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Microbial ecology of fresh pork stored under modified atmosphere at −1, 4.4 and 10°C

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The prevalent bacteria on fresh pork packaged in modified atmosphere with elevated CO_2 were determined by selection of representative colonies from the greatest dilution of meat samples. The pork samples were stored in two packaging films of different oxygen permeability at three storage temperatures. Strains were classified and those identified as lactic acid bacteria were screened for production of inhibitory substances. The types of bacteria isolated from samples stored in the two packaging films were similar. Storage temperature influenced the type of bacteria that dominated the microbial population. At 10°C the prevalent microflora consisted of aeromonads, Enterobacteriaceae and lactic acid bacteria but at 4.4 and −1°C, aeromonads, *Brochothrix thermosphacta* and lactic acid bacteria dominated. Listeriae were detected as part of the prevalent microflora on samples stored at −1°C, but not on samples stored at 4.4 or 10°C. Species of lactic acid bacteria dominating the microflora were influenced by growth medium. The majority of isolates taken from Plate Count agar were carnobacteria whereas those from Lactobacilli MRS agar were homofermentative lactic acid bacteria. Of the 538 lactic acid bacteria isolates screened for production of inhibitory substances, 162 strains showed deferred inhibition toward a range of lactic acid bacteria and nonlactic acid bacteria indicator strains.

Key words: Modified atmosphere package (MAP); Pork; Microflora; Bacteriocin; Lactic acid bacteria

Introduction

Storage life of chilled fresh pork is markedly extended when retail cuts are packaged in plastic film with low gas transmission rate, under vacuum or in modified atmosphere with elevated carbon dioxide (MAP) and lowered oxygen (Spahl et al., 1981; Gill and Harrison, 1989; McMullen and Stiles, 1991). Initial microbial load, gas permeability of the packaging film and storage temperature significantly affect the storage life of the meat (Gardner et al., 1967; Adams and Huffman, 1972; McMullen and Stiles, 1991). Prevalence of lactic acid bacteria in place of the putrefactive microflora of aerobically packaged meats is responsible for the extended storage life. However, the lactics and other bacteria such as

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Brochothrix thermosphacta and members of the Enterobacteriaceae have been implicated in the spoilage of MAP meats (Egan et al., 1989; Gill and Harrison, 1989; Lambert et al., 1991). Increased knowledge of the microbiology of MAP meats is essential for a more complete understanding of the dynamics of extended storage and safety of these products.

Studies of the lactic acid bacteria of chilled, vacuum-packaged fresh meats revealed the prevalence of homo- and heterofermentative lactic acid bacteria, mainly *Lactobacillus*-type bacteria or leuconostocs (Hitchener et al., 1982; Shaw and Harding, 1984; Schillinger and Lücke, 1987). Some strains were identified as atypical lactobacilli that have been established as a new genus, *Carnobacterium* (Collins et al., 1987). Lactic acid bacteria are the prevalent microflora of modified atmosphere packaged (MAP) meats because of their resistance to the CO_2 environment, their growth rate at chill temperatures and production of numerous inhibitory substances including organic acids, hydrogen peroxide and naturally produced proteinaceous antagonistic substances (bacteriocins). In recent years, the antagonistic activity of a variety of lactic acid bacteria has been studied with major emphasis on the production of bacteriocins by these organisms (Harris et al., 1989; Ahn and Stiles, 1990; Schillinger and Holzapfel, 1990; Hastings and Stiles, 1991). It has been suggested that bacteriocins play a role in the prevalence of lactic acid bacteria in vacuum and modified atmosphere packaged meats (Klaenhammer, 1988; Hastings and Stiles, 1991). However, few researchers have examined the incidence of bacteriocin-producing lactic acid bacteria on modified atmosphere packaged meats throughout storage, and their role in the prevalence of lactic acid bacteria on chill stored meats is unclear. In this study we determined the nature of the prevailing microflora during storage of pork cuts at different storage temperatures in two packaging films and examined the prevalent lactic acid bacteria for antimicrobial activity and bacteriocin production.

