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ORIGINAL ARTICLE

Effect of packaging atmosphere and pH on the virulence and growth of *Yersinia enterocolitica* on pork stored at 4°C

P. W. Bodnaruk[1] and F. A. Draughon[2]*

Growth and virulence of pathogenic Yersinia enterocolitica *were investigated on high (pH> 6·0) and normal (pH<5·8) pH pork packaged in modified atmospheres and stored at 4°C. Modified atmospheres used in the study were vacuum packaging and saturated CO_2. Pork was packaged in a high gas barrier packaging film and examined over a 30-day period. Phenotypic characteristics were used to detect the presence of the virulence plasmid of* Y. enterocolitica *after exposure to the pork packaging and storage regimen. Phenotypic characteristics of* Y. enterocolitica *isolates from pork loin stored at 4°C for 30 days that were studied included Congo red uptake, calcium dependence and autoagglutination in methyl red Voges–Proskauer broth and tissue culture medium. Numbers of* Y. enterocolitica *on the lean surface of high pH pork slices increased approximately 2·7 log cfu cm^{-2} when vacuum packaged and stored at 4°C for 30 days. Storage of inoculated normal pH pork in 100% CO_2 resulted in* Y. enterocolitica *remaining in the lag phase over the storage period. Virulence of* Y. enterocolitica *was maintained in 25 to 35% of isolates following storage for 30 days at 4°C in vacuum- and CO_2-packaged meats and was not affected by pH of the pork loin.*

Introduction

Refrigerated storage (0 to 4°C) is one of the most important methods of preservation used in the meat industry (Lambert et al. 1991). When coupled with modified atmosphere (MA) packaging, spoilage of fresh meat can be delayed by inhibiting spoilage flora and retarding enzymatic degradation (Young et al. 1988). Aerobic spoilage organisms usually warn consumers of meat spoilage, but MA packaging may inhibit spoilage flora and may allow the growth of psychrotrophic pathogens such as *Yersinia enterocolitica* and *Listeria monocytogenes* (Grau and Vanderlinde 1990). Centralized prepackaging of meat in MA has been proposed as an effective method for retail sale (McMullen and Stiles 1991). Production of pre-cut, MA-packaged fresh meat that is ready for retail sale appears to be a current trend in the North American meat industry (Nunes 1995). Vacuum- or MA-packaging of meat of normal (pH<5·8) and high (pH>6·0) pH may lengthen the shelf-life when compared with aerobic storage, but may also increase the risk of foodborne illness (Farber 1991). *Y. enterocolitica* is frequently associated with pigs and pork products and can be transmitted to humans by consumption of raw, undercooked or recontaminated processed meats (Manu-Tawaih et al. 1993, Rodriguez et al. 1994).

*Corresponding author.

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[1]*Armour Swift-Eckrich, 3131 Woodcreek Drive, Downers Grove, IL 60515, USA*
[2]*Department of Food Science and Technology, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1071, USA*

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Pathogenic strains of *Y. enterocolitica* harbor a 40–50-megadalton (Md) plasmid that confers virulence on the infecting organism (Prpic et al. 1985). Several phenotypic characteristics of *Y. enterocolitica* have been described and are associated with the virulence plasmid. These characteristics include Congo red binding, calcium dependence, crystal violet binding, autoagglutination in tissue culture medium RPMI-1640 and autoagglutination in methyl red Voges–Proskauer broth. Loss of the plasmid results in loss of the virulence-associated phenotype (Bhaduri and Turner-Jones 1993). Phenotypic characteristics associated with virulence are widely used as screening tests of virulence of *Y. enterocolitica* as they are both rapid and economical (Bhaduri and Turner-Jones 1993). Both virulent and avirulent strains of *Y. enterocolitica* have been isolated from foods (Stern 1982). Effects of consuming avirulent varieties may be no more hazardous than consuming non-pathogenic coliform bacteria (Stern 1982). Bhaduri and Turner-Jones (1993) attempted to assess the stability of the virulence plasmid in *Y. enterocolitica* under various anaerobic conditions found in food processing. No plasmid loss with virulent *Y. enterocolitica* in logarithmic and stationary phase was observed when the organisms were exposed to a vacuum or to an atmosphere of 94% CO_2:6% H_2 for 24 h at 28°C.

