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ORIGINAL ARTICLE

The glutamate decarboxylase acid resistance mechanism affects survival of *Listeria monocytogenes* LO28 in modified atmosphere-packaged foods

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Abstract

Aims: The contribution of the glutamate decarboxylase (GAD) acid resistance system to survival and growth of *Listeria monocytogenes* LO28 in modified atmosphere-packaged foods was examined.

Methods and Results: The survival and growth of the wild-type LO28 and four GAD deletion mutants (Δ*gadA*, Δ*gadB*, Δ*gadC*, Δ*gadAB*) in packaged foods (minced beef, lettuce, dry coleslaw mix) during storage at 4, 8 and 15°C were studied. Survival and growth patterns varied with strain, product type, gas atmosphere and storage temperature. In minced beef, the wild-type LO28 survived better ($P < 0{\cdot}05$) than the GAD mutant strains at 8 and 15°C. In both packaged vegetables at all storage temperatures, the wild-type strain survived better ($P < 0{\cdot}05$) than the double mutant Δ*gadAB*. The requirement for the individual *gad* genes varied depending on the packaged food. In the case of lettuce, *gadA* played the most important role, while the *gadB* and *gadC* genes played the greatest role in packaged coleslaw (at 15°C).

Conclusions: This work demonstrates that elements of the GAD system play significant roles in survival of *L. monocytogenes* LO28 during storage in modified atmosphere-packaged foods.

Significance and Impact of the Study: A better understanding of how *L. monocytogenes* behaves in modified atmosphere-packaged foods, and how it responds to elevated carbon dioxide atmospheres.

Introduction

Modified atmosphere packaging (MAP) involves packaging foods in a modified gaseous mixture chosen to control the product's biochemical and enzymatic reactions, reduce microbial spoilage and extend the shelf-life. The elevated carbon dioxide (CO_2) concentrations within modified atmosphere (MA) packages of food may inhibit the growth of a number of micro-organisms (Daniels *et al.* 1985). The extent of inhibition by CO_2 varies with the micro-organism, CO_2 concentration, temperature of incubation and type of food (Enfors *et al.* 1979; Dixon and Kell 1989; Hudson *et al.* 1994).

A number of studies have indicated that MAP can enhance the survival and growth of facultatively anaerobic pathogens, such as *Listeria monocytogenes* (Hintlian and Hotchkiss 1986; Brackett 1994; Francis and O'Beirne 1997). In addition, *L. monocytogenes* has been shown to survive and grow on a range of packaged meats (Wimpfheimer *et al.* 1990; Devlieghere *et al.* 2001; Mataragas *et al.* 2003) and minimally processed vegetables (Beuchat and Brackett 1990; Carlin *et al.* 1995; Francis and

O'Beirne 1997, 2001a; Jacxsens *et al.* 1999) during storage at refrigeration temperatures.

In addition to its ability to grow at low temperatures (Bell and Kyriakides 1998), *L. monocytogenes* is able to survive a variety of other environmental stresses, including low pH. One well-characterized adaptive response is to acid stress, the so-called acid tolerance response (ATR), in which *L. monocytogenes* cells exposed to pH 5·5 for a short period can withstand a normally lethal pH environment (Hill *et al.* 1995; Hill and Gahan 2000). The ability of *L. monocytogenes* to induce an ATR has been shown to play an important role in its survival in acidic foods and in its survival *in vivo* (Gahan *et al.* 1996; Gahan and Hill 1999; Hill *et al.* 2002). In addition, acid-adapted *L. monocytogenes* cells demonstrated enhanced survival in MA packages of vegetables which had elevated in-pack CO_2 levels (i.e. 30%) (Francis and O'Beirne 2001b).

Listeria monocytogenes is one of a relatively small number of bacteria known to possess a glutamate decarboxylase (GAD)-dependent stress response system that plays a major role in its acid tolerance. When the cell is exposed to low pH, GAD catalyses the conversion of glutamate to γ-amino butyrate and CO_2, thus removing a proton from the cytoplasm and resulting in an increase in cytoplasmic pH. Previous investigations have shown that *L. monocytogenes* LO28 possesses a GAD system that contains at least two independently regulated GAD, encoded by the *gadA* and *gadB* genes, and a putative glutamate/γ-amino butyrate antiporter, encoded by *gadC* (Cotter *et al.* 2001a). More recently, Cotter *et al.* (2005) demonstrated that strain LO28 possesses two additional *gad* genes, and contains a total of three decarboxylase enzymes and two antiporters, as does the sequenced *L. monocytogenes* EGDe (Glaser *et al.* 2001).

Analysis of a set of four GAD deletion mutants demonstrated a dramatic decrease in the level of GAD activity in the order of wild-type LO28 > Δ*gadA* > Δ*gadB* = Δ*gadC* > Δ*gadAB*, with a significantly enhanced acid sensitivity of the mutants, indicating that the GAD system may play an important role in gastric transit, and is necessary for prolonged survival of *L. monocytogenes* in acid foods (Cotter *et al.* 2001a,b). In a recent study, Jydegaard-Axelsen *et al.* (2004) observed an increase in expression of the *gad* genes (*gadA*, *gadB*, *gadC*) when strain LO28 was grown in CO_2, suggesting that strain LO28 responds to CO_2 as it would to acid using the GAD system.