Materials and Methods

The method of meat packaging and storage has been described (McMullen and Stiles, 1991). At weekly intervals for up to 4 weeks for samples stored at 10°C, and up to 9 or 10 weeks for samples stored at -1 and 4.4°C, samples were removed from storage and prepared for microbial analysis, including total aerobic plate count on Plate Count agar (PCA; Difco Laboratories Inc., Detroit, Michigan) incubated aerobically at 25°C; and presumptive lactic acid bacteria count on nonacidified Lactobacilli MRS agar (MRS; Difco) incubated at 25°C in an atmosphere of 10% CO_2 and 90% N_2. Colonies for identification in this study were selected from samples stored in foil laminate with negligible oxygen transmission and in plastic film with oxygen transmission of 25 $ml \cdot m^{-2} \cdot 24\ h^{-1} \cdot atm^{-1}$ at 23°C and 0% relative humidity. Colonies were picked from the greatest dilution of the samples that generally contained 20–50 colonies, and were selected to represent the principal colony types growing on the plates. Where appropriate, two colonies of each type were picked.

TABLE I

Tests used to classify Gram-negative, catalase-positive bacteria to the generic or family level [a]

Genus or Family	Oxidase	Motility	Flagellation pattern	Fermentation of glucose [b]
Pseudomonas	+/−	+	polar	O
Flavobacterium	+	−	−	O
Moraxella	+	−	−	NS
Alcaligenes	+	+	peritrichous	NS
Acinetobacter	−	−	−	NS
Alteromonas	+	+	single polar	O
Aeromonas	+	+	single polar	O/F
Enterobacteriaceae	−	+/−	peritrichous	O/F

[a] Based on Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1986).
[b] O, oxidative; NS, nonsaccharolytic; O/F, oxidative and fermentative.

The selected colonies were inoculated into soft APT agar plugs (0.4% agar; Difco). Strains were subcultured in basal medium (BM) broth (Wilkinson and Jones, 1977), examined for purity, and differentiated by Gram stain and catalase and oxidase reactions. Catalase-positive strains were characterized according to the criteria in Tables I and II. Motility was tested in APT plugs made with 0.4% agar and incubated at 25°C. Flagella staining was done using Ryu stain (Heimbrook et al., 1989). Oxidative/fermentative utilization of glucose was done using OF medium (Difco) containing 1% filter-sterilized glucose. Strains were inoculated into two tubes, one of which was overlayed with approximately 2 ml of sterile mineral oil. For Gram-positive strains, additional tests included the determination of oxygen requirement by observation of growth under an APT plug, ability to grow in BM broth at 35°C, and ability to grow on streptomycin thallous acetate agar (STAA; Gardner, 1966).

The Gram-positive, catalase-negative strains were subdivided according to the scheme proposed by Schillinger and Lücke (1987) with the following changes: (1) carnobacteria were differentiated from heterofermentative lactobacilli by the formation of predominantly L(+)-lactate (Collins et al., 1987), and were further

TABLE II

Tests used to differentiate Gram-positive, catalase-positive bacterial species [a]

Bacterial genus or species	Oxidase	Motility	Flagellation pattern	Oxygen requirement	Growth at 35°C	Growth on STAA [b]
B. thermosphacta	−	−	−	facultative	−	+
Listeria spp.	−	+	peritrichous	facultative	+	−
Kurthia spp.	+	+	peritrichous	strict aerobe	+	−
Micrococcus spp.	−	−	−	facultative	+	−

[a] Based on Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1986).
[b] Streptomycin thallous acetate agar (Gardner, 1966) for differientation of *B. thermosphacta*.

TABLE III

Carbohydrate fermentation patterns used to differentiate *Carnobacterium* species [a]

Carbohydrate [b]	*C. divergens*	*C. gallinarum*	*C. mobile*	*C. piscicola*
Inulin	–	–	+	+
Mannitol	–	–	–	+
Melibiose	–	–	–	+
Melezitose	v	+	–	v
Xylose	–	+	–	–

[a] Adapted from Collins et al. (1987).
[b] –, no acid production; +, acid production; v, variable acid production.

differentiated by the carbohydrate fermentation patterns shown in Table III; (2) strains that produced gas from glucose, no ammonia from arginine, and greater than 80% of lactate as the D(–)-isomer, were classified as leuconostocs and subdivided according to the scheme in Fig. 1; and (3) homofermentative lactobacilli were differentiated on the basis of fermentation patterns for arabinose, cellobiose, gluconate, lactose, maltose, mannitol, melibiose, raffinose, rhamnose and trehalose, based the criteria of Kandler and Weiss (1986).