The objective of this study was to investigate the growth and survival of pathogenic *Y. enterocolitica* on MA-packaged fresh pork of normal and high pH stored at 4°C, and to examine possible changes in virulence following exposure of *Y. enterocolitica* to a food-processing regimen. Normal and high pH meat was chosen owing to the differences in rates of spoilage when packaged without O_2 (Gill and Newton 1979).

Materials and methods

Bacterial strains and preparation of inoculum

Three strains of *Y. enterocolitica*, YE133 (serotype 0:8), YE228 (serotype 0:3) and YE321 (serotype 0:20), obtained from Peter Feng, United States Food and Drug Administration, Washington DC, were used for inoculation studies. Stock cultures were stored on trypticase soy agar (TSA; BBL, Cockeysville, MD, USA) slants at 4°C. Cultures were carefully selected to represent diverse serotypes and diverse environmental characteristics.

Pure culture of *Y. enterocolitica* were grown (individually) for 48 h at 25°C in trypticase soy broth (TSB; BBL, Cockeysville, MD, USA). Cultures were pooled (1:1:1) and added to 1 l of 0·1% peptone water (PW; Difco, Detroit, MI, USA) to give an approximate final concentration of 10^6 cfu ml^{-1}. The diluted culture of *Y. enterocolitica* was used for meat inoculation with a target inoculum of log 3 to log 4 cfu cm^{-2}.

Preparation of meat samples

Pork striploins (*M. longissimus dorsi*) were obtained from a local retailer on three separate occasions. Muscle tissue was trimmed of fat and divided into steaks approximately 1·5 cm thick. Steaks were tested for initial microbial load at the onset of each replication to determine the acceptability of each pork loin for the study. Initial aerobic plate count for pork loins was initially log 3·0 to log 3·5 cfu cm^{-2}. High and normal pH muscles were selected based on lean tissue color. Normal pH pork has a pale pink color while high pH pork has a dark red appearance (Wilson et al. 1981). All muscle pH values were checked in the laboratory using a Corning 350 pH/ion analyzer (Corning, NY). Pork steaks were inoculated with *Y. enterocolitica* by immersion for 30 sec in the diluted culture. This gave a surface inoculum level of approximately 10^3 cfu cm^{-2}. Uninoculated control slices of pork were dipped in sterile water. Steaks were allowed to dry for 10 min on sterile racks.

Meat packaging

The packaging film used was B-900 (Cryovac, Duncan, SC, USA), a multilayered polyolefin with gas barrier properties (O_2 transmission rate 3–6 ml m^{-2} 24 h^{-1} at 4·4°C and 0% RH). A Multivac (Koch, Kansas City, MO, USA) gas-

flushing machine was used to achieve the desired atmosphere. Two packaging atmospheres were used, vacuum and 100% CO_2. Slices of inoculated and uninoculated pork were either vacuum packaged or packaged in 100% CO_2.

Storage and sampling

Pork loins were tested for the presence of *Y. enterocolitica* at the time of packaging. Packaged pork samples were stored at 4°C. On each sampling day the pH of pork steaks from each packaging treatment was measured and the gas composition of CO_2 packages was determined using a Hewlett Packard gas chromatograph, model 5890-series II (Hewlett Packard, Wilmington, DE, USA) equipped with a thermal conductivity detector (TCD) and a stainless steel CTRI column (Alltech Associates, Inc., Deerfield, IL, USA). Column and TCD temperatures were set at 25°C, the quantity of sample injected was 5 ml and the total run time was 3 min. Helium was used as the carrier gas at a flow rate of 40 ml min^{-1}. No gas composition analysis was made on vacuum-packaged pork slices because weep present in the package interfered with sample collection.

