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The Effect of Acid Adaptation Conditions on Heat Resistance of *Escherichia coli* O157: H7

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Abstract

The objective of this study was to determine the effect of acid adaptation conditions on heat resistance of *E. coli* O157: H7 932. *E. coli* O157: H7 was adapted to acid by exposing the cells to pH 4.5 (2h), pH 5.0 (1h), and pH 5.5 (1 h) in tryptic soy broth. D and z values of the acid adapted and control cultures at 54°C, 56°C, and 58°C were determined in E buffer. The heat resistance of *E. coli* O157: H7 increased significantly ($p<0.05$) after acid adaptation at pH 4.5 or pH 5.0. *E. coli* O157: H7 adapted to acid at pH 4.5 for 2 h had the highest D values at all temperatures tested (20.3–10.7–3.3 min) while D values of culture adapted to acid at pH 5.0 for 1 h were 18.2, 7.9, and 2.6 min at 54°C, 56°C and 58°C, respectively. Heat resistance of culture adapted to acid at pH 5.5 for 1 h and the control culture was not significantly different ($P<0.05$). Culture adapted to acid at pH 4.5 had the highest z value (5.10°C), whereas control culture had the lowest z value (4.33°C). This study showed that the magnitude of heat tolerance changed with the adaptation pH and at low adaptation pH, *E. coli* O157: H7 showed maximum heat resistance. Acid adaptation at pH 4.5 or 5.0 provides *E. coli* O157: H7 with cross-protection against heat treatments, and that this factor must be considered to estimate this pathogen's thermal tolerance accurately.

Key words: *E. coli* O157:H7, acid adaptation, heat resistance.

Introduction

E. coli O157: H7 is an important food-borne pathogen that causes the disease syndromes of hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in humans (Griffin and Tauxe, 1991). It was identified in 1982 and outbreaks of food-borne illness due to *E. coli* O157:H7 have been reported with increasing frequency since that time (Bell, 2002). Undercooked ground beef has been implicated most often in outbreaks of food-borne illness caused by *E. coli* O157:H7, but more acidic foods such as unpasteurized apple juice (McCarty, 1996), apple cider (Besser *et al.*, 1993), mayonnaise (Weagant *et al.*, 1994) and yoghurt (Morgan *et al.*, 1993) have also been implicated in outbreaks.

The acid tolerance property may allow *E. coli* O157:H7 to survive in highly acidic foods. The extended survival of enteric pathogens in acidic foods and the increased tolerance of acid adapted cells to unfavourable growth conditions is well established (Arnold and Kaspar, 1995; Leyer *et al.*, 1995; Semanchek and Golden, 1996). Briefly acid adaptation or acid tolerance is a phenomenon by which microorganisms show an increased resistance to environmental stress after the exposure to a moderate acid environment (Foster, 1991). Acid adaptation may also increase the heat resistance of pathogens. Leyer and Johnson (1993) showed that acid adaptation of *Salmonella typhimurium* resulted in increased thermal tolerance. Farber and Pagotto (1992) also reported that acid adapted *Listeria monocytogenes* enhanced thermal tolerance.

Previously in our laboratory we have demonstrated that *E. coli* O157:H7 has the ability to survive at extremely low pH (pH 3.0) if first adapted to mild pH (pH 4.5–5.5). Furthermore, it was also noted that the

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extent of increased acid tolerance was affected by the adaptation pH. Maximum acid tolerance was observed in *E. coli* O157:H7 adapted to acid at pH 4.5, subsequently to pH 5.0 and pH 5.5 (Tosun, 2003).

The objective of this study was to determine the effect of acid adaptation conditions on heat resistance of *E. coli* O157:H7.

Experimental

Materials and Methods

Bacterial strain and media. *E. coli* O157:H7 932 strain (a clinical isolate) was provided by M. P. Doyle (Center for Food Safety and Quality Enhancement. Department of Food Science and Technology, The University of Georgia, Griffin, USA).

