubated statically for 18 h at 37°C to induce acid resistance *(16).* Each culture was washed twice by centrifugation at 3,500 × *g* for 10 min at 10°C, and finally suspended in physiological saline (8.5 g/liter of NaCl). The optical density at 600 nm of each cell suspension was adjusted to 0.8 (ca. 10^9 cells per ml). Actual starting concentrations were confirmed by plating serial dilutions on TSA.

Preparation of acetic acid solution. The acetic acid solutions were prepared essentially as described, but with a few modifications *(11).* The ionic strength in the acetic solutions (400 mM) was held constant by adjusting the NaCl concentration based on acid anion concentrations as determined by using pHtools, a MATLAB routine *(24).* The pH of the acetic acid solutions was adjusted with HCl and NaOH. For the treatment of the acid solution in the presence of glutamic acid, the concentration of glutamic acid was 2 mM.

Acid challenge studies. For the pure (individual) culture experiments, aliquots of 200 μl of each cell suspension were added into 1.8 ml of acetic acid solutions (400 mM, pH 3.3) with or without glutamic acid in 12-well tissue culture plates (flat-bottom plates; no. 351172 Falcon, Franklin Lakes, NJ) and incubated aerobically at 30°C for 25 min. After incubation, the cells were immediately diluted 10-fold to rapidly neutralize the pH prior to plating. This was done by transferring 20 μl of the acid treated cell suspension into 180 μl of 0.1 M 3-(*N*-morpholino)propanesulfonic acid buffer (pH 7.2; Sigma, St. Louis, MO) with 0.85% saline in a 96-well microplate. Numbers of viable cells were determined after additional dilution and plating on TSA supplemented with 1% glucose, with a spiral plater (model 4000, Spiral Biotech, Inc., Norwood, MA). Plates were incubated for 24 to 48 h at 37°C, and an automatic plate reader (QCount, Spiral Biotech) was used to count the colonies.

For the strain mixture study, equal volumes of bacterial cell suspensions of four to six strains from each isolation source (identification numbers shown in boldface in Table 1) were pooled and mixed prior to the acid challenge studies. The procedure of the acetic acid challenge for the mixed strains was the same as that for individual strains, but additional conditions were tested, including different pH levels (pH 3.3, 3.7, and 4.3), different temperatures (15, 22, and 30°C), and different atmospheric conditions (aerobic and anaerobic). Anaerobic solutions were incubated at room temperature in an anaerobic chamber (COY, Grass Lake, MI) with a mixed anaerobic gas (5% carbon dioxide, 10% hydrogen, and the balance nitrogen) for 24 h prior to use, reducing dissolved oxygen from 5 mg/liter (typical of tap water) to less than 0.05 mg/liter as determined with a dissolved oxygen probe *(31).*

Genomic fingerprint analysis. *E. coli* O157 isolate fingerprints were generated and analyzed by pulsed-field gel electrophoresis (PFGE) of *Spe*I-digested genomic DNA by using the PulseNet procedure (http://www.cdc.gov/pulsenet/). Pulsed-field gel certified agarose was obtained from Bio-Rad (Hercules, CA); Tris-borate-EDTA running buffer and lysozyme were purchased from Sigma. *Spe*I was purchased from New England Biolabs (Beverly, MA). Lambda concatemers (Bio-Rad) were used as size markers. *E. coli* banding patterns were analyzed and comparisons were made by using Molecular Analyst software (Bio-Rad), employing the Dice similarity coefficient in conjunction with the unweighted pair group method by using arithmetic averages for clustering.

Statistical analysis. Three or more replications of the tests for each strain were carried out. Strains were grouped by source. Data analysis was done with a one-way analysis of variance by using SAS (version 6.12 software, SAS Institute, Inc., Cary, NC). Differences ($P < 0.05$) between means of log reduction for the various groupings of strains were analyzed by using the least-significant difference test.