Using a cork borer, four samples (each 3·5 $cm^2 \times c$. 0·5 cm deep) were taken from the lean surface at 5-day intervals throughout the 30-day storage period. The lean tissue samples were blended with 0·1% PW in a Model 400 Stomacher Lab Blender (Steward, London, UK) for 1 min. Aliquots (0·1 ml) of serial dilutions were surface plated on to appropriate solid media for micro-organism enumeration and identification. Inoculated pork slices were sampled for *Y. enterocolitica* only, while uninoculated pork slices were sampled for normal spoilage flora. The media and conditions for enumeration and isolation of micro-organisms were as follows: *Y. enterocolitica*, cefsulodin irgasan novobiocin agar (CIN; BBL, Cockeysville, MD, USA) incubated at 30°C for 24–48 h; aerobic mesophiles, tryptone yeast extract soy glucose agar (TYSG; Grau et al. 1985) incubated at 25°C for 72 h; lactic acid bacteria, De man, Rogosa, Sharpe agar (MRS; Oxoid, Basingstoke, UK) incubated at 25°C for 72 h; *Brochothrix thermosphacta*, streptomycin thallous acetate actidione agar (STAA; Gardner 1966) incubated at 25°C for 72 h; Gram-negative bacteria, peptone agar (PEPA; Grau 1983) incubated at 25°C for 48 h.

Identification of micro-organisms

Presumptive *Y. enterocolitica* colonies on CIN agar were confirmed as positive if they were Gram-negative, motile at 25°C, non-motile at 37°C, sucrose, urea and mannitol positive, and rhamnose, raffinose, melibiose, and Simmons citrate negative (Weagant et al. 1992). Gram-positive, catalase-negative colonies selected from MRS were assumed to be lactic acid bacteria. Oxidase-negative colonies selected from STAA were identified as *B. thermosphacta* (Gardner 1966). To determine the count of Gram-negative bacteria, colonies picked from PEPA were tested for Gram reaction using the method of Buck (1982). Colonies counted on TYSG were recorded as an aerobic plate count (APC).

Virulence testing

Virulence of *Y. enterocolitica* is associated with a 40–50-Md plasmid (Prpic et al. 1985). To simplify testing for the virulence plasmid, isolates of *Y. enterocolitica* were tested for phenotypic characteristics associated with virulence (Riley and Toma 1989, Kwaga and Iversen 1991) before inoculation on to pork and after packaging and storage. Following 30-days storage at 4°C, *Y. enterocolitica* isolates were confirmed biochemically and randomly selected from CIN agar plates for virulence screening tests. Where possible a maximum of 20 isolates were chosen. The reliability of indicator tests for virulence has been investigated by several authors (Prpic et al. 1985, Kwaga and Iversen, 1991). As no individual virulence-associated phenotypic characteristic is a reliable single indicator of virulence (Kwaga and Iversen 1991), several testing methods were used as confirmation in our study for screening of virulence of *Y. enterocolitica* isolates: Congo red uptake and calcium dependence (Riley and Toma, 1989), autoagglutination (AA) in methyl red Voges–-Proskauer media (Weagant et al. 1992), and

AA in tissue culture media RPMI-1640 (Laird and Cavanaugh 1980).

Statistical analysis

The General Linear Models (GLM) procedure was used to analyze the main effects and interactions of packaging atmospheres, storage time and lean tissue pH on the differences in mean microbial numbers (SAS Institute, Cary, NC). When significant ($P<0{\cdot}05$), means were separated by the LSMeans procedure. Three replicates were performed for all experiments. Each replication was designed as a complete randomized block with approximately six weeks between replications.