The methods of Wilkinson and Jones (1977) were used to test for growth in 6.5% NaCl, for growth at 10, 15, 35 and 45°C, and for production of dextran (slime) from sucrose. Gas production from glucose, carbohydrate fermentation tests and production of ammonia from arginine were done according to methods described by Shaw and Harding (1985). To determine the proportion of the lactate isomers formed, strains were grown in BM broth for 48 h, heated at 80°C for 15 min, cooled, centrifuged at 14900 × *g* for 5 min and the supernatant analyzed for D(–)- (Gawehn, 1984), and L(+)-lactate (Noll, 1984) using D- and L-lactate dehydrogenase, NAD and glutamate pyruvate transaminase (Boehringer Mannheim, Montreal, Que.). The method of Kempler and McKay (1980) was used for detection of citrate dissimilation.

All strains classified as lactic acid bacteria were screened for antibacterial activity by the deferred antagonism technique (Hastings and Stiles, 1991) against the indicator strains listed in Table IV. To broaden the range of lactic acid

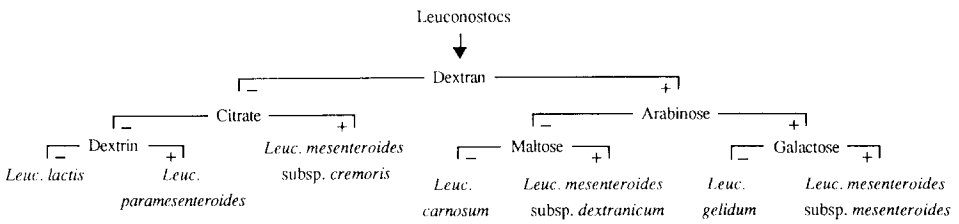

Fig. 1. Scheme used for the differentiation of *Leuconostoc* spp., adapted from Shaw and Harding (1989) and Garvie (1986). Dextran: production of dextran from sucrose; citrate: dissimilation of citrate; arabinose, dextrin, maltose, galactose: production of acid from the respective carbohydrate sources.

TABLE IV

Bacterial strains used in this study and their sources

Species	Strain and Source
Carnobacterium piscicola	LV17 (Shaw [a])
Carnobacterium divergens	LV13 (Shaw)
Aciduric *Lactobacillus* strain	LV69 (Shaw)
Lactobacillus plantarum	ATCC [b] 4008
Pediococcus parvulus	ATCC 19371
Enterococcus faecalis	ATCC 19433
Enterococcus faecium	ATCC 19434
Brochothrix thermosphacta	ATCC 11509
Brochothrix thermosphacta	UA Meat Isolate 41
Listeria monocytogenes	ATCC 15313
Listeria monocytogenes	Scott A
Listeria sp.	UA 42 [c]
Listeria innocua	ATCC 33090
Staphylococcus aureus	S6 [d]
Staphylococcus aureus	ATCC 13565
Bacillus macerans	ATCC 7048
Bacillus cereus	ATCC 14579
Clostridium bifermentans	ATCC 19299

[a] Strains obtained from Dr B.G. Shaw, Institute of Food Research, Bristol, UK.
[b] American Type Culture Collection, Rockville, MD.
[c] University of Alberta Food Microbiology Culture Collection.
[d] Obtained from Dr P. Ewan, Health Protection Branch, Ottawa.

bacteria used as indicators, each set of 12 strains was screened against the other lactic strains within the set. To test the nature of the inhibitory substance, a 24-h culture grown in APT broth was centrifuged at $6000 \times g$ for 3 min, the supernatant was neutralized to pH 6.5 with 10 N NaOH and either unheated (control) or heated at 65°C for 30 min, divided and untreated (control), or treated with pronase E (Sigma; 1 mg/ml) or catalase (Sigma; 100 units/ml). After 1 h incubation, 10 μl of each sample was spotted onto the surface of APT plates that had been overlayed with soft APT agar inoculated with 1% of an overnight culture of either *Carnobacterium piscicola* LV17 or *C. divergens* LV13. Inhibitory activity was estimated by spotting doubling dilutions of supernatant onto overlayed APT agar as described above. The reciprocal of the highest dilution showing inhibition was taken as a measure of arbitrary activity units (AU) adjusted per ml of supernatant fluid (Hastings and Stiles, 1991).