E. coli O157:H7 was stored at +4°C in tryptic soy agar (TSA, Oxoid) and subcultured every month. Culture was activated from stock culture after two successive transfers of the test organism in tryptic soy broth (TSB, Oxoid) at 37°C for 24 h. This activated culture was used in experimental studies.

Acid adaptation of test organism. To prepare the acid adapted cells of *E. coli* O157:H7 the procedure described by Tosun (2003) was followed. Cells were grown overnight at 37°C in TSB at pH 7.0 and 40 millilitres of the cultures was taken and dispensed equally to four centrifuge tubes. Cultures were centrifuged at 5000 rpm. The supernatant was discarded, and the cell pellets were suspended in 10 ml of pH 4.5, 5.0 and 5.5 TSB (pH adjusted with 6N HCI) for acid adapted cells and other cell pellets were suspended in 10 ml of pH 7.0 TSB for nonadapted cells (control culture). Culture adapted to acid at pH 4.5 was incubated at 37°C for 2 h. Other acid adapted and control cultures were incubated at 37°C for 1 h.

Calculation of D and z values. Acid adapted and control cultures were centrifuged at 5000 rpm. The supernatant was discarded and the cell pellets were washed once with 10 ml of E buffer (Vogel and Bonner, 1956) pH 7.0, centrifuged and resuspended in the same amount of E buffer. E buffer was prepared at 50x strength as follows. In distilled water (670 ml) were dissolved successively, $MgSO_4 \times 7H_2O$ (10 g), citric acid $\times H_20$ (100 g), $K_2HPO_4 \times$ anhydrous (500 g), and $NaNH_4HPO4 \times 4H_2O$ (175 g), the final volume being about 1 liter. After 50-fold dilution with distilled water, the resulting single strength medium has pH of 7.00. 1 ml inoculum was taken from acid adapted and control culture and added to 100 ml of E buffer heated at 54°C, 56°C and 58°C in 250 ml Erlenmeyer flasks in a water bath. The surface of E buffer in the flask was 2 cm below the level of the water surface during heat treatment. Uninoculated sample was used for temperature control by using a thermometer.

D values (time to inactivate 90% of the population) were calculated by plotting the log number of survivors against time for each heating temperature. The line of best fit for survivors plots was determined by linear regression analysis.

Z values (change in heating temperature needed to change the D value by 90%) were estimated by plotting the log D values versus heating temperatures.

Enumeration of *E. coli* O157:H7. Viable cells of acid adapted and control cells were enumerated immediately at every 4 or 10 minute of heating. Serial decimal dilutions in 0.1% peptone water were prepared. The viable populations of *E. coli* O157:H7 were than determined by plating 0.1 ml of the serially diluted samples on sorbitol MacConkey agar (SMAC, Oxoid) and incubated at 37°C fo2r 4–48 h. *E. coli* O157:H7 formed colourless colonies on SMAC agar.

Statistical analysis. Experiments were run in duplicates. Data were analysed using the SPSS 9.0 statistical software (SPSS Inc., Chicago, IL, USA) for analysis of variance and Duncan's multiple range test.

Results

Thermal inactivation curves for acid adapted and control *E. coli* O157:H7 cells heated in E buffer at 54°C, 56°C and 58°C are shown in Figure 1. D values for acid adapted and control cultures are listed in Table I. *E. coli* O157:H7 adapted to acid at pH 4.5 for 2 h had the highest D values of all temperatures

Table I
D values and correlation coefficients of thermal inactivation curves for acid adapted and control culture of *E. coli* O157:H7