Results

Microflora isolated from vacuum- and 100% CO_2-packaged normal and high pH pork are shown in Table 1. Aerobic plate counts (APC) increased significantly ($P<0{\cdot}05$) in all treatments over the 30-day storage period. APC of vacuum- and carbon dioxide-packaged pork were not significantly different ($P>0{\cdot}05$) for most treatments. Meat examined prior to inoculation was found to be free of *Y. enterocolitica*. At 4°C, *Y. enterocolitica* grew on high pH pork slices reaching 5·8 log cfu cm^{-2} after 30-days storage on vacuum-packaged pork and 4·8 log cfu cm^{-2} on pork packaged under CO_2 (Table 1). Normal pH pork supported growth of *Y. enterocolitica* when vacuum packaged, reaching 5·2 log cfu cm^{-2} after 25-days storage at 4°C. *Y. enterocolitica* packaged in CO_2 on normal pH pork survived in an extended lag phase, maintaining a level of approximately 3 log cfu cm^{-2}.

Lactic acid bacteria became the dominant component of the flora in all packages after 30-days storage at 4°C, reaching between 6·8 and 7·6 log cfu cm^{-2} (Table 1). Lactic acid bacteria increased by 4·7 to 4·9 log cfu cm^{-2} during storage of normal pH pork at 4°C, whereas on high pH pork lactics increased by 3·0 to 3·7 log cfu cm^{-2}. This difference was probably due to the higher initial population of lactic acid bacteria on the high pH pork as both high and normal pH pork supported the same final populations. A large component of the aerobic mesophilic count was lactic acid bacteria, since lactic counts increased up to 8 log cfu cm^{-2} after 20-days storage in all packaging treatments (Table 1).

B. thermosphacta grew slowly on high pH pork packaged in vacuum and CO_2, increasing between 0·6 and 1·0 log cfu cm^{-2} over the 30-day storage period. Numbers of *B. thermosphacta* were significantly ($P<0{\cdot}05$) higher overall on high pH pork than on normal pH pork. However, increases were too small to have much practical importance. No increase in growth could be detected on normal pH pork packaged under CO_2 or vacuum (Table 1).

Gram-negative bacteria reached populations in excess of 6·0 log cfu cm^{-2} on meat packaged under vacuum (Table 1). Meat packaged under CO_2 supported a maximum Gram-negative bacterial count of around 5·0 log cfu cm^{-2} after 30-days storage. Numbers of Gram-negative bacteria on high pH pork were consistently ($P<0{\cdot}05$) higher than on corresponding normal pH pork whether vacuum packaged or packaged in CO_2.

Gas analysis data showed that the percentage of residual O_2 in CO_2-packaged bags for inoculated and uninoculated pork increased slightly over time (<1%), but an atmosphere in excess of 99% CO_2 was maintained over 30-days storage at 4°C. A change in gas headspace of less than 1% over 30 days at 4°C was not significant statistically ($P>0{\cdot}05$) or of practical importance.

Meat samples showed no significant change ($P>0{\cdot}05$) in pH over storage time for each of the packaging treatments (Table 2). The pH of high and normal pH pork loin was significantly different ($P<0{\cdot}05$). High pH meat maintained a pH above 6·0 and normal pH meat remained between pH 5·4 and 5·7. Values at day 0 were not different ($P>0{\cdot}05$) for each of the packaging atmospheres. The pH of loins was not significantly ($P>0{\cdot}05$) affected by inoculation or packaging atmospheres ($P>0{\cdot}05$).

Indicator tests for virulence of confirmed *Y. enterocolitica* isolates from vacuum- and CO_2-packaged pork of normal and high pH are shown in Table 3. These data show that between 25 and 35% of colonies tested

Table 1. Microflora (log cfu cm^{-2} (s.d.[a])) isolated from vacuum- and CO_2-packaged normal and high pH pork stored at 4°C[b]

