Fresh-Cut Lettuce in Modified Atmosphere Packages Stored at Improper Temperatures Supports Enterohemorrhagic *E. coli* Isolates to Survive Gastric Acid Challenge

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ABSTRACT: Incidences of foodborne outbreaks involving enterohemorrhagic *Escherichia coli* strains with mutations in a key regulatory gene, *rpoS*, have been reported. Incentives, if any, for losing this regulatory function are not clear since the RpoS regulator is required for the expression of several environmental stress tolerance genes. RpoS also positively regulates 2 of the 3 acid-resistance systems of *E. coli* under aerobic growth conditions and enables the pathogen to survive gastric acid challenge. We selected 7 enterohemorrhagic *E. coli* isolates, 6 of which are known to carry defective *rpoS* gene, and then analyzed resistance to synthetic gastric juice after the strains were inoculated on fresh-cut lettuce and stored under modified atmosphere packaging (MAP) conditions. Subatmospheric oxygen partial pressures in MAP enabled all 6 *rpoS*-defective isolates to induce acid resistance over the 8-d storage period if the temperature was ≥ 15 °C. No acid resistance was induced for MAP-stored lettuce left at temperatures ≤ 10 °C or for lettuce packed and stored under aerobic conditions. The data underscore the impending danger of abusive storage temperatures especially with regard to the application of MAP to extend the shelf life of fresh produce. The results also highlight the biological significance of having multiple acid-resistance pathways and the complex regulatory network of enterohemorrhagic *E. coli* strains.

Keywords: food safety, shelf life, temperature abuse

Introduction

Ready-to-eat produce is rapidly gaining popularity and it is meeting the needs of time-strapped, health-conscious customers (Clemens 2004; Lin 2004). Producers mainly rely on produce sanitation washes, refrigeration temperatures, and, more recently, modified atmosphere packaging (MAP) to extend shelf life and to reduce microbial load (Rico and others 2007). Current fresh-cut packaging practices have enabled the industry to maintain fresh-like appearance for long (approximately 14 d) periods of time (Yucan 2003; Kim and others 2005). However, the ready-to-eat produce is not subjected to any killing steps such as surface pasteurization or cooking. Thus, the combination of extended shelf life along with the fact that it is often eaten raw has made this commodity a potentially new challenge to food safety (Sivapalasingam and others 2004; Bhagwat 2006; Froder and others 2007). Several instances of foodborne illness associated with fresh produce have been reported involving outbreaks of *Salmonella,* Shiga-toxigenic and enterotoxigeneic *E. coli*, *Shigella, Yersinia pseudotuberculosis*, and lettuce was the single most frequently implicated commodity (Sivapalasingam and others 2004; Rangel and others 2005).

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In spite of diverse virulence characteristics of enteric human pathogens, one common trait that is very evident is their ability to survive passage through the acidic environment of the stomach so that they may colonize the mammalian gastrointestinal tract and cause disease (Waterman and Small 1996; Foster 2004). *S. flexneri* and diarrheagenic *E. coli* strains when grown to stationary growth phase induce 2 acid-resistance pathways which require an RNA polymerase sigma-factor, RpoS (Lin and others 1995; Bhagwat 2003). The 1st acid-resistance system (AR1) is referred to as the glucose-repressible oxidative pathway and protects cells from acidic stress above pH 3.0 (Slonczewski and Foster 1996). The 2nd acid resistance system (AR2) is glutamate dependent (GDAR) and can protect cells from acidic stress below pH 2.5 (Hersh and others 1996; Slonczewski and Foster 1996). In spite of the central regulatory role of RpoS (Cheville and others 1996; Price and others 2000), clinical and foodborne isolates of pathogenic *E. coli* with *rpoS* mutations have been reported (Waterman and Small 1996; Ferenci 2003; King and others 2004; Bhagwat and others 2005). This observation needs further investigation since advantages, if any, in losing RpoS are not obvious.

The fate of *E. coli* O157:H7 cells introduced onto packaged lettuce has been examined in several studies and recently summarized (Delaquis and others 2007). Although there is a large body of literature describing how MAP affects microbial load on various produce (Novak and others 2003; Matthews 2006), little is known about how growth under subatmospheric oxygen partial pressures would impact the enteric pathogens' ability to breach the gastric stomach barrier and increase the risk of disease. There is still a major concern about produce safety associated with the

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use of MAP, mainly due to the extension of shelf life, possibly creating opportunities for human pathogens to grow, survive, and produce toxins (Larson and Johnson 1999; Soliva-Fortuny and Martin-Belloso 2003; Rico and others 2007). The cut (wounded) plant tissue releases nutrients that become available for microbial growth (Rajkowski and Baldwin 2003; Luo 2007). The objectives of this study were to evaluate if MAP would provide appropriate low oxygen partial pressures for anaerobic growth and if fresh-cut lettuce surfaces have the necessary sugars to induce fermentative growth, the 2 critical factors needed to induce acid-resistance in RpoS-deficient *E. coli* and *S. flexneri* strains (Bhagwat and Bhagwat 2004; Bhagwat and others 2005). The study highlights the importance of proper storage temperatures for MAP-stored produce as well as the biological significance of multiple acid-resistance pathways of enterohemorrhagic *E. coli*.