RESULTS

The survival of *E. coli* strains, measured as log-CFU-per-milliliter reduction of each individual strain exposed to an acetic acid solution typical of nonpasteurized acidified vegetable products (400 mM acetic acid, pH 3.3, ionic strength 0.34) for 25 min, with or without glutamic acid (2 mM), at 30°C is shown in Table 1. The mean value of the viable cell reductions of the eight bovine carcass isolates after acetic acid treatment in the absence of glutamic acid was 1.18 log CFU/ml, showing the highest resistance compared with isolates from other sources. The survival of bovine carcass isolates was significantly ($P < 0.05$) different from that of food isolates (the mean reduction was 2.17 log CFU/ml) and from human isolates, which had a mean reduction of 3.18 log CFU/ml ($P < 0.0001$), but not significantly different from the bovine feces isolates (which included the non-O157 isolates; the mean reduction was 1.4

TABLE 1. Escherichia coli *strains used in the study and survival in 400 mM acetic acid solutions (pH 3.3 at 30°C for 25 min)*

Isolates[a]				Reduction in log CFU/ml[b]	
ID no.[c]	Original ID no.	Serotype	Source (reference)	Without glutamic acid	With glutamic acid
B0201	SRCC 1675	O157:H7	Apple cider, October 2002	3.56 ± 0.16	2.66 ± 0.20
B0349	NMSLD-1	O157:H7	Spinach	2.91 ± 0.22	0.02 ± 0.01
B0264	RM1484	O157:H7	Apple juice, associated with 1996 outbreak	2.80 ± 0.20	0.02 ± 0.01
B0204	SRCC 1941	O157:H7	Pork, September 2002	2.52 ± 0.16	0.1 ± 0.03
B0202	SRCC 1486	O157:H7	Salami, October 2002	2.14 ± 0.06	2.89 ± 0.06
B0203	SRCC 2061	O157:H7	Ground beef, October 2002	1.22 ± 0.32	0.09 ± 0.07
B0348	38094	O157:H7	Salami	1.12 ± 0.13	0.04 ± 0.03
B0350	960212	O157:H7	Sakai	1.11 ± 0.27	0.03 ± 0.02
B0243	3004-98	O157:H7	Bovine carcass	1.63 ± 0.24	0.29 ± 0.06
B0257	234AB1	O157	Bovine carcass	1.62 ± 0.11	0.06 ± 0.09
B0235	5A-1	non-O157	Bovine feces *(6)*	1.50 ± 0.13	0.07 ± 0.07
B0256	183 (H18-1)	O157	Bovine carcass	1.41 ± 0.20	0.02 ± 0.01
B0242	233AC1	O157:H7	Bovine carcass *(4, 25)*	1.31 ± 0.12	0.11 ± 0.06
B0240	294RC1	O157:H7	Bovine carcass *(4, 25)*	1.17 ± 0.15	0.17 ± 0.08
B0239	258AC1	O157:H7	Bovine carcass *(4, 25)*	1.13 ± 0.35	0.39 ± 0.10
B0237	1C-3	non-O157	Bovine feces *(6)*	0.95 ± 0.15	0.08 ± 0.01
B0238	97AC1	O157:H7	Bovine carcass *(4, 25)*	0.64 ± 0.07	0.03 ± 0.02
B0241	28RC1	O157:H7	Bovine carcass *(4, 25)*	0.46 ± 0.11	0.08 ± 0.10
B0258	F6B-2	O157:H7	Bovine feces *(5)*	1.64 ± 0.09	0.16 ± 0.10
B0234	3A-1	non-O157	Bovine feces *(6)*	1.60 ± 0.19	0.02 ± 0.02