In this paper, the contribution of the GAD system to the survival and growth of *L. monocytogenes* LO28 during storage in MA-packaged food products was examined. The effects of product type (minced beef, lettuce, coleslaw mix), gas atmospheres (varying CO_2 levels) and storage temperatures (4, 8 and 15°C) were also investigated.

Materials and methods

Preparation of the food products

Minced beef

The meat sample (lean beef from the shin and shank) was minced in the on-site abattoir. It was then transferred back to the laboratory in a sterile sealed bag to prevent cross-contamination.

Lettuce and dry coleslaw mix

Heads of Irish Iceberg lettuce were purchased from a local supplier. Outer and damaged leaves and the core of the lettuce heads were removed and discarded. Inner leaves were sliced manually using a sharp knife to approximately 10-mm strips. Dry coleslaw mix, consisting of 80% shredded cabbage and 20% shredded carrot, was obtained from a local supplier.

Strains used

The wild-type strain was *L. monocytogenes* LO28. Nonpolar mutants of *gadA*, *gadB* and *gadC* with 336, 405 and 444 bp deletions respectively, were created individually (Cotter *et al.* 2001a). LO28 Δ*gadAB* was a double mutant in which both *gadA* and *gadB* genes had internal deletions, resulting in negligible GAD activity. The culture medium for bacterial growth was tryptone soya broth (CM 129), supplemented with 0·6% yeast extract (Oxoid L21, TSB-YE).

Preparation of inocula

Listeria monocytogenes strains were maintained at −20°C in TSB-YE supplemented with 15% (v/v) glycerol. Resuscitation was achieved by thawing cultures at room temperature (17–22°C) followed by a loop transfer in TSB-YE (10 ml) and overnight incubation at 37°C. Cultures were centrifuged (5000 ***g***, 15 min), the cells were washed once with sterile phosphate-buffered saline (PBS; Oxoid BR014), resuspended and diluted in PBS to the desired concentrations to allow for contamination of food products at initial levels of approximately 10^5 CFU g^{-1} of the product.

Inoculation of meat and vegetable products and storage

Minced beef

Meat portions (750 g) and 30 ml of the cell suspensions were transferred aseptically into a sterile blender and

blended to ensure an even distribution of the inoculum. Inoculated meat portions (25 g) were transferred aseptically into bags (12 cm × 12 cm), composed of oriented polypropylene packaging film (Cannings Packaging Ltd., Dublin, Ireland), which were later heat-sealed after flushing bags with 20% CO_2 and 80% oxygen (O_2).

Lettuce and dry coleslaw mix

Vegetable portions (25 g) were transferred aseptically into bags (10 cm × 10 cm), composed of 35 μm oriented polypropylene packaging film (Cannings Packaging Ltd.). After appropriate dilution, ten 10-μl aliquots of the cell suspensions were distributed over the vegetables contained within the packages. After inoculation, packages were heat-sealed and were gently shaken to assist inoculum distribution. Immediately after sealing, all food samples were transferred to storage temperatures of 4, 8 and 15°C and stored for a period of 12 days.

Microbiological analyses

Microbiological analyses were carried out on day 0 (day of inoculation) and at regular intervals throughout the storage period. At each sampling, duplicate packs from the same experiment were analysed for populations of *L. monocytogenes* and mesophilic bacteria. The 25-g sample from each pack was aseptically transferred into a stomacher bag. Samples were homogenized for 2 min at high speed with 225 ml of sterile PBS using a Seward laboratory stomacher (Model 400; AGB Scientific, Ireland). Serial dilutions of each homogenized sample were made in PBS and were surface spread (100 μl per plate) in duplicate onto appropriate media. Numbers of *L. monocytogenes* were determined on *Listeria*-selective agar (LSA; Oxoid CM 856 and SR140) after incubation for 48 h at 35°C. Total counts of mesophilic bacteria were enumerated on plate count agar (PCA; Oxoid, CM 325) after incubation at 35°C for 48 h.

Gaseous atmospheres inside the packages

On each sampling date, gases within three of each package type were analysed using an O_2 and CO_2 gas analyser (Model TIA-III LV, PBI-Dansensor; PBI Development, Ringsted, Denmark).

pH of food samples

On each of the sampling dates, the pH of the food samples was analysed. A 10-g sample from each package was homogenized for 2 min with 90 ml of distilled water using a Waring blender. The pH of the homogenized sample was measured during storage using a digital pH meter (pH meter 3310; Jenway Ltd, Dunmow, UK).

Measurement of levels of free glutamate in foods

The levels of free L-glutamic acid in the food samples were measured using an L-glutamic acid food analysis kit (Boehringer Mannheim, Indianapolis, USA) according to the manufacturer's instructions.

Statistical analyses

All experiments were carried out in duplicate and replicated thrice. On each analysis date, duplicate samples were serially diluted and plated in duplicate. Reported populations therefore represent the means of six values. Figures show the means of six values ± standard deviations (SD). Results were analysed by analysis of variance and least significant difference testing at the $P < 0{\cdot}05$ level.

Results

In order to determine whether the GAD system plays a role in the survival of *L. monocytogenes* in MAP foods, the wild-type strain LO28 and a set of four mutant strains were inoculated onto MAP meats and vegetables, and differences in their survival rates were followed during storage. Figure 1 shows the survival of the wild-type strain, and the GAD deletion mutants on packaged minced beef during storage at 4, 8 and 15°C. At 4°C, the GAD mutants behaved similar to the wild-type strain, with all populations decreasing during storage (Fig. 1a). At 8 and 15°C, however, the wild-type strain survived significantly better ($P < 0{\cdot}05$) than the mutant strains, but there was no major difference between mutants.