Materials and Methods

Bacterial strains and culture conditions

We selected 7 enterohemorrhagic *E. coli* strains belonging to 5 serogroups, obtained from the Natl. Food Safety and Toxicological Center, Michigan State Univ., East Lansing, Mich., U.S.A. and either associated with foodborne illness or from clinical studies as described previously (Bhagwat and others 2005). The strains belonged to serogroups O157:H7 (strains 52, 253, and 256), O26:H11 (strain 200), O55:H7 (strain 219), O91:H21 (strain 221), and O111:H12 (strain 230). Except for strain 52, the strains were known to carry a mutation in the *rpoS* gene (Bhagwat and others 2005). Frozen stocks maintained at −75 °C were streaked on Luria–Bertani (LB) agar and after overnight growth at 37 °C, a single colony was inoculated into LB growth medium buffered with 100 mM MES (morpholineethanesulfonic acid, pH 5.5). For fermentative growth under anaerobic conditions, cultures were inoculated in brain heart infusion broth with 0.4% glucose (BHIG, 3 mL) in 13 × 100 mm test tubes. The test tubes were incubated without shaking for 18 to 20 h in anaerobic bags, which achieve < 2 kPa O_2, and > 4 kPa CO_2 within 2 h of incubation at 37 °C (Becton Dickinson, Sparks, Md., U.S.A.) (Bhagwat and Bhagwat 2004).

Preparation of fresh-cut lettuce, modified atmosphere packaging (MAP), measurement of CO_2 and O_2 partial pressures, and storage

Romaine lettuce was obtained (on 5 different occasions keeping the vendor and the variety unchanged) from a local wholesale market and processed immediately. Fresh romaine lettuce leaves were cut into approximately 2.5 × 2.5 cm pieces, washed to remove endogenous microflora in 100 mg/mL total chlorine solution (NaOCl) adjusted to pH 6.5 with HCl for 1 min, and centrifuged using a salad centrifugal dryer (model T-304; Meyer Machine Co.) at 650 rpm (approximately 111 g_n) for 2.5 min to remove excess water. Fresh-cut romaine lettuce samples (25 g) were transferred into packages (10 × 10 cm) prepared from a low oxygen transmission rate (OTR) film (see below). To establish an initial package atmosphere of 1 kPa O_2, the packages were vacuum evacuated to 1.5 kPa, flushed with nitrogen gas to 64.0 kPa, heat sealed for 2 s, and vented to atmospheric pressure using an automated chamber machine (model A300/42, Multivac Inc., Kansas City, Mo., U.S.A.).

To store under aerobic conditions, 3 0.5-mm holes were punched in some of the packaged fresh-cut lettuce samples using a 26-gauge syringe needle.

The OTR of the film used to prepare the packages was 16.6 pmol/s/m^2/Pa as determined by the film manufacturer (Packaging Concepts, Salinas, Calif., U.S.A.) at 23 °C using a MOCON apparatus to measure the steady-state rate of dry O_2 gas transmission through plastic films, that is, according to ASTM International procedure D 3985-81 (ASTM Intl. 1986). At 10 °C, the film OTR was 9.0 pmol/s/m^2/Pa as determined using an exponential decay method for determining OTRs through plastic films in a static cell (Moyls and others 1992) with slight modifications (Kim and others 2005).

Inoculation of fresh-cut lettuce with *E. coli*

Fresh-cut lettuce packages were inoculated individually with different strains of hemorrhagic *E. coli*. Bacterial cultures from the LB-MES growth medium were suspended in phosphate-buffered saline to get a cell concentration of approximately 4.5 × 10^6 CFU in 100 μL. The 100-μL suspension was then placed on preweighed fresh-cut lettuce in the form of several drops and packaged within the next 1 h. Bacterial cell count was confirmed by plating 10-fold serial dilutions of cell suspensions on LB agar media and enumerating colonies following 24 h incubation at 37 °C.