Temperature	Cell type	Correlation coefficient	D value
54°C	acid adapted (pH 4.5)	0.86	20.3
	acid adapted (pH 5.0)	0.80	18.2
	acid adapted (pH 5.5)	0.84	14.0
	control culture (pH 7.0)	0.86	15.9
56°C	acid adapted (pH 4.5)	0.96	10.7
	acid adapted (pH 5.0)	0.99	7.90
	acid adapted (pH 5.5)	0.97	5.58
	control culture (pH 7.0)	0.94	7.41
58°C	acid adapted (pH 4.5)	0.93	3.3
	acid adapted (pH 5.0)	0.98	2.6
	acid adapted (pH 5.5)	0.99	2.3
	control culture (pH 7.0)	0.98	1.9

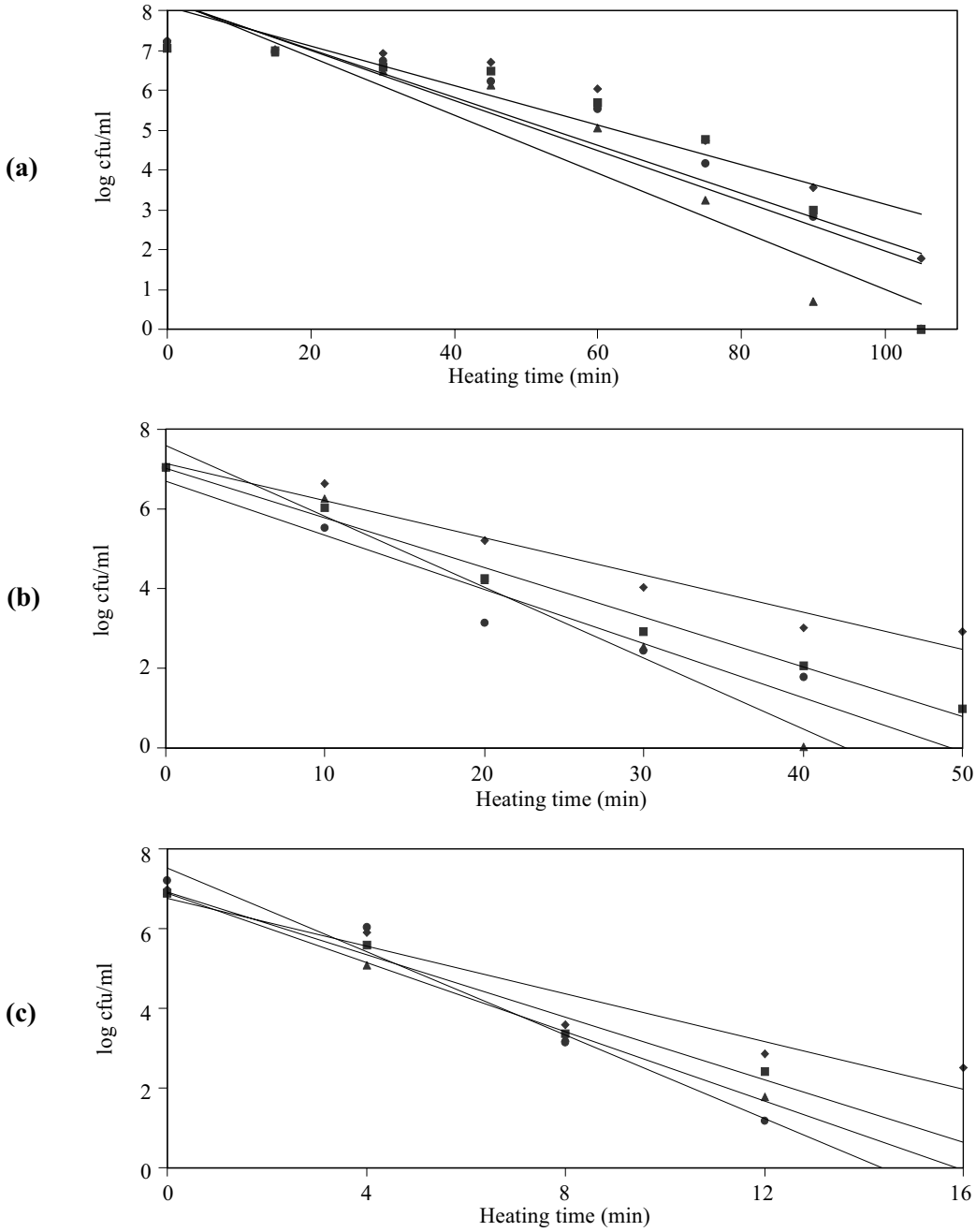

Fig. 1. Thermal inactivation curves for *E. coli* O157:H7 adapted to acid at various pH.
pH 4.5 (◆), pH 5.0 (■), pH 5.5 (▲), control culture (●); heated at 54°C (a), 56°C (b) and 58°C (c) in E buffer

tested. There was no statistically significant difference between the D values of culture adapted to acid at pH 5.5 and control culture ($P<0.05$). However D values of culture adapted to acid at pH 5.0 for 1 h were higher than those of culture adapted to acid at pH 5.5 or those of control culture ($P<0.05$).

Table II
Z values and correlation coefficients of thermal death time curves for acid adapted and control culture of *E. coli* O157:H7

Cell type	Correlation coefficient	Z value (°C)
Acid adapted (pH 4.5)	0.97	5.10
Acid adapted (pH 5.0)	0.99	4.71
Acid adapted (pH 5.5)	0.99	5.09
Control culture (pH 7.0)	0.97	4.33

Regression statistics and z values are listed in Table II. *E. coli* O157:H7 adapted to acid at pH 4.5 for 2 h had the highest z value (5.10°C), whereas control culture had the lowest z value (4.33°C).

Acid adaptation of *E. coli* O157: H7 at pH 4.5 or 5.0 significantly increased the heat resistance of *E. coli* O157: H7 in all temperatures tested ($P<0.05$). However heat resistance of *E. coli* O157: H7 adapted to acid at pH 5.5 for 1 h was not significantly different from that of the control ($P<0.05$).

Discussion