Figure 2 shows survival and growth of the wild-type and GAD mutant strains on packaged lettuce during storage at 4 and 8°C. At 4°C, the Δ*gadB* and Δ*gadC* mutants behaved similar to the wild-type strain, with populations not changing significantly ($P > 0{\cdot}05$) during storage on lettuce (Fig. 2a). However, growth of the Δ*gadA* and Δ*gadAB* mutants was significantly ($P < 0{\cdot}05$) reduced during storage, with Δ*gadAB* demonstrating a 1-log reduction in populations during storage. At 8°C, there was no significant difference ($P > 0{\cdot}05$) in the survival and growth of the wild-type or Δ*gadB* and Δ*gadC* strains; populations of all strains increased by approximately 1-log cycle during storage (Fig. 2b). However, Δ*gadA* and Δ*gadAB* did not grow as well as the other mutants. Populations of Δ*gadA* increased between days 0 and 2, however, numbers declined in the storage thereafter to initial population densities. Populations of the Δ*gadAB* mutant, which had the two listerial GAD homologues deleted, remained at initial levels throughout the storage period.

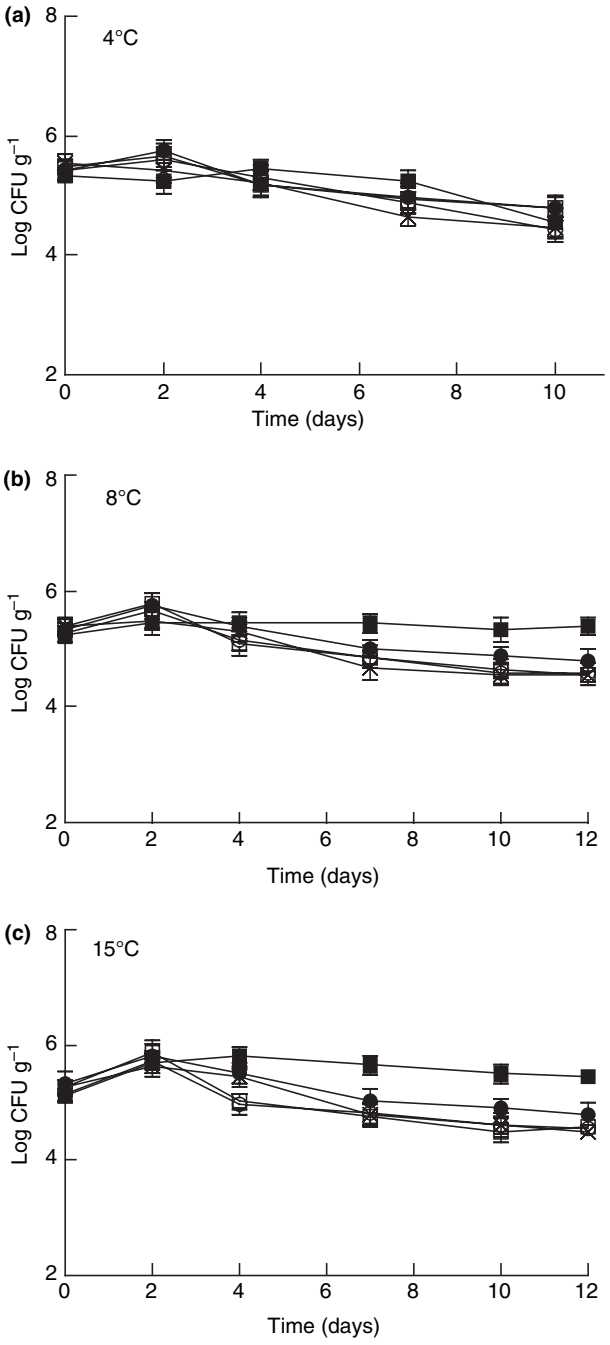

Figure 1 Survival of *Listeria monocytogenes* LO28 (■) and the glutamate decarboxylase (GAD) deletion mutants, Δ*gadA* (●), Δ*gadB* (□), Δ*gadC* (○) and Δ*gadAB* (×) on modified atmosphere-packaged minced beef during storage at: (a) 4°C (b) 8°C and (c) 15°C.