Acid-challenge assays

Acid-challenge assays for pure cultures of *E. coli*: for the oxidative, glucose-repressed, acid-resistance system (AR1), cells were diluted (1:200) directly from the growth media (LB-MES broth) to EG growth medium pH 3.0 and challenged for 2 h at 37 °C. For the glutamate-dependent acid-resistance system (AR2) and arginine-dependent acid-resistance system (AR3), the cells were diluted (1:200) directly from the growth media (BHI broth with 0.4% glucose) to EG growth medium containing either 1.5 mM glutamate (AR2) or 1.5 mM arginine (AR3) at pH 2.0 and challenged at 37 °C for 2 h (Bhagwat and others 2005). Before adding cells, acid-challenge media were warmed to 37 °C and pH was adjusted with 6 N HCl.

For acid challenge involving bacteria inoculated on fresh-cut lettuce, the fresh-cut lettuce pieces from individual packages were placed in a stomacher bag with a filter lining and pummeled in 225 mL synthetic gastric juice (pH 2.0). The composition of the synthetic gastric juice was 8.3 g proteose-peptone, 3.5 g glucose, 2.05 g NaCl, 0.6 g KH_2PO_4, 0.11 g $CaCl_2$, 0.37 g KCl, 0.05 g porcine bile, 0.1 g lysozyme, 13.3 mg pepsin, and 1 L Millipore-filtered water, pH adjusted to 2.0 with 6 N HCl. (Beumer and others 1992; Cui and others 2001). Initial viable counts were determined immediately by diluting cells in phosphate buffered saline (50 mM, pH 7.2) and plating on LB agar. Samples were mixed 1 more time at the end of 2-h acid challenge incubation before taking aliquots for determining viable cell counts. At least 3 replicates were included for each acid-challenge assay and data from all replicates were included to plot the survival percentages.

Statistical analysis

For all statistical analyses, SigmaStat 3.0 software (Ashburn, Va., U.S.A.) was used. Data were analyzed by 1-way analysis of variance (ANOVA) test to determine statistical differences between means of treatments.

Results

RpoS status and resistance to synthetic gastric juice

rpoS encodes a transcriptional sigma factor which positively regulates 2 of the 3 acid-resistance pathways in *E. coli*. All strains (except strain 52) showed a low level of AR1 induction when cells were challenged after an overnight aerobic growth in LB-MES medium (Table 1). Aerobically grown cells were unable to induce a glutamate-dependent acid-resistance system (AR2) as they showed poor survival (< 0.001%) after acid challenge at pH 2.0. We observed

that when cells were grown under reduced oxygen and in the presence of glucose, RpoS (sigma factor) was no longer necessary for induction of AR2 (Table 1). Anaerobic growth also induced a 3rd acid-resistance pathway (AR3), which is dependent on arginine. Accordingly, all *E. coli* strains were able to induce AR2 and AR3 pathways when grown anaerobically. A sizable fraction of the population survived when challenged at pH 2.0 either in the presence of glutamate (AR2; 33% to 79% survival) or arginine (AR3; 7% to 13% survival) (Table 1). A similar acid challenge administered to aerobically grown cells resulted in poor survival (< 0.001% survival for AR2 pathway).

Modified atmosphere packaging and induction of acid resistance

We further examined whether or not MAP storage conditions provided the necessary low O_2 partial pressures and if available metabolites from the fresh-cut lettuce were sufficient to promote fermentative growth of *E. coli* and induction of AR2. We examined the acid-resistance capability of *E. coli* strains by inoculating the aerobically grown cells onto fresh-cut lettuce. The lettuce packages under MAP were stored at 5, 10, 15, and 20 °C over a period of 7 d. At 5 °C, wild-type *E. coli* strain 52 maintained acid-resistance (>30% survival), while the *rpoS*-mutant strain 253 failed to induce acid resistance and remained acid sensitive (0.35% survival) after 7-d storage (Figure 1). The relatively low CO_2 transmission rate of the packaging film allowed for rapid accumulation of CO_2 during the 1st 24 h and thereafter the CO_2 partial pressure stabilized at approximately 4 kPa for the rest of the experiment (Figure 1). The O_2 partial pressure steadily increased to 2.6 kPa over the period of 7 d. Total aerobic viable cell counts (for noninoculated fresh-cut lettuce) increased from $2.0 \pm 3.7 \times 10^3$ to $5.0 \pm 7.1 \times 10^4$ cells for every 25 g of lettuce during 7-d storage at 5 °C. Similarly, the viable count of *E. coli* strain 253 increased only slightly from $4.5 \pm 5.1 \times 10^6$ at the time of inoculation to $2.0 \pm 1.2 \times 10^7$ after 7-d storage at 5 °C (data not shown). However, increases in the viable counts were not statistically significant ($P = 0.2$) and more likely reflect variation due to handling of packages during preparation and generating modified atmosphere as well as the time taken by the produce to attain the storage temperature of 5° C. No microbial growth of any *E. coli* strain was observed in LB broth left at 5° C for 7 d (data not shown).