Microflora		Storage time (days)													
		0		5		10		15		20		25		30	
Vacuum-packaged pork															
Aerobic plate count	N[c]	3·4c	(0·1)	5·7e	(0·2)	7·0g	(0·2)	6·9f	(0·6)	7·9g	(0·1)	8·5g	(0·3)	7·7g	(0·2)
	H[d]	4·1d	(0·1)	5·8e	(0·6)	6·4f	(0·5)	6·7f	(0·2)	7·6g	(0·2)	8·1g	(0·5)	7·6g	(0·1)
Lactic acid bacteria	N	2·7b	(0·7)	5·6e	(0·1)	6·4f	(0·6)	6·9f	(0·1)	7·6g	(0·1)	8·0g	(0·2)	7·6g	(0·2)
	H	3·8c	(1·2)	5·7e	(0·6)	6·3f	(0·4)	6·7f	(0·2)	7·2g	(0·2)	6·3f	(1·9)	7·5g	(0·1)
Gram-negative bacteria	N	3·4c	(0·1)	4·0c	(0·2)	4·8d	(0·1)	5·5e	(0·7)	5·5e	(0·8)	5·7e	(0·4)	6·2f	(0·3)
	H	3·5c	(0·2)	4·9d	(0·4)	4·8d	(0·3)	6·1ef	(0·6)	6·5f	(0·2)	7·0g	(0·6)	6·4f	(0·2)
Brochothrix thermosphacta	N	–[e] a	(–)	1·9a	(0·2)	1·8a	(0·2)	1·9a	(0·4)	2·0ab	(0·5)	2·3ab	(0·2)	–a	(–)
	H	2·0b	(0·6)	3·2c	(0·1)	2·9b	(0·2)	2·8b	(0·2)	2·6b	(0·1)	2·6b	(0·3)	2·7b	(0·1)
Yersinia enterocolitica	N	3·5c	(0·4)	3·7c	(0·7)	4·6d	(0·7)	4·1cd	(0·2)	4·6d	(1·5)	5·2e	(1·4)	3·8c	(1·6)
	H	3·1c	(0·7)	4·1d	(0·1)	4·7d	(1·1)	5·8e	(0·3)	5·9e	(0·4)	5·7e	(0·2)	5·8e	(0·5)
CO-packaged pork															
Aerobic plate count	N	3·4c	(0·1)	4·9d	(0·1)	5·4e	(0·3)	6·2f	(0·1)	7·6g	(0·3)	7·8g	(0·1)	7·6g	(0·3)
	H	3·8c	(1·2)	5·6e	(0·7)	6·2f	(0·4)	6·0ef	(0·4)	7·0fg	(0·7)	7·6g	(0·4)	7·3g	(0·5)
Lactic acid bacteria	N	2·7b	(0·7)	4·7de	(0·2)	5·3e	(0·3)	6·1f	(0·1)	6·9fg	(0·7)	7·5g	(0·2)	7·5g	(0·2)
	H	3·8c	(1·2)	5·4e	(0·6)	6·0f	(0·4)	6·2f	(0·2)	6·8fg	(0·7)	7·6g	(0·4)	6·8fg	(0·2)
Gram-negative bacteria	N	3·4c	(0·1)	2·7b	(0·9)	2·9b	(0·2)	2·8b	(0·2)	3·7c	(0·6)	4·1cd	(0·1)	4·9d	(0·1)
	H	3·5c	(0·2)	4·5d	(0·7)	4·2d	(0·5)	5·5e	(0·2)	5·1de	(0·1)	5·0de	(0·3)	5·2de	(0·1)
Brochothrix thermosphacta	N	–a	(–)	–a	(–)	1·9a	(0·4)	2·3ab	(0·5)	2·4ab	(1·2)	–a	(–)	2·0ab	(0·6)
	H	2·0b	(0·6)	3·2c	(0·2)	2·9bc	(0·1)	3·3c	(0·2)	2·8b	(0·2)	2·3b	(1·0)	3·1c	(0·3)
Yersinia enterocolitica	N	3·5c	(0·4)	3·1c	(0·3)	3·3c	(0·6)	3·2c	(0·2)	3·1c	(0·7)	2·9b	(0·6)	3·3c	(0·4)
	H	3·1c	(0·7)	3·6c	(0·3)	4·7d	(0·6)	4·3d	(0·3)	4·0d	(0·1)	4·2cd	(0·2)	4·8d	(0·7)

[a]Standard deviation of three replicates.
[b]Means followed by different letters throughout table are significantly different ($P<0·05$).
[c]Normal pH pork.
[d]High pH pork.
[e]None detected (for statistical calculations the value of 0·1 was used instead of zero since zero is not a valid log number.