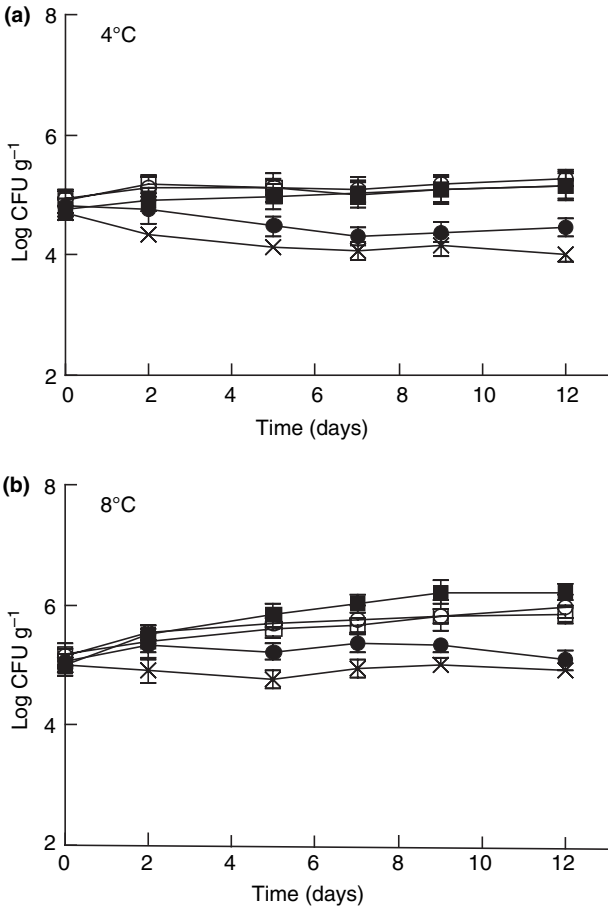

Figure 2 Survival of *Listeria monocytogenes* LO28 (■) and the glutamate decarboxylase (GAD) deletion mutants, Δ*gadA* (●), Δ*gadB* (□), Δ*gadC* (○) and Δ*gadAB* (×) on modified atmosphere-packaged lettuce during storage at: (a) 4°C and (b) 8°C.

The survival of the wild-type and mutant strains on MAP coleslaw mix was also examined during storage at 4, 8 and 15°C (Fig. 3). For all storage temperatures, populations of the wild-type and mutant strains decreased ($P < 0{\cdot}05$) during storage. At 4°C, the Δ*gadA*, Δ*gadB* and Δ*gadC* mutants behaved similar to the wild-type strain, with all populations declining by approximately 1-log cycle during storage (Fig. 3a). However, populations of the Δ*gadAB* mutant were significantly lower ($P < 0{\cdot}05$) than the wild-type or other mutant strains, with numbers decreasing by 2-log cycles during the storage period. At 8°C, Δ*gadA* behaved similar to the wild-type strain with numbers declining by 1·5-log cycles during storage. The Δ*gadB* and Δ*gadC* mutants behaved similar to the wild-type strain up to day 9; however, by day 12, numbers were 1-log cycle lower. Populations of Δ*gadAB* were significantly lower ($P < 0{\cdot}05$) than the wild-type or other mutant strains, with numbers declining by 3-log cycles during the storage period. During storage at 15°C, GAD deletion mutations resulted in a dramatic decrease in survival on packaged coleslaw, where the order of survival was LO28 > Δ*gadA* > Δ*gadC* > Δ*gadB* > Δ*gadAB*. Viable populations of the wild-type LO28 remained at the end

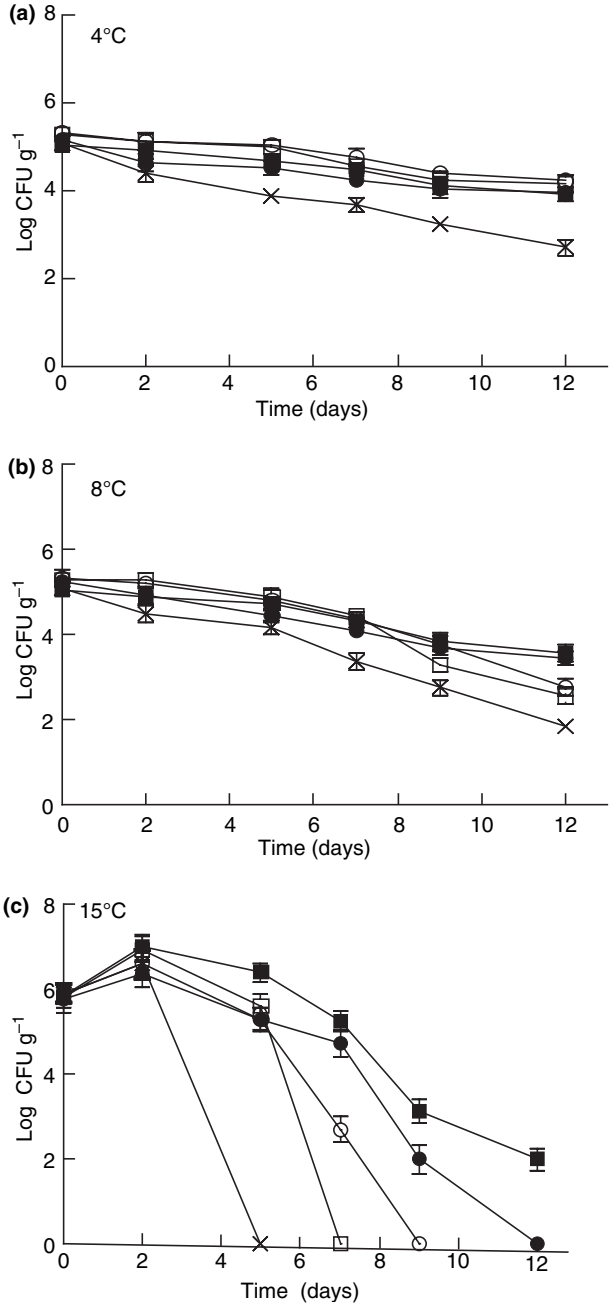

Figure 3 Survival of *Listeria monocytogenes* LO28 (■) and the glutamate decarboxylase (GAD) deletion mutants, Δ*gadA* (●), Δ*gadB* (□), Δ*gadC* (○) and Δ*gadAB* (×) on modified atmosphere-packaged dry coleslaw mix during storage at: (a) 4°C, (b) 8°C and (c) 15°C.

of the 12-day storage period. However, viable populations of Δ*gadC* were undetectable on day 9, while populations of Δ*gadB* and Δ*gadAB* could not be detected on days 7 and 5, respectively. The results indicate that the wild-type strain survived and/or grew significantly better ($P < 0{\cdot}05$) than the double mutant Δ*gadAB* on both packaged vegetables at all storage temperatures. Thus, the GAD system plays an important role in the survival of *L. monocytogenes* during storage in MAP vegetables.