B0259	F7B-1	O157:H7	Bovine feces *(5)*	1.56 ± 0.33	0.32 ± 0.19
B0254	106-4-1	O157	Bovine feces	1.54 ± 0.24	0.29 ± 0.13
B0262	NS-P410	O157	Bovine feces	1.53 ± 0.01	0.08 ± 0.07
B0260	F15B-1	O157	Bovine feces *(5)*	1.42 ± 0.12	0.28 ± 0.13
B0236	3C-3	non-O157	Bovine feces *(6)*	1.40 ± 0.20	0.02 ± 0.01
B0261	902-2	O157	Bovine feces *(7)*	1.33 ± 0.17	0.16 ± 0.19
B0253	1307-8(8-1)	O157	Bovine feces	1.19 ± 0.20	0.23 ± 0.06
B0255	207-10-2	O157	Bovine feces	1.12 ± 0.45	0.28 ± 0.15
B0233	1A-5	non-O157	Bovine feces *(6)*	1.08 ± 0.23	0.07 ± 0.02
B0301	RM5630	O157:H7	Water *(18)*	3.57 ± 0.32	0.12 ± 0.00
B0307	RM5875	O157:H7	Water *(18)*	2.41 ± 0.40	0.03 ± 0.02
B0306	RM5850	O157:H7	Water *(18)*	2.38 ± 0.42	0.04 ± 0.03
B0309	RM5714	O157:H7	Water *(18)*	2.13 ± 0.20	0.20 ± 0.15
B0302	RM5667	O157:H7	Water *(18)*	1.40 ± 0.12	0.10 ± 0.04
B0297	RM5450	O157:H7	Water *(18)*	1.66 ± 0.15	0.14 ± 0.03
B0299	RM5607	O157:H7	Water *(18)*	1.66 ± 0.38	0.35 ± 0.39
B0285	RM4886	O157:H7	Water *(18)*	1.38 ± 0.22	0.07 ± 0.02
B0275	RM4859	O157:H7	Water *(18)*	1.36 ± 0.36	0.34 ± 0.08
B0305	RM5754	O157:H7	Water *(18)*	1.35 ± 0.45	0.05 ± 0.05
B0281	RM4876	O157:H7	Water *(18)*	1.28 ± 0.13	0.11 ± 0.11
B0289	RM5036	O157:H7	Water *(18)*	1.21 ± 0.16	0.05 ± 0.04
B0280	RM4864	O157:H7	Water *(18)*	1.10 ± 0.07	0.10 ± 0.00
B0287	RM4888	O157:H7	Water *(18)*	0.80 ± 0.15	0.12 ± 0.06
B0283	RM4884	O157:H7	Water *(18)*	0.50 ± 0.33	0.05 ± 0.00
B0269	RM4263	O157:H7	Human, outbreak, 2000, waterborne	4.71 ± 0.08	0.34 ± 0.34
B0273	RM4688	O157:H7	Human, outbreak, 2002, leafy vegetable	4.13 ± 0.46	0.07 ± 0.07
B0247	3159-98	O157:H7	Human outbreak *(22)*	3.63 ± 0.34	0.08 ± 0.08
B0296	RM5279	O157:H7	Human, outbreak, 2005, leafy vegetable	3.58 ± 0.32	0.17 ± 0.10
B0311	RM6011	O157:H7	Human, outbreak, 2006, leafy vegetable	3.43 ± 0.13	0.35 ± 0.03
B0246	3139-98	O157:H7	Human outbreak *(22)*	3.35 ± 0.21	0.05 ± 0.01
B0271	RM4406	O157:H7	Human, outbreak, 2003, leafy vegetable	3.15 ± 0.34	3.75 ± 0.06
B0250	3261-98	O157:H7	Human outbreak	2.90 ± 0.31	0.11 ± 0.13
B0263	RM1242	O157:H7	Human, sporadic, 1997	2.85 ± 0.37	0.09 ± 0.11
B0251	3361-91	O157:H7	Human outbreak *(22)*	2.82 ± 0.52	0.09 ± 0.11
B0249	3187-95	O157:H7	Human outbreak	2.75 ± 0.25	0.07 ± 0.09
B0266	RM2189	O157:H7	Human, outbreak, 1999, taco meat	2.73 ± 0.51	0.19 ± 0.20
B0245	3055-93	O157:H7	Human outbreak *(22)*	2.72 ± 0.29	0.28 ± 0.04
B0265	RM1918	O157:H7	Human, outbreak, 1999, lettuce	2.57 ± 0.34	0.11 ± 0.15
B0244	3014-93	O157:H7	Human outbreak *(22)*	2.43 ± 0.04	0.11 ± 0.15