Effect of abusive storage temperatures on induction of acid resistance

We analyzed the effect of higher storage temperatures on the acid-resistance capacity of enterohemorrhagic *E. coli* strains under MAP conditions. There was no significant difference in the

Table 1 — Acid resistance properties of various enterohemorrhagic *E. coli* strains grown under aerobic and anaerobic conditions.

	Strength of various acid-resistance pathways (% surviving population after growth and acid challenge in specified media)			
	Aerobic growth (LB-MES, pH 5.5)		Anaerobic growth in presence of glucose (BHIG, <2 kPa O_2, >4kPa CO_2)	
Strain and serotype	AR1 (pH 3.0)[a]	AR2 (pH 2.0 + glutamate)[b]	AR2 (pH 2.0 + glutamate)[b]	AR3 (pH 2.0 + arginine)[c]
52 (O157:H7)	46.4 ± 4.9 (A)	88.6 ± 9.2	77.1 ± 5.6 (A)	9.52 ± 2.3 (A)
253 (O157:H7)	0.81 ± 0.6 (C)	< 0.001	65.21 ± 9.6 (A)	12.65 ± 3.1 (A)
256 (O157:H7)	5.59 ± 1.9 (B)	< 0.001	79.15 ± 8.3 (A)	9.52 ± 2.3 (A)
200 (O26:H11)	5.73 ± 2.2 (B)	< 0.001	63.5 ± 5.1 (A)	8.14 ± 1.8 (A)
219 (O55:H7)	3.23 ± 1.1 (B)	< 0.001	86.03 ± 9.8 (A)	13.3 ± 4.1 (A)
221 (O91:H21)	9.33 ± 3.9 (B)	< 0.001	33.32 ± 6.6 (B)	7.7 ± 3.3 (A)
230 (O111:H12)	5.59 ± 1.6 (B)	< 0.001	60.23 ± 7.6 (A)	6.83 ± 1.3 (A)

Mean values ($n = 3$) in each column that are not followed by the same letter in the parenthesis indicate significant ($P < 0.05$) differences.
[a]Cells were acid challenged in minimal EG medium, pH 3 for 2 h at 37 °C.
[b]Cells were acid challenged in minimal EG medium + 1.5 mM glutamate, pH 2 for 2 h at 37 °C.
[c]Cells were acid challenged in minimal EG medium + 1.5 mM arginine, pH 2 for 2 h at 37 °C.

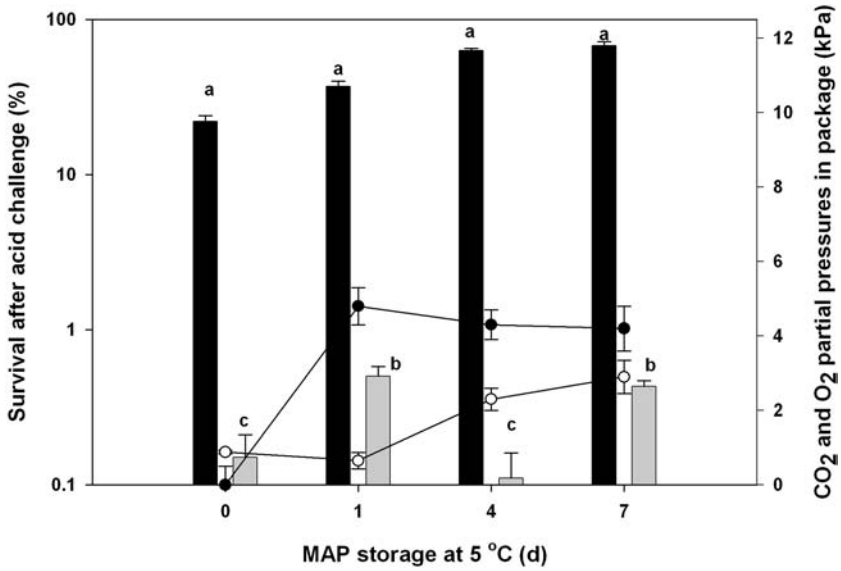

Figure 1 — Ability of enterohemorrhagic *E. coli* strains to induce acid resistance on fresh-cut lettuce stored under MAP at 5 °C. *E. coli* strains 52 (black bars) or 253 (grey bars) were inoculated onto fresh-cut lettuce and stored under MAP at 5 °C. At the specified storage times, the partial pressures of CO_2 (•) and O_2 (○) were measured before performing acid challenge in synthetic gastric juice (pH 2.0 for 2 h at 37 °C). Surviving population was determined by performing viable count measurements on LB agar plates. Mean values ($n = 4$) for the survival data with different letters indicate significant ($P < 0.05$) difference in % survival after 2-h acid challenge.