The fresh-cut vegetables were sealed in packages initially enclosing air. During storage, the gas atmospheres within the packages were modified, mainly as a result of the respiration of the vegetables. The concentrations of O_2 and CO_2 achieved within packs varied with the packaged product and storage temperature. With packages of lettuce stored at 4 and 8°C, O_2 levels fell to 2–5% and 1–3%, and CO_2 levels increased to 9–10% and 12–13%, respectively. Dry coleslaw mix had a higher respiration rate and higher levels of CO_2 were reached in packs of coleslaw than those attained in packs of lettuce. With coleslaw, CO_2 levels rose to 12–13% during storage at 4°C, and to 25–27% and 34–35% during storage at 8 and 15°C, respectively. In the case of minced beef, packages were flushed with 20% CO_2 and 80% O_2 and these gas levels persisted during storage.

The total aerobic mesophilic microflora proliferated on all products during storage (Fig. 4). Generally, increasing the storage temperature from 4°C to 15°C increased the rate and extent of microbial growth. Initial microbial counts on all products were approximately 6 log CFU g^{-1}. Populations on minced beef increased to 7·5, 8·5 and 9·0 log CFU g^{-1} during storage at 4, 8 and 15°C, respectively. On lettuce, microbial counts reached final population densities of *c.* 7·3 log CFU g^{-1} at 4 and 8°C, and 8·0 log CFU g^{-1} at 15°C. On coleslaw mix, counts of mesophilic microflora also increased more rapidly at the higher temperatures; however, no difference in final population densities was observed.

Changes in pH of food samples were measured during storage and they varied depending on the packaged product (Fig. 5). The initial pH of minced beef was *c.* 6·0 and it did not vary over the duration of storage, regardless of storage temperature. The pH of the lettuce samples increased during storage from 5·7 to a maximum of 6·6, and there was no effect of storage temperature on pH changes. In the case of coleslaw mix, however, storage temperature had a major effect on changes in pH. At 4°C, the initial pH was *c.* 6·2 and it remained at this approximate value throughout the storage period. At 8°C, the pH gradually dropped from *c.* 6·2 on day 0 to pH 5·8 on day 5, and to pH 4·1 on day 12. At 15°C, the pH decreased rapidly from *c.* 6·2 to pH 4 on day 5 and it stayed at pH 4 for the remaining storage period.

The glutamate levels were also measured; free glutamate levels per 100 g were 11, 10 and 7 mg, respectively for coleslaw, minced beef and lettuce.

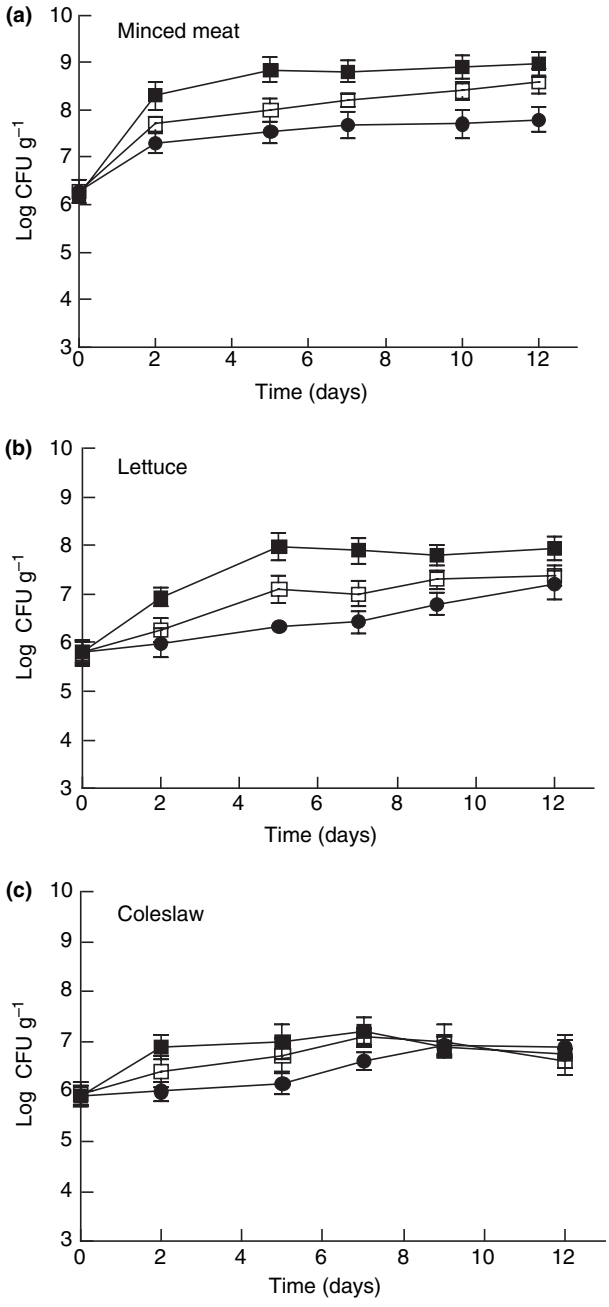

Figure 4 Effects of storage temperature on growth of total aerobic mesophilic microflora on modified atmosphere-packaged (a) minced beef, (b) lettuce and (c) dry coleslaw mix. ●, 4°C; □, 8°C; ■, 15°C.