Microbial adaptation responses to one stress can lead to cross protection against another stress. It has been long recognised that pH can affect microbial thermal resistance. Increased thermal tolerance resulting from bacterial responses to exposure to an acidic environment has been demonstrated in *L. monocytogenes* (Farber and Pagotto, 1992). Leyer and Johnson (1993) also reported that acid adapted *S. typhimurium* cells had higher heat tolerance than nonadapted counterparts.

Cheville *et al.* (1996) reported that exposure of *E. coli* O157: H7 to an environment induced acid shock resulting in expression of new genes, regulated by the alternative sigma factor that is encoded by the *rpoS* locus. Perhaps, RpoS regulated proteins cross-protection against heat.

Ryu and Beuchat (1998) and Mazzotta (2001) reported that acid adaptation significantly increased the heat resistance of *E. coli* O157: H7. In these studies acid tolerance was determined for only one pH value, however in our current study heat tolerance of *E. coli* O157: H7 adapted to acid at different pH value was determined. In this study we showed that the magnitude of heat tolerance changed with the adaptation pH and lower the adaptation pH the greater the heat tolerance.

At each heating temperature the D values of cells adapted to acid at pH 4.5 or 5.0 were significantly higher than D values of control cells. The level of heat tolerance in *E. coli* O157: H7 was influenced by adaptation pH.

Splittstoesser *et al.* (1996) reported that D values of *E. coli* O157: H7 was 2.5 min when heated in apple cider (pH 4.5) at 58°C. $D_{58°C}$ value of our test strain adapted to acid at pH 4.5 observed in our study was considerably higher than the value of 2.5 min reported by these researches. Doyle and Schoeni (1984) reported that D values for *E. coli* O157: H7 (strain 932) in ground beef were 39.83 min at 54.4°C and 4.5 min at 57.2°C. Our study shows that $D_{54°C}$ values of strain 932 were 20.3, 18.2, 14.0 and 15 min according to adaptation pH. Our test strain has lower D values because TSB contains no fat to protect cells which might occur in ground beef.