acid-resistance levels of *E. coli* strain 253 cells at the end of 7-d storage at 5 °C (Figure 1) and 10 °C (data not shown). However, at temperatures higher than 10 °C (for example, 15 °C), *rpoS*-defective strain (strain 253) acquired acid resistance under MAP conditions (Figure 2, data indicated by grey bars). At 15 °C there was a rapid increase in the CO_2 partial pressure with a concomitant decrease in O_2. *E. coli* strain 52 maintained its acid-resistance ability throughout storage at 15 °C. Similar induction levels for acid resistance were observed for fresh-cut lettuce packages in MAP left at 20 °C but the produce quality deteriorated rapidly after 6-d storage at this temperature (data not shown). Induction of acid resistance observed at higher temperatures occurred in the presence of MAP storage conditions (Figure 2, data indicated by grey bars), but not when packaged lettuce was stored aerobically at the identical storage temperature (Figure 3, data indicated by grey bars). As expected for packages with 3 macropores, there were no appreciable changes in the CO_2 and O_2 partial pressures within the packages and near atmospheric conditions were maintained throughout the storage period. Strain 52 maintained its high acid-resistance levels during aerobic storage conditions.

Ability to induce acid resistance in other enterohemorrhagic *E. coli* strains

Fresh-cut lettuce packed in MAP was inoculated with individual strains and stored at 15 °C for a period of 7 d. As expected, the initial acid-resistance levels of all strains (except strain 52) were low and only 0.1% of the initial inoculum survived acid challenge at pH 2.0 (Figure 4). However, all strains developed the AR2-mediated acid tolerance (synthetic gastric juice, pH 2.0) after 7-d storage at 15 °C while AR2 resistance of strain 52 was maintained at high level (Figure 4). The aerobic plate count for noninoculated fresh-cut lettuce packed in MAP after 7-d storage was 8×10^8 cells per 25 g; however, surviving population (if any) after acid challenge at pH 2.0 in the synthetic gastric juice was below the detection limit ($< 5.0 \times 10^4$ cells per 25 g), which extrapolates to < 0.006% survival. The data indicate the acid-sensitive nature of the resident microflora of the noninoculated lettuce as well as its inability to induce acid-resistance under MAP-storage conditions even at abusive temperature of 15 °C.

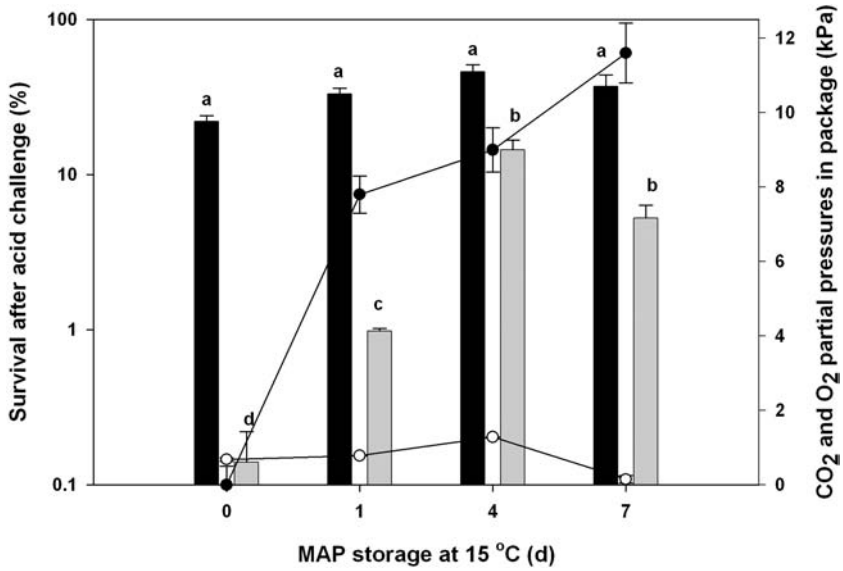

Figure 2—Ability of enterohemorrhagic *E. coli* strains to induce acid resistance on fresh-cut lettuce stored under MAP at 15 °C. *E. coli* strains 52 (black bars) or 253 (grey bars) were inoculated onto fresh-cut lettuce and stored under MAP at 15 °C. At the specified storage times, the partial pressures of CO_2 (•) and O_2 (○) were measured before performing acid challenge in synthetic gastric juice (pH 2.0 for 2 h at 37 °C). Surviving population was determined by performing viable count measurements on LB agar plates. Mean values ($n = 4$) for the survival data with different letters indicate significant ($P < 0.05$) difference in % survival after 2-h acid challenge.