[a] ID, identification.

[b] Values are means ± standard deviations of the results from three independent replicate tests for each strain.

[c] The strains in boldface were selected to make mixtures representing the corresponding sources.

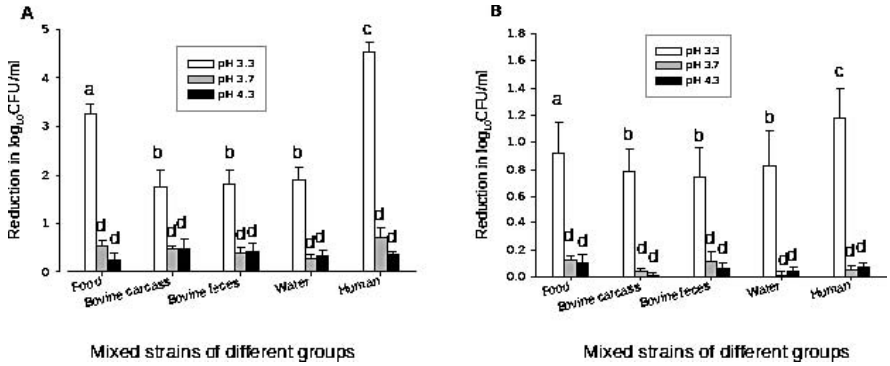

FIGURE 1. *The effect of 400 mM acetic acid on the survival of the mixtures of* E. coli *O157:H7 strains from different sources at different pH values (white bars, pH 3.3; gray bars, pH 3.7; black bars, pH 4.3) and different temperatures. (A) 30°C. (B) 22°C. The bars indicate the mean values, and the error bars indicate the standard deviations (*n *= 3). Different letters indicate a significant (*P *< 0.05) difference.*

log CFU/ml, $P = 0.998$) or the water isolates (mean reduction was 1.65 log CFU/ml, $P = 0.75$). Surprisingly, the human isolates were the most sensitive to the acetic acid solution, and their survival was significantly ($P < 0.0001$) lower than that of any of the other four groups of isolates (to any group). The sensitivity of each of the 15 human isolates was significantly greater than that of the eight bovine carcass isolates and 13 bovine feces isolates.

To further investigate the survival of the *E. coli* strains from different sources, four to five strains from each source were selected to make a mixture representing their corresponding source (the selected strain identification numbers are those shown in boldface in Table 1) and were treated under additional conditions chosen for their relevance to acidified foods. The effect of 400 mM acetic acid on the survival of the cocktail of *E. coli* O157:H7 strains from different sources at different pH values, temperatures, and atmospheric conditions are illustrated in Figures 1 and 2.

The resistance of the mixed-strain suspensions representing the five different source groups to the acetic acid solution (400 mM, pH 3.3, and 30°C for 25 min) had the same trend as that of the individual strains from the corresponding groups (Table 1 and Figure 1). When the mixed cells of human outbreak isolates were exposed to the acetic acid solution, the viable cell density was decreased by approximately 4.5 orders of magnitude, making this the group most sensitive to the acid solution under these conditions. The viable cell densities for the mixtures of food, bovine carcass, bovine feces, and water isolates were reduced by 3.3, 1.8, 1.8, and 1.9 log CFU/ml, respectively. However, the sensitivity of *E. coli* O157:H7 to the acetic acid solution was significantly decreased when pH increased to 3.7 and 4.3 at 30°C. There was no significant ($P \geq 0.05$) difference in the survival of the isolate mixtures from different sources (Fig. 1A) at a relatively higher pH (3.7 or 4.3). When the challenge test was performed at lower temperatures, the sensitivity of *E. coli* O157:H7 to the 400 mM acetic acid solution was also significantly reduced. At 22°C, the acetic acid solution reduced the population of the human isolate mixture by approximately 1.2 log CFU/ml. Fewer than 90% of the cells in the strain mixtures from the other four groups were inactivated (Fig. 1B). At 15°C, there was little or no detectable inhibitory effect(s) on any mixtures of *E. coli* O157:H7 strains, regardless of the pH values (data not shown).

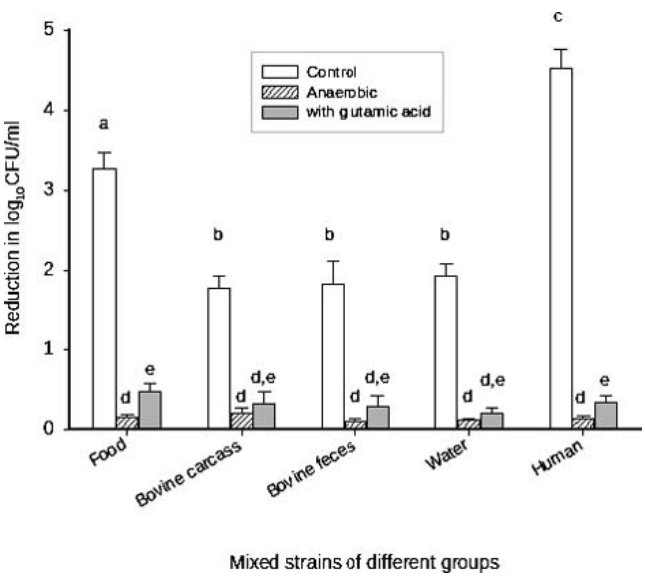

FIGURE 2. *The effect of anaerobic (striped bars) incubation or addition of glutamic acid (gray bars) on the acetic acid resistance of the mixture of* E. coli *O157:H7 strains from different sources at pH 3.3 and 30°C. The white bars represent the control treatment (aerobic, no glutamic acid) as shown in Figure 1A (pH 3.3). The bars indicate the mean values and the error bars indicate the standard deviations (*n *= 3). Different letters indicate a significant (*P *< 0.05) difference.*