In summary, maximum heat resistance was observed when *E. coli* O157: H7 was adapted to acid at pH 4.5 for 2 h. These findings may have important implications in the food industry because most of fermented foods have pH value near 4.5. In addition, hot water and organic acid sprays are frequently used for the decontamination of beef carcasses in the meat industry. Increased tolerance of acid adapted *E. coli* O157: H7 cells to heat could have practical implications when establishing mild thermal processing schemes to eliminate the organism from fermented foods.

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Literature

Arnold K.W. and C.W. Kaspar. 1995. Starvation and stationary phase induced acid tolerance in *Escherichia coli* O157: H7. *Appl. Environmen. Microbiol.* **61**: 2037–2039.

Bell C. 2002. Approach to the control of enterohaemorrhagic *Escherichia coli* (EHEC). *Int. J. Food Microbiol.* **78**: 197–216.

Besser R.E., S.M. Lett and J.T. Weber. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157: H7 in fresh-pressed apple cider. *J. American. Med. Associat.* **269**: 2217–2220.

Cheville A.M., D.W. Arnold., C. Buchrieser, C.M. Cheng and C.W. Kaspar. 1996. RpoS regulation of acid, heat, and salt tolerance in *Escherichia coli* O157: H7. *Appl. Environ. Microbiol.* **62**: 1822–1824.

Doyle M.P. and J.L. Schoeni. 1984. Survival and growth characteristics of *E. coli* associated with hemorrhagic colitis. *Appl. Environ. Microbiol.* **48**: 855–856.

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.

Foster J.W. 1991. *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. *J. Bacteriol.* **173**: 6896–6902.

Griffin P.M. and R.V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli* and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* **13**: 60–97.

Leyer G.J. and E.A. Johnson. 1993. Acid adaptation induces cross protection against environmental stresses in *Salmonella typhimurium*. *Appl. Environmen. Microbiol.* **59**: 1842–1847.

Leyer G.J., L. Wang and E.A. Johnson. 1995. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. *Appl. Environmen. Microbiol.* **61**: 3752–3755.

Mazzotta A.S. 2001. Thermal inactivation of stationary phase and acid adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. *J. Food Protect.* **64**: 315–320.

McCarty M. 1996. *Escherichia coli* O157: H7 outbreak in USA traced to apple juice. *Lancet* **348**: 1299–1312.

Morgan D., C.D. Newman, D.N. Hutchinson, A.M. Walker, B. Rowe and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157:H7 infection associated with the consumption of yoghurt. *Epidemiol. Infect.* **111**: 181–187.

Ryu J.H. and L.R. Beuchat. 1998. Influence of acid tolerance responses on survival, growth, and thermal cross protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. *Int. J. Food. Microbiol.* **45**: 185–193.

Semanchek J.J. and D.A. Golden. 1996. Survival of *Escherichia coli* O157:H7 during fermentation of apple cider. *J. Food Protect.* **59**: 1256–1259.

Splittstoesser D.F., M.R. McLellan and J.J. Churey. 1996. Heat resistance of *Escherichia coli* O157:H7 in apple juice. *J. Food Protect.* **59**: 226–229.

Tosun H. 2003. Acid tolerance response of some pathogen bacteria and its importance in food industry. PhD thesis. Ege University, İzmir, Turkey.

Vogel H.N. and D.M. Bonner. 1956. Acettylornithinase of *Escherichia coli*. Partial purification and some proporties. *J. Biol. Chemist.* **93**: 273–284.

Weagant S.D., J.L. Bryant and D.H. Bark. 1994. Survival of *Escherichia coli* O157: H7 in mayonnaise and mayonnaise based sauces at room and refrigerated temperatures. *J. Food. Protect.* **57**: 629–631.