Table 2. Effect of storage time on pH (mean[a] (s.d.[b])) of normal and high pH pork vacuum-packaged or packaged under CO_2

Storage time (days)	Vacuum-packaged				Packaged under CO_2			
	Normal pH pork		High pH pork		Normal pH pork		High pH pork	
0	5·57a[c]	(0·02)	6·21b	(0·09)	5·62a	(0·06)	6·28b	(0·02)
5	5·65a	(0·04)	6·25b	(0·04)	5·57a	(0·06)	6·15b	(0·06)
10	5·60a	(0·02)	6·16b	(0·03)	5·61a	(0·03)	6·13b	(0·08)
15	5·52a	(0·07)	6·22b	(0·09)	5·58a	(0·04)	6·25b	(0·06)
20	5·36a	(0·09)	6·24b	(0·02)	5·57a	(0·05)	6·07b	(0·03)
25	5·51a	(0·08)	6·21b	(0·05)	5·54a	(0·06)	6·14b	(0·05)
30	5·58a	(0·03)	6·19b	(0·06)	5·58a	(0·04)	6·30b	(0·03)

[a]Mean of three replicates from uninoculated pork.
[b]Standard deviation.
[c]Means followed by different letters are significantly different ($P<0·05$).

retained the virulence plasmid following packaging and storage at 4°C (Table 3). Virulence of *Y. enterocolitica* was not influenced by pH of pork loins or the packaging condition ($P>0{\cdot}05$). Different phenotypic assays showed small variations in the percentage found positive; however, differences were not significant ($P>0{\cdot}05$).

Discussion

Except for normal pH pork packaged in CO_2, *Y. enterocolitica* grew in all packaging treatments at 4°C. Several authors have reported the growth of *Y. enterocolitica* in vacuum-packaged meats and meats stored under atmospheres of saturated CO_2 (Gill and Reichel 1989, Manu-Tawaih et al. 1993, Doherty et al. 1995). Gill and Reichel (1989) observed the growth of *Y. enterocolitica* on high pH beef stored under 100% CO_2 at 5 and 10°C. Doherty et al. (1995) noted almost a 4 log cfu g^{-1} increase of *Y. enterocolitica* inoculated on to lamb pieces and packaged in 100% CO_2 during a 28-day storage period at 5°C. It is evident that *Y. enterocolitica* may be a potential problem on MA-packaged meats. Results of the present study show that normal pH pork packaged in 100% CO_2 and stored at 4°C provided an environment that suppresses the growth of *Y. enterocolitica*. Packaging high pH pork in either a vacuum or 100% CO_2 did not inhibit *Y. enterocolitica* growth. Lactate production in *post rigor* muscle is responsible for the decrease in pH observed in meat. In muscle of pH 5·55, lactate concentrations of 115 to 145 mM have been measured, whereas for muscle of pH 6·1 concentrations of lactate between 65 and 105 mM have been recorded (Grau 1981). As lactate is an effective inhibitor of *Y. enterocolitica* growth, increased numbers observed on high pH meat are likely to be due to lower lactate concentrations (Little et al. 1992). Numbers of *Y. enterocolitica* on vacuum-packaged high pH pork increased more rapidly compared to other treatments, possibly owing to a less inhibitory environment created by lower lactate concentrations and to reduced CO_2 concentrations.

Gram-negative bacteria grew most rapidly on high and normal pH pork packaged under vacuum. Packaging pork slices in a high concentration of CO_2 appeared to have an inhibitor effect on Gram-negative bacterial populations, particularly in normal pH pork. Further inhibition of Gram-negative bacteria may have been imposed by the high numbers of lactic acid bacteria present on the CO_2-packaged pork. A similar effect was noted by Gill and Penney (1988) on CO_2-packaged beef.