We compared the sensitivity of *E. coli* O157:H7 strains from different sources in 400 mM acetic acid solution (pH 3.3, 30°C for 25 min) in the presence and absence of dissolved oxygen. Under anaerobic conditions (Fig. 2), the *E. coli* O157:H7 cells from all five sources showed little or no reduction in cell numbers, as we have previously observed *(31).* There was a significant ($P < 0.05$) difference in acetic acid resistance among the mixtures of *E. coli* O157:H7 strains from different sources between anaerobic and aerobic conditions, however. We also determined the survival of the mixtures of *E. coli* O157:H7 strains from different sources in 400 mM acetic acid containing 2 mM glutamic acid (Fig. 2). Acetic acid containing glutamic acid showed minimal inhibitory effects on all of the *E. coli* O157:H7 strains, irrespective of pH values or temperatures, and there was no significant difference in acetic acid resistance among the groups tested with glutamic acid.

PFGE was applied for molecular typing of 24 *E. coli* O157:H7 isolates from different sources (Fig. 3). Using digestion with *Spe*I, a total of 20 restriction enzyme digestion

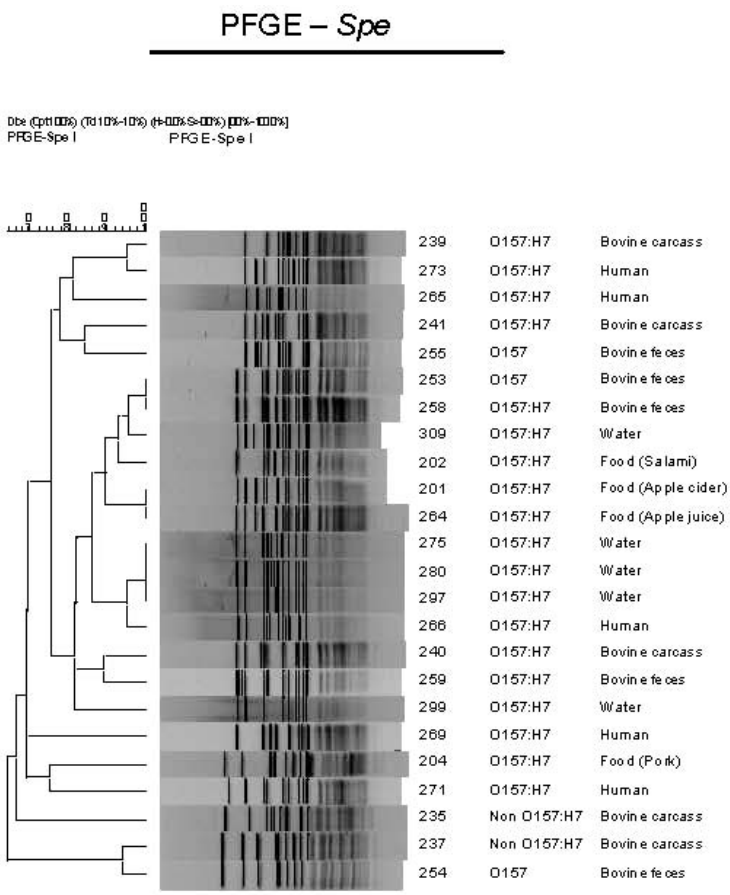

FIGURE 3. *Dendogram of PFGE data for the isolates used for the group analysis. The alignment of the bands and the dendogram were generated with Molecular Analyst software, as described in the text. The numbers next to each lane are strain designations (B0-numbers from Table 1), which are followed by serotype and source isolation.*

patterns were identified. The diversity index of PFGE typing was 0.83 (20 types identified among 24 isolates: 20 of 24 = 0.83). Isolates 275, 280, and 297, with the same PFGE-*Spe*I pattern, were isolated from water; similarly, isolates 253 and 258 from bovine feces, and isolates 201 and 264 from food (apple cider) were matched based on source. However, all of the five human clinical isolates showed different PFGE–*Spe*I types. The 10 isolates from bovine sources were found to have 8 different types. These data indicated that isolates from the same source having similar acid resistance do not show an obvious trend to be grouped together by PFGE pattern.