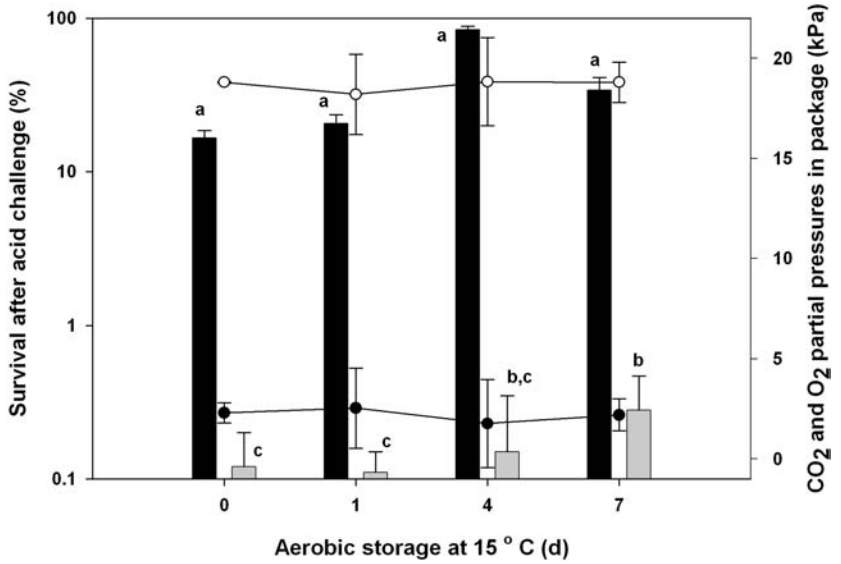

Figure 3—Ability of enterohemorrhagic *E. coli* strains to induce acid resistance on fresh-cut lettuce stored under aerobic conditions at 15 °C. *E. coli* strains 52 (black bars) or 253 (grey bars) were inoculated onto fresh-cut lettuce and stored in macroperforated packages (that is, aerobically) at 15 °C. At the specified storage times, the partial pressures of CO_2 (•) and O_2 (○) were measured before performing acid challenge in synthetic gastric juice (pH 2.0 for 2 h at 37 °C). Surviving population was determined by performing viable count measurements on LB agar plates. Mean values ($n = 3$) for the survival data with different letters indicate significant ($P < 0.05$) difference in % survival after 2-h acid challenge.

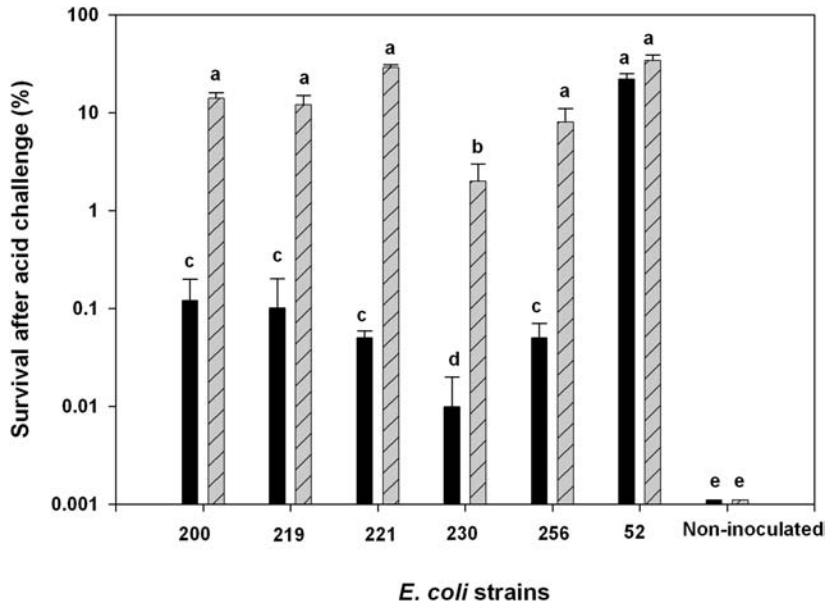

Figure 4—Ability of various enterohemorrhagic *E. coli* strains to induce acid-resistance on fresh-cut lettuce stored under MAP at 15 °C for 7 d. Various *E. coli* strains were inoculated onto fresh-cut lettuce and stored under MAP at 15 °C. Acid challenge was performed in synthetic gastric juice (pH 2.0 for 2 h at 37 °C) at the time of inoculation (day 0, filled bars) and after 7-d storage (hatched bars). Surviving population was determined by performing viable count measurements on LB agar plates. Mean values ($n = 3$) for the survival data with different letters indicate significant ($P < 0.05$) difference in % survival after 2-h acid challenge.