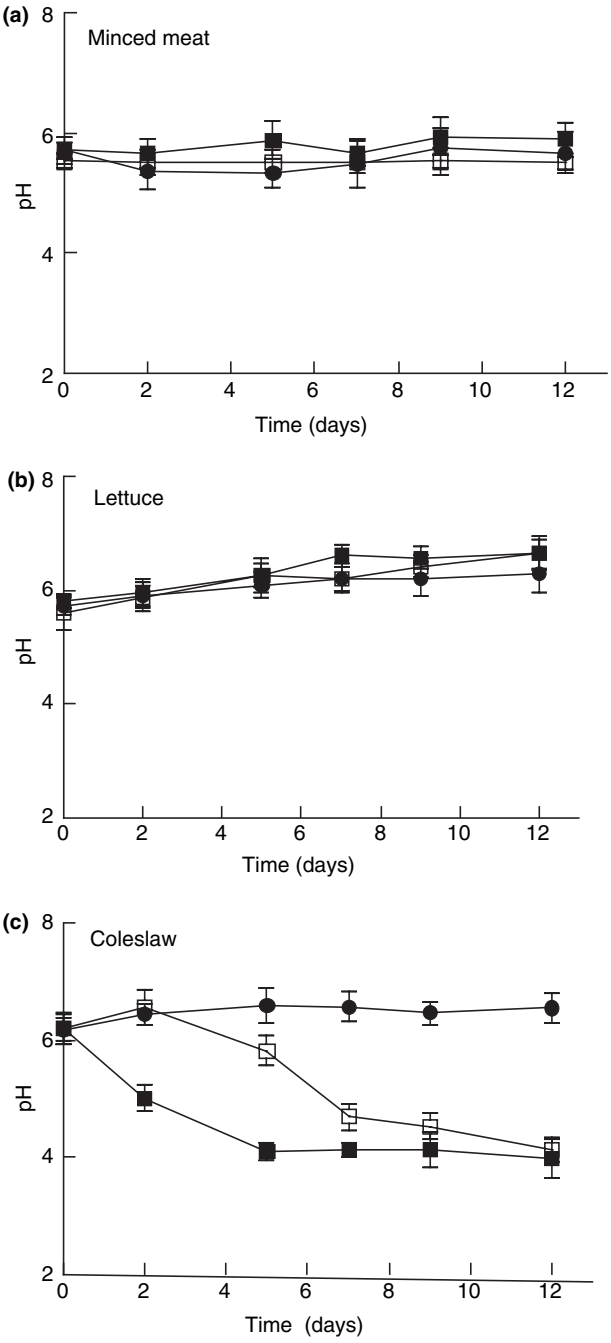

Figure 5 Effects of storage temperature on changes in pH of modified atmosphere-packaged (a) minced beef, (b) shredded lettuce and (c) dry coleslaw mix. ●, 4°C; □, 8°C; ■, 15°C.

Discussion

This work demonstrates reduced survival and/or growth of the GAD mutant strains in a number of MAP foods, especially in those containing elevated CO_2 levels. Fernández *et al.* (1997) reported that elevated CO_2 concentrations were inhibitory to *L. monocytogenes* at refrigeration temperatures. CO_2 inhibits the growth of bacteria by: (i) affecting cellular enzymes and decreasing the rate of metabolic reactions (Ranson *et al.* 1960); (ii) CO_2 product repression of carboxylases and decarboxylases (King and Nagel 1975); (iii) disrupting cell membrane structural integrity and/or specific functions (Sears and Eisenberg

1961); (iv) decreasing the substrate and intra-cellular pH (Wolfe 1980), or by a combination of these mechanisms (Dixon and Kell 1989).

The survival/growth patterns of the wild-type LO28 and the mutant strains varied depending on the type of packaged food. The reasons for the differences in survival in the various foods may be related to a number of factors including, different CO_2 levels, different product pH values, different substrate compositions, nutrient (glutamate) concentrations and availability and/or the indigenous microflora and associated competitive interactions on foods. Differences in survival between the wild-type LO28 and the mutants were especially evident in packaged coleslaw during storage at 15°C, where the order of survival was LO28 > Δ*gadA* > Δ*gadC* > Δ*gadB* > Δ*gadAB*. The reasons for the reduced survival of the mutants were presumably because of direct inhibitory effects of CO_2, as well as indirect effects of CO_2 resulting in acidification of the coleslaw. From the gas analysis and pH studies, the high CO_2 levels (*c.* 35%) within the coleslaw packages at 15°C resulted in rapid decrease of coleslaw pH. An important factor contributing to bacterial survival in acidic and other stressful environments is the ability to regulate their intracellular pH (pH_i) and membrane potential, a process primarily driven by the controlled movement of cations across the cell membrane (Hill *et al.* 1995). If CO_2 growth inhibition is caused by acidification of the food, the acid-tolerant strain LO28 most likely used the GAD system to re-establish pH_i, and differences in survival of the various GAD mutants were presumably because of differences in acid resistance and ability to control pH_i. Jydegaard-Axelsen *et al.* (2004) observed an increase in expression of GAD genes (*gadA*, *gadB*, *gadC*) in *L. monocytogenes* LO28 grown in atmospheres of 100% CO_2 or 100% nitrogen (N_2). The similarity in response towards CO_2 and N_2 indicated that *L. monocytogenes* LO28 responded to CO_2 as it would to acid using the GAD system, and indicates overlap between the CO_2 response and the response to acidity and anaerobiosis, respectively.