DISCUSSION

Differences in acid tolerance among isolates of *E. coli* have been reported *(9, 21, 26, 33, 37, 42),* but it is difficult to compare these data, because the studies were performed with different microorganisms, growth media, physiological status of bacteria, organic acids, pH, and incubation temperatures. Our studies focused on the acetic acid resistance of a mixture of *E. coli* O157:H7 isolates from different sources. Interestingly, the non-O157:H7 strains from bovine feces had acid resistance characteristics similar to the O157:H7 strains (Table 1). Acetic acid is the most common organic acid present in acidified foods. Contrary to our results, McKellar and Knight *(37)* reported that human outbreak *E. coli* O157:H7 strains were significantly ($P < 0.05$) better able to survive acid treatment than were strains from animal or human sources. However, our study used the procedure of Buchanan and Edelson *(16)* to induce acid resistance during preparation of the bacterial cells. Our results showed that temperature, pH, and atmosphere are the significant factors that can alter acid resistance of *E. coli* O157:H7 in acetic acid solution. Cheville et al. *(19)* showed that resistance to heat and acid challenges can be influenced by the incubation temperature of the microorganism prior to an acid challenge. Previously, we determined that dissolved oxygen influences significantly the acid sensitivity of *E. coli* O157:H7 *(31).* Wild-type *E. coli* O157:H7 cultures that were grown aerobically required less acetate to induce extreme acid resistance than did those grown anaerobically, and the addition of the reducing agent cysteine to the media greatly increased the requirement for acetate *(23).* Small et al. *(41)* reported that anaerobic growth under acidic conditions restored acid resistance of a nonpathogenic *E. coli rpoS* mutant.

For comparisons between bacterial strains isolated from different sources, we held temperature, pH, and ionic strength constant to control protonated acid concentrations, as described previously *(11).* The adaptation of *E. coli* O157:H7 strains for growth or survival in different environments, including rumen, meat, water, or human gastrointestinal tract, may contribute to the differences in sensitivity to acetic acid that we observed. Exposure to environmental stresses other than acid may lead to altered acid resistance due to cross-protection *(19, 20).* Because the genes regulating responses to acid stress are controlled by global regulatory systems *(29, 32, 39),* it is possible that

changes in expression of key regulatory genes, such as *rpoS* may be responsible for the stain differences observed. Lin et al. *(35, 36)* developed assays to separate three different acid resistance mechanisms by which *E. coli* can survive in low-pH environments for extended periods: arginine and glutamate decarboxylase–dependent systems and a glucose catabolite-repression system. They suggested that these mechanisms promote survival in low-pH environments, such as stomach and acid foods. There was no significant difference between the groups in overall decarboxylate-dependent system of acetic acid resistance (Table 1, column 6); however, several strains (B0201, B0202, and B0271 listed in Table 1) apparently lacked the glutamate decarboxylation system *(30),* or if it were present, may have not been functional. *E. coli* O157:H7 strains lacking the glutamate decarboxylase system were also found by Bhagwat et al. *(9).* These strains are currently the subject of further research, and the role of the glutamate decarboxylase system in survival *of E. coli* strains in food systems remains unclear *(40).* Our results confirmed that significant differences in acid resistance among *E. coli* O157:H7 isolates from different sources exist. For example, the human outbreak strains, in general, were more sensitive in the absence of glutamic acid than were any other group of strains (Table 1). One outbreak strain, B0271, apparently lacked detectable glutamic acid decarboxylase activity (Table 1). It is interesting that the human isolates were less acid resistant than were the environmental isolates tested in this study, because acid resistance is a virulence factor for *E. coli* O157:H7, aiding in transit of the organism through the stomach *(20).*

It is worth noting also that the outbreak strains were diverse based on spatial, temporal, and genotypic differences. Eight of the human outbreaks strains (Table 1, the strains designated ''RM'') represented eight different outbreaks occurring between 1997 and 2006, and associated with different food or environmental sources (taco meat, various leafy vegetables, and water), and multiple genotypes *(22).* Whiting and Golden *(42)* reported previously on the amount of variation existing among strains of *E. coli* O157:H7, showing *D*-values in acid and preservative treatments at pH 4.8 ranging from 24 to 189 h. Large et al. *(33)* observed that the strain-to-strain variation within a clonal group was larger than variation between replicates or due to experimental error. These data are consistent with our results indicating no significant correlation of resistance with genotype. Genetic analysis of several of these isolates (RM strains) has shown differences between PFGE and multilocus variable-number tandem-repeat analysis studies *(22).* Thus, conclusions regarding the genetic differences in the acid resistance of *E. coli* O157:H7 groups of bacteria will require examination of additional isolates from a variety of sources.