Discussion

E. coli O157:H7 is a highly acid-resistant foodborne pathogen that survives in the bovine and human gastrointestinal tracts and in acidic foods such as apple cider (Zhao and others 1993; Price and others 2004). This property is thought to contribute to the low infectious dose of the organism (Audia and others 2001; Foster 2004). In this study, we observed that several enterohemorrhagic *E. coli* strains, in spite of their dysfunctional *rpoS* locus, were able to utilize MAP conditions and metabolites available from the fresh-cut lettuce surfaces to induce acid resistance at growth permissive temperatures (that is, ≥ 15 °C) (Figure 2 and 4). On the other hand, no acid resistance was induced for MAP-stored lettuce left at temperatures ≤ 10 °C (Figure 1), or for lettuce packed and stored under aerobic conditions (Figure 3). The data underscore the impending danger of abusive storage temperatures especially with regard to the application of MAP to extend the shelf life of fresh produce.

In this study, we examined how the film oxygen transmission rate and respiring surface area recommended for fresh-cut lettuce would affect one of the critical physiological properties (that is, resistance to gastric acidity) of enterohemorrhagic *E. coli* strains. MAP has a distinct advantage in delaying spoilage of the wounded plant tissue so that the produce will have a fresh-like appearance for an extended period of time (Yucan 2003; Matthews 2006). The U.S. Food and Drug Administration (FDA) uniform food code requires that all refrigerated hazardous foods be stored at 5 °C or below. Maintaining proper storage temperatures is often most difficult at the retail level, due to the increased handling and the need to display the product in a visually appealing manner. Unfortunately, temperature abuse occurs frequently at several stages from farm to fork, that is, during fresh-cut-product shipping, transportation, and retail display as well as at home settings (Bruhn 2006). In a study where over 1400 perishable food items (produce and meat products) were examined for temperature abuse at retail locations, over 87% of the samples were kept at above the recommended temperatures (LeBlanc and others 1996; Marklinder and others 2004). This study highlights the potential food safety hazards associated with packaged fresh-cut lettuce if packages are left at abusive temperatures. The tenacious acid-resistance ability of enterohemorrhagic *E. coli* strains having multiple pathways and complex regulatory network needs to be taken into consideration especially for foods packed to extend their fresh-like appearance and which are most likely consumed raw.

Conclusions

This study addresses concerns regarding produce safety associated with the use of MAP, mainly due to the extension of shelf life which may be creating opportunities for human pathogens to grow, survive, and produce toxins. The data presented here underscore the impending danger of abusive storage temperatures, especially with regard to the application of MAP to extend the shelf life of fresh produce. The results also highlight the biological significance of having multiple acid-resistance pathways and complex regulatory network that enterohemorrhagic *E. coli* strains use to overcome gastric acid barrier.

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References

ASTM Intl. 1986. Standard test method for oxygen gas transmission rate through plastic film and sheeting using coulometric sensor. In: Annual book of ASTM standards. Philadelphia, Pa.: ASTM Publisher. p 387–93.

Audia JP, Webb CC, Foster JW. 2001. Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. Int J Med Microbiol 291:97–106.

Beumer RR, de Vries J, Rombouts FM. 1992. *Campylobacter jejuni* non-culturable coccoid cells. Int J Food Microbiol 15:153–63.

Bhagwat AA. 2003. Regulation of the glutamate-dependent acid-resistant system of diarrheagenic *Escherichia coli* strains. FEMS Microbiol Lett 227:39–45.

Bhagwat AA. 2006. Microbiological safety of fresh-cut produce: where are we now? In: Matthews KR, editor. Microbiology of fresh produce. Washington, D.C.: American Society for Microbiology. p 121–65.

Bhagwat AA, Bhagwat MA. 2004. Comparative analysis of transcriptional regulatory elements of glutamate-dependent acid-resistance systems of *Shigella flexneri* and *Escherichia coli* O157:H7. FEMS Microbiol Lett 234:139–47.

Bhagwat AA, Chan L, Han R, Tan J, Kothary M, Jean-Gilles J, Tall BD. 2005. Characterization of enterohemorrhagic *Escherichia coli* strains based on acid resistance phenotypes. Infect Immun 73:4993–5003.

Bruhn CM. 2006. Consumer handling of fresh produce. In: Matthews KR, editor. Microbiology of fresh produce. Washington, D.C.: ASM Press. p 221–31.