The requirement for the individual *gad* genes varied depending on the food and/or storage conditions. The packaged coleslaw contained anti-listerial shredded carrots (Francis and O'Beirne 2001a), had low pH values and high in-pack CO_2 levels (at 15°C) and under these stressful conditions, the *gadB* and *gadC* genes played an important role. In the case of packaged lettuce, *gadA* played the most obvious role. The packaged lettuce may be considered a less stressful environment for *Listeria*, as it had a higher pH and the packages contained lower CO_2 levels and in this environment, *gadA* appeared to play the greatest role. Cotter *et al.* (2005) reported similar results; *gadA* played a role in survival/growth at relatively mild acidic conditions, whereas the *gadB* and *gadC* genes were required for survival in low pH conditions. While the CO_2 levels within packs of lettuce were not very high, they appeared to be high enough to reduce the growth of the Δ*gadA* and *gadAB* mutants. Other factors such as the presence of competitive microflora on lettuce, dominated mainly by enterobacteriaceae and pseudomonads (Francis and O'Beirne 1998a; Jacxsens *et al.* 2003), may have caused additional stress and reduced survival/growth of these mutants (Francis and O'Beirne 1998a,b).

The GAD mutants behaved similar to the wild-type strain on minced beef stored at 4°C, but their survival was impaired at 8 and 15°C. The reason for reduced survival of the mutants at 8 and 15°C was presumably because of direct inhibitory effects of elevated CO_2 levels, although it is unclear why no inhibitory effects were observed at 4°C. The additional impact of the rapidly growing competitive mesophilic microflora on the meat at 8 and 15°C possibly contributed to the generation of more stressful conditions for the mutants because of nutrient depletion, changes in the chemical composition of the product and/or production of bactericidal agents. The high levels of CO_2 within packages of meat combined with the higher temperatures may have selected for growth of lactic acid bacteria (LAB) (Francis and O'Beirne 1998b), and allowed the more acid-resistant wild-type to survive better than the acid-sensitive mutants (Vescovo *et al.* 1996; Francis and O'Beirne 1998a,b).

This study demonstrates that the GAD acid resistance system plays an important role in the survival and/or growth of *L. monocytogenes* LO28 in MAP foods, especially those containing elevated CO_2 atmospheres. The reasons for reduced survival/growth of the mutants in the different packaged products appear because of a number of reasons including, direct inhibitory effects from high CO_2 levels, indirect effects of CO_2 resulting in acidification of the product and possible promotion of growth of competitive microflora such as LAB, and/or general inhibitory effects from the competitive indigenous microflora of foods. Each *gad* gene played a different role and the requirement for individual *gad* genes varied depending on the food or environment. In more stressful environments (i.e. higher CO_2 conditions), the *gadB* and *gadC* genes played the greatest role, whereas in less stressful environments (i.e. lower CO_2 levels) *gadA* appears to be more important.

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References

Bell, C. and Kyriakides, A. (1998) Background. In *Listeria: A Practical Approach to the Organism and its Control in Foods* ed. Bell, C. and Kyriakides, A. pp. 3–29. London: Chapman and Hall.

Beuchat, L.R. and Brackett, R.E. (1990) Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *J Food Sci* **55**, 755–758.

Brackett, R.E. (1994) Microbiological spoilage and pathogens in minimally processed refrigerated fruits and vegetables. In *Minimally Processed Refrigerated Fruits and Vegetables* ed. Wiley, R.C. pp. 269–312. New York: Chapman and Hall.

Carlin, F., Nguyen-the, C. and Abreu da Silva, A. (1995) Factors affecting the growth of *Listeria monocytogenes* on minimally processed fresh endive. *J Appl Bacteriol* **78**, 636–646.

Cotter, P.D., Gahan, C.G.M. and Hill, C. (2001a) A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. *Mol Microbiol* **40**, 465–475.

Cotter, P.D., O'Reilly, K. and Hill, C. (2001b) Role of the glutamate decarboxylase acid resistance system in the survival of *Listeria monocytogenes* LO28 in low pH foods. *J Food Prot* **64**, 1362–1368.

Cotter, P.D., Ryan, S., Gahan, C.G.M. and Hill, C. (2005) Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated with an ability to grow at low pH. *Appl Environ Microbiol* **71**, 2832–2839.

Daniels, J.A., Krishnamurthi, R. and Rizvi, S.S.H. (1985) A review of the effect of carbon dioxide on microbial growth and food quality. *J Food Prot* **48**, 532–537.