The development of acid tolerance by foodborne pathogenic bacteria may be significant at several points along the farm-to-table continuum of food production. It is important to understand how previous environment and processing conditions can affect the acid tolerance status of foodborne *E. coli* O157:H7 in order to devise strategies for better control of the occurrence, growth, or survival of this organism in foods.

ACKNOWLEDGMENTS

The authors acknowledge Dr. Weiming Gu for his technical support of PFGE procedure, Ms. Sandra Parker for excellent secretarial assistance, and Diana Carychao for characterization of some of the strains. This investigation was supported in part by a research grant from Pickle Packers International, Inc., Washington, DC, Kangwon National University, and U.S. Department of Agriculture, Cooperative State Research, Education, and Extension Service project number 2006-01240.

REFERENCES

1. Ahamad, N., and E. H. Marth. 1989. Behavior of *Listeria monocytogenes* at 7, 13, 21 and 35°C in tryptose broth acidified with acetic, citric and lactic acids. *J. Food Prot.* 52:688–695.
2. Arnold, K. W., and C. W. Kaspar. 1995. Starvation- and stationary-phase–induced acid tolerance in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 61:2037–2039.
3. Anonymous. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami. *Morb. Mortal. Wkly. Rep.* 44:157–160.
4. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
5. Berry, E. D., and G. A. Barkocy-Gallagher. 2002. Acid resistance status of *Escherichia coli* O157 in bovine feces as shed from naturally contaminated cattle. American Dairy Science Association–American Society of Animal Science–Canadian Society of Animal Science Joint Meeting, Quebec City, Quebec, Canada, 21 to 25 July 2002.
6. Berry, E. D., G. A. Barkocy-Gallagher, and G. R. Siragusa. 2004. Stationary-phase acid resistance and injury of recent bovine *Escherichia coli* O157 and non-O157 biotype 1 *Escherichia coli* isolates. *J. Food Prot.* 67:583–590.
7. Berry, E. D., and D. N. Miller. 2005. Cattle feedlot soil moisture and manure content. II. Impact on *Escherichia coli* O157. *J. Environ. Qual.* 34:656–663.
8. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. *JAMA* 269:2217–2220.
9. Bhagwat, A. A., L. Chan, R. Han, J. Tan, M. Kothary, J. Jean-Gilles, and B. D. Tall. 2005. Characterization of enterohemorrhagic *Escherichia coli* strains based on acid resistance phenotypes. *Infect. Immun.* 73:4993–5003.
10. Booth, I. R., and R. G. Kroll. 1989. The preservation of foods by low pH, p. 119–160. *In* G. W. Gould (ed.). Mechanisms of action of food preservation procedures. Elsevier Science Publishers, New York.
11. Breidt, F., 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.
12. Breidt, F., J. S. 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.
13. 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.
14. Brudzinski, L., and M. A. Harrison. 1998. Influence of incubation conditions on survival and acid tolerance response of *Escherichia coli* O157:H7 and non-O157:H7 isolates exposed to acetic acid. *J. Food Prot.* 61:542–546.
15. Brul, S., and P. Coote. 1999. Preservative agents in foods: mode of action and microbial resistance mechanism. *Int. J. Food Microbiol.* 50:1–17.