Cheville AM, Arnold KW, Buchrieser C, Cheng CM, Kaspar CW. 1996. *rpoS* regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7. Appl Environ Microbiol 62:1822–4.
Clemens R. 2004. The expanding U.S. market for fresh produce. Iowa Agric Rev 10:1–4.
Cui S, Meng J, Bhagwat AA. 2001. Availability of glutamate and arginine during acid challenge determines cell density-dependent survival phenotype of *Escherichia coli* strains. Appl Environ Microbiol 67:4914–8.
Delaquis P, Bach S, Dinu L. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. J Food Prot 70:1966–74.
Ferenci T. 2003. What is driving the acquisition of *mutS* and *rpoS* polymorphisms in *Escherichia coli*? Trends Microbiol 11:457–61.
Foster JW. 2004. *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat Rev Microbiol 2:898–907.
Froder H, Martins CG, DeSouza KLO, Landgraf M, Franco BDGM, Destro MT. 2007. Minimally processed vegetable salads: microbial quality evaluation. J Food Prot 70:1277–80.
Hersh BM, Farooq FT, Barstad DN, Blankenhorn DL, Slonczewski JL. 1996. A glutamate-dependent acid resistance gene in *Escherichia coli*. J Bacteriol 178:3978–81.
Kim JG, Luo Y, Saftner RA, Gross KC. 2005. Delayed modified atmosphere packaging of fresh-cut romaine lettuce: effects on quality maintenance and shelf-life. J Amer Soc Hort Sci 130:116–23.
King T, Ishihama A, Kori A, Ferenci T. 2004. A regulatory trade-off as a source of strain variation in the species *Escherichia coli*. J Bacteriol 186:5614–20.
Larson AE, Johnson EA. 1999. Evaluation of botulinal toxin production in packaged fresh-cut cantaloupe and honeydew melons. J Food Prot 62:948–52.
LeBlanc DI, Stark R, MacNeil B, Goguen B, Beaulieu C. 1996. Perishable food temperatures in retail stores. In: New developments in refrigeration and food safety and quality. Intl. Inst. of Refrigeration, Paris. p 42–51.
Lin BH. 2004. Fruits and vegetables consumption: looking ahead to 2010. Washington, D.C.: U. S. Dept. of Agriculture, Economic Research Service. p 1–4.
Lin J, Lee IS, Frey J, Slonczewski JL, Foster JW. 1995. Comparative analysis of extreme acid survival in *Salmonella typhimurium, Shigella flexneri*, and *Escherichia coli*. J Bacteriol 177:4097–104.
Luo Y. 2007. Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in romaine lettuce. HortScience 42:1413–9.
Marklinder IM, Lindbald M, Eriksson LM, Finnson AM, Lindqvist R. 2004. Home storage temperatures and consumer handling of refrigerated foods in Sweden. J Food Prot 67:2570–7.
Matthews KR. 2006. Microbiology of fresh produce. Washington, D.C.: ASM Press.
Moyls L, Hocking R, Beveridge T, Timbers G. 1992. Exponential decay method for determining gas transmission rate of films. Trans ASAE 35:1259–65.
Novak JS, Sapers GM, Juneja VK. 2003. Microbial safety of minimally processed foods. Boca Raton, Fla.: CRC Press.
Price SB, Cheng CM, Kaspar CW, Wright JC, DeGraves FJ, Penfound TA, Castanie-Cornet MP, Foster JW. 2000. Role of *rpoS* in acid resistance and fecal shedding of *Escherichia coli* O157:H7. Appl Environ Microbiol 66:632–7.
Price SB, Wright JC, DeGraves FJ, Castanie-Cornet M-P, Foster JW. 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: 4792–9.
Rajkowski KT, Baldwin EA. 2003. Concerns with minimal processing in apple, citrus, and vegetable products. In: Novak JS, Sapers GM, Juneja VK, editors. Microbial safety of minimally processed foods. New York: CRC Press. p 35–52.
Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis 11:603–9.
Rico D, Martin-Diana AB, Barat JM, Barry-Ryan C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends Food Sci Technol 18:373–86.
Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973–1997. J Food Prot 67:2342–53.
Slonczewski JL, Foster JW. 1996. pH-regulated genes and survival at acidic pH. In: Neidhardt FC, Curtiss R III, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE, editors. *Escherichia coli* and *Salmonella* cellular and molecular biology. Washington, D.C.: ASM Press. p 1539–49.
Soliva-Fortuny RC, Martin-Belloso O. 2003. New advances in extending the shelf-life of fresh-cut fruits: a review. Trends Food Sci Technol 14:341–53.
Waterman SR, Small PLC. 1996. Characterization of the acid resistance phenotype and *rpoS* alleles of shiga-like toxin-producing *Escherichia coli*. Infect Immun 64: 2808–11.
Yucan JTC. 2003. Modified atmosphere packaging for shelf-life extension. In: Novak JS, Sapers GM, Juneja VK, editors. Microbial safety of minimally processed foods. Washington, D.C.: CRC Press. p 205–19.
Zhao T, Dolye MP, Besser RE. 1993. Fate of enterohemorrhagic *Escherichia coli* 0157:H7 in apple cider with and without preservatives. Appl Environ Microbiol 59:2526–30.