Devlieghere, F., Geeraerd, A.H., Versyck, K.J., Vandewaetere, B., Van Impe, J. and Debevere, J. (2001) Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: a predictive model. *Food Microbiol* **18**, 53–66.

Dixon, N.M. and Kell, D.B. (1989) The inhibition by CO_2 of the growth and metabolism of micro-organisms: a review. *J Appl Bacteriol* **67**, 109–136.

Enfors, S.O., Molin, G. and Ternström, A. (1979) Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. *J Appl Bacteriol* **47**, 197–208.

Fernández, P.S., George, S.M., Sills, C.C. and Peck, M.W. (1997) Predictive model of the effect of CO_2, pH, temperature and NaCl on the growth of *Listeria monocytogenes*. *Int J Food Microbiol* **37**, 37–45.

Francis, G.A. and O'Beirne, D. (1997) Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. *Int J Food Sci Technol* **32**, 141–151.

Francis, G.A. and O'Beirne, D. (1998a) Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of *Listeria innocua*. *Int J Food Sci Technol* **33**, 477–488.

Francis, G.A. and O'Beirne, D. (1998b) Effects of storage atmosphere on *Listeria monocytogenes* and competing microflora using a surface model system. *Int J Food Sci Technol* **33**, 465–476.

Francis, G.A. and O'Beirne, D. (2001a) Effects of vegetable type, package atmosphere and storage temperature on growth and survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *J Ind Microbiol Biotechnol* **27**, 111–116.

Francis, G.A. and O'Beirne, D. (2001b) Effects of acid adaptation on the survival of *Listeria monocytogenes* on modified atmosphere packaged vegetables. *Int J Food Sci Technol* **36**, 477–487.

Gahan, C.G.M. and Hill, C. (1999) The relationship between acid stress responses and virulence in *Salmonella typhimurium* and *Listeria monocytogenes*. *Int J Food Microbiol* **50**, 93–100.

Gahan, C.G.M., O'Driscoll, B. and Hill, C. (1996) Acid adaptation of *Listeria monocytogenes* can enhance survival in acidic foods and during milk fermentation. *Appl Environ Microbiol* **62**, 3128–3132.

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H., *et al.* (2001) Comparative genomics of *Listeria* species. *Science* **294**, 849–852.

Hill, C. and Gahan, C.G.M. (2000) *Listeria monocytogenes*: role of stress in virulence and survival in food. *Ir J Agricul Food Res* **39**, 195–201.

Hill, C., O'Driscoll, B. and Booth, I. (1995) Acid adaptation and food poisoning microorganisms. *Int J Food Microbiol* **28**, 245–254.

Hill, C., Cotter, P.D., Sleator, R.D. and Gahan, C.G.M. (2002) Bacterial stress response in *Listeria monocytogenes*: jumping the hurdles imposed by minimal processing. *Int Dairy J* **12**, 273–283.

Hintlian, C.B. and Hotchkiss, J.H. (1986) The safety of modified atmosphere packaging: a review. *Food Technol* **40**, 70–76.

Hudson, J.A., Mott, S.J. and Penney, N. (1994) Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere packaged sliced roast beef. *J Food Prot* **57**, 204–208.

Jacxsens, L., Devlieghere, F., Falcato, P. and Debevere, J. (1999) Behaviour of *Listeria monocytogenes* and *Aeromonas* spp. on fresh-cut produce packaged under equilibrium-modified atmosphere. *J Food Prot* **62**, 1128–1135.

Jacxsens, L., Devlieghere, F., Ragaert, P., Vanneste, E. and Debevere, J. (2003) Relation between microbiological quality, metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. *Int J Food Microbiol* **83**, 263–280.

Jydegaard-Axelsen, A.-M., Høiby, P.E., Holmstrøm, K., Russel, N. and Knøchel, S. (2004) CO_2- and anaerobiosis-induced changes in physiology and gene expression of different *Listeria monocytogenes* strains. *Appl Environ Microbiol* **70**, 4111–4117.

King, A.D. Jr. and Nagel, C.W. (1975) Influence of carbon dioxide upon the metabolism of *Pseudomonas aeruginosa*. *J Food Sci* **40**, 362–366.

Mataragas, M., Drosinos, E.H. and Metaxopoulos, J. (2003) Antagonistic activity of lactic acid bacteria against *Listeria monocytogenes* in sliced cooked cured pork shoulder stored under vacuum or modified atmosphere at 4 ± 2°C. *Food Microbiol* **20**, 259–265.

Ranson, S.L., Walker, D.A. and Clarke, I.D. (1960) Effects of carbon dioxide on mitochondrial enzymes from *Ricinus*. *Biochem J* **76**, 216–221.

Sears, D.F. and Eisenberg, R.M. (1961) A model representing a physiological role of CO_2 at the cell membrane. *J Gen Physiol* **44**, 869–887.

Vescovo, M., Torriani, S., Orsi, C., Macchiarolo, F. and Scolari, G. (1996) Application of antimicrobial-producing lactic acid bacteria to control pathogens in ready-to-use vegetables. *J Appl Bacteriol* **81**, 113–119.

Wimpfheimer, L., Altman, N.S. and Hotchkiss, J. H. (1990) Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under MAs and in air. *Int J Food Microbiol* **11**, 205–214.

Wolfe, S.K. (1980) Use of CO- and CO_2-enriched atmospheres for meats, fish and produce. *Food Technol* **34**, 55–58.