16. Buchanan, R. L., and S. G. 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.
17. Buchanan, R. L., and S. G. Edelson. 1999. pH-dependent stationary-phase acid resistance response of enterohemorrhagic *Escherichia coli* in the presence of various acidulants. *J. Food Prot.* 62:211–218.
18. Centers for Disease Control and Prevention. 1999. Public health dispatch: outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington County Fair—New York, 1999. *Morb. Mortal. Wkly. Rep.* 48:803–804.
19. Cheville, A., K. W. Armold, C. M. Cheng, and C. W. Kasper. 1996. *rpoS* regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 62:1822–1824.
20. Chung, H. J., W. Bang, and M. A. Drake. 2006. Stress response of *Escherichia coli*. *Comp. Rev. Food Sci. Food Saf.* 5:52–64.
21. Conner, D. E., and J. S. Kotrola. 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. *Appl. Environ. Microbiol.* 61:382–385.
22. Cooley, M., D. Carychao, L. Crawford-Miksza, M. T. Jay, C. Myers, C. Rose, C. Keys, J. Farrar, and R. E. Mandrell. 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. *PloS ONE* 2:e1159.
23. Diez-Gonzalez, F., and J. B. Russell. 1999. Factors affecting the extreme acid resistance of *Escherichia coli* O157:H7. *Food Microbiol.* 16:367–374.
24. Dougherty, D. P., E. Ramos Da Conceicao Neta, R. F. McFeeters, S. R. Lubkin, and F. Breidt, Jr. 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.
25. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
26. Entani, E., M. Asai, S. Tsujihata, Y. Tsukamoto, and M. Ohta. 1998. Antimicrobial action of vinegar against foodborne pathogenic bacteria including *Escherichia coli* O157:H7. *J. Food Prot.* 61:953–959.
27. Farber, J. M., and F. Pagotto. 1992. The effect of acid shock on the heat resistance of *Listeria monocytogenes*. *Lett. Appl. Microbiol.* 15: 197–201.
28. Glass, K. A., J. M. Loeffelholz, J. P. Ford, and M. P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. *Appl. Environ. Microbiol.* 58: 2513–2516.
29. Hengge-Aronis, R. 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*. *Cell* 72: 165–168.
30. Hersh, B. M., F. T. Farooq, D. N. Barstad, D. L. Blankenshorn, and J. L. Slonczewski. 1996. A glutamate-dependent acid resistance gene in *Escherichia coli*. *J. Bacteriol.* 178:3978–3981.
31. Kreske, A. C., K. Bjornsdottir, F. Breidt, and H. Hassan. 2008. Effects of pH, dissolved oxygen, and ionic strength on the survival of *Escherichia coli* O157:H7 in organic acid solutions. *J. Food Prot.* 71:2404–2409.
32. Lange, R., and R. Hengge-Aronis. 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. *Mol. Microbiol.* 5:49–59.
33. Large, T. M., S. T. Walk, and T. S. Whittam. 2005. Variation in acid resistance among Shiga toxin–producing clones of pathogenic *Escherichia coli*. *Appl. Environ. Microbiol.* 71:2493–2500.
34. Leyer, G. J., and E. A. Johnson. 1993. Acid adaptation induces cross-protection against environmental stress in *Salmonella typhimurium*. *Appl. Environ. Microbiol.* 59:1842–1847.
35. Lin, J., I. Lee, J. Frey, J. L. Slonczewski, and J. W. Foster. 1995. Comparative analysis of extreme acid survival in *Salmonella typhimurium, Shigella flexneri,* and *Escherichia coli*. *J. Bacteriol.* 177: 4097–4104.
36. Lin, J., M. P. Smith, K. C. Chapin, H. S. Baik, G. N. Bennett, and J. W. Foster. 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl. Environ. Microbiol.* 62:3094–3100.
37. McKellar, R., and K. Knight. 1999. Growth and survival of various strains of enterohemorrhagic *Escherichia coli* in hydrochloric and acetic acid. *J. Food Prot.* 62:1466–1469.
38. Miller, L. G., and C. W. Kasper. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. *J. Food Prot.* 57:460–464.
39. Price, S. B., C. M. Cheng, C. W. Kasper, J. C. Wright, F. J. DeGraves, T. A. Penfound, M. P. Castanie-Cornet, and J. W. Foster. 2000. Role of *rpoS* in acid resistance and fecal shedding of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 66:632–637.
40. Price, S. B., J. C. Wright, F. J. DeGraves, M. P. Castanie-Cornet, and J. W. Foster. 2004. Acid resistance systems required for survival of *Escherichia coli* O157:H7 in the bovine gastrointestinal tract and in apple cider are different. *Appl. Environ. Microbiol.* 70:4792–4799.
41. Small, P., D. Blankenhorn, D. Welty, E. Zinser, and J. L. Slonczewski. 1994. Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: role of *rpoS* and growth pH. *J. Bacteriol.* 176:1729–1737.
42. Whiting, R. C., and M. H. Golden. 2002. Variation among *Escherichia coli* O157:H7 strains relative to their growth, survival, thermal inactivation, and toxin production in broth. *Int. J. Food Microbiol.* 75:127–133.