Table 3. Effect of packaging conditions on the stability of the *Yersinia enterocolitica* virulence plasmid following 30-days storage at 4°C

Packaging conditions	Total number of colonies selected from CIN[a] agar	No. (%) of colonies AA[b] in MR-VP[c]	No. (%) of colonies AA in RPMI-1640[d]	No. (%) of CRMOX+[e] colonies
Normal pH pork vacuum-packaged	41	10 (25)	12 (29)	12 (29)
Normal pH pork 100% CO_2-packaged	44	14 (32)	15 (34)	13 (30)
High pH pork vacuum-packaged	55	16 (29)	17 (31)	16 (29)
High pH pork 100% CO_2-packaged	60	19 (32)	21 (35)	17 (28)

[a]Cefsulodin irgasan agar.
[b]Autoagglutination.
[c]Methyl red Voges–Proskauer broth.
[d]Tissue culture fluid RPMI-1640.
[e]Congo red binding, calcium dependence positive.

Packaging normal pH pork in 100% CO_2 produces an inhibitory environment for some Gram-negative bacteria. *Y. enterocolitica* were inhibited for the duration of the storage period on normal pH CO_2-packaged pork, while total Gram-negative bacteria remained in lag phase for 15 days before growth phase was initiated. The reason for this inhibition is unclear. Several theories have been presented to account for the inhibitory effects of CO_2 on aerobic spoilage bacteria. These include bacterial intracellular pH alteration, disruption of cell membranes caused by dissolution of CO_2 and inhibition of non-decarboxylating enzymes (Lambert et al. 1991). Regardless of the mode of action of CO_2, the combination of a normal pH meat environment and a saturated CO_2 atmosphere resulted in growth inhibition of *Y. enterocolitica*.

CO_2 is used in MA packaging to retard bacterial growth by increasing the lag phase and the generation time (Daniels et al. 1985). Several investigators have found that 100% CO_2 does not exert an inhibitory effect on lactic acid bacteria (Blickstad and Molin 1983, Rousset and Renerre 1991). Our results also indicate that packaging atmosphere had no significant effect ($P>0{\cdot}05$) on the growth of lactic acid bacteria at most storage times. Initially, lactics were higher in high pH pork (Table 1) using either atmosphere (log 2·7 normal, log 3·7 high pH). Meat pH significantly affected ($P<0{\cdot}05$) growth of lactics during storage at 4°C with the greatest mean increase in lactic acid bacteria numbers occurring on normal pH pork after 25 days.

The growth of the spoilage bacterium *B. thermosphacta* on beef is controlled by a combination of meat pH and availability of oxygen (Campbell et al. 1979). On normal pH beef packaged anaerobically, *B. thermosphacta* is not able to grow (Campbell et al. 1979). Our results indicate that *B. thermosphacta* does not grow or increases only slightly on CO_2- or vacuum-packaged normal pH pork while growth on high pH pork was evident (Table 1). Population levels of *B. thermosphacta* were higher ($P<0{\cdot}05$) on high pH pork than normal pH pork although final levels attained were unlikely to increase the spoilage rate of high pH pork. Inhibition of *B. thermosphacta* by lactobacilli has been reported by Roth and Clark (1975) and this factor may have had an influence on the final levels of *B. thermosphacta* observed on high pH pork.

Virulence in *Y. enterocolitica* is encoded by virulence genes that increase the organism's pathogenic potential. Characteristic of pathogenic *Y. enterocolitica* is the presence of a 40–50-Md plasmid that carries essential virulence genes (Prpic et al. 1985). Culturing at 37°C and prolonged storage of *Y. enterocolitica* can result in loss of the virulence plasmid and hence loss of virulence (Kwaga and Iversen 1991, Bhaduri and Turner-Jones 1993). Storage of virulent *Y. enterocolitica* in anaerobic atmospheres has been shown not to affect carriage of the virulence plasmid (Bhaduri and Turner-Jones 1993). Data in Table 3 show that between 25 and 35% of virulent *Y. enterocolitica* isolates retained their virulence-associated phenotypes, indicating that the virulence plasmid can be maintained following passage through a pork packaging and storage regimen and exposure to a saturated CO_2 atmosphere.

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