Results

Treatment variables for the pork samples for isolation of strains for this study included two packaging types, aseptic and commercial meat preparation and three storage temperatures. A total of 1220 strains was selected, of which 1049 strains

(86%) remained viable and were characterized. The method of strain selection ensured that the selected strains are those prevalent in the meat microflora, but they do not represent the proportions of these strains on the samples. The types of bacteria growing on the samples prepared either aseptically or commercially and stored in the two packaging films were similar, therefore data were pooled across method of meat preparation and package type.

Of the characterized strains, a total of 652 (62.1%) was lactic acid bacteria. Strains isolated from MRS included 20% nonlactics that were primarily identified as yeast, based on microscopic identification; whereas strains isolated from PCA included 49% nonlactics, of which 52 (19%) of 278 strains were identified as yeast.

Classification of nonlactic strains isolated from PCA is shown in Table V. From samples stored at −1 and 4.4°C, the frequency of detection of Gram-negative bacteria and micrococci decreased with time of storage, whereas the frequency of detecting aeromonads and *Brochothrix thermosphacta* increased. The majority of the *B. thermosphacta* strains were isolated from samples stored in plastic film with the higher oxygen transmission rate. At −1°C, listeriae were detected as one of the prevalent strains isolated from the samples throughout storage, indicating that they grew in proportion to the total population of the meat; however, listeriae were not detected among the strains isolated from samples stored at 4.4 or 10°C. Enterobacteriaceae were isolated as part of the prevalent microflora of samples stored at 4.4 and 10°C, but not from samples stored at −1°C.

The classification of the lactic strains isolated from PCA and MRS are summarized in Table VI. From PCA, the majority of the strains (85%) isolated were carnobacteria and homofermentative lactobacilli; whereas on MRS the majority of strains (62%) isolated were homofermentative lactobacilli, while carnobacteria were infrequent isolates.

Temperature of storage had little effect on the types of lactic acid bacteria present on the pork samples, with the exception of the homofermentative lactobacilli and pediococci. The majority of the homofermentative strains isolated on PCA and MRS from pork stored at −1°C had carbohydrate fermentation patterns that matched *L. alimentarius, L. farciminis* and *L. sake*. From samples stored at 4.4°C there was a greater variety of homofermentative lactobacilli including: *L. alimentarius, L. curvatus, L. farciminis, L. plantarum* and *L. sake*. From samples stored at 10°C the homofermentative lactobacilli included: *L. alimentarius, L. casei* subsp. *casei, L. coryneformis* subsp. *coryneformis, L. farciminis, L. plantarum* and *L. sake*. A large proportion (89 strains; 38%) of homofermentative strains could not be identified from their fermentation patterns. The heterofermentative lactic acid bacteria isolated from both PCA and MRS were *L. viridescens* and *L. minor*. All of the carnobacteria isolated were identified as *C. divergens*. The leuconostoc strains were *L. gelidum* except for a few strains of *L. mesenteroides* subsp. *mesenteroides*. A few strains of *Pediococcus* sp. were isolated at all three storage temperatures, but the majority were isolated from MRS agar from samples stored at 10°C.

A total of 538 lactic cultures was tested for inhibitory activity against the range of indicator organisms listed in Table IV, and against different sets of lactic acid

TABLE V

Classification of nonlactic acid bacteria isolated from Plate Count agar plates from pork samples stored at −1, 4.4 and 10°C

Storage time [a] (weeks)	Number of strains																	
	Gram-negative bacteria			Aeromonads			Listeriae			Micrococci			Enterobacteriaceae			*Brochothrix thermosphacta*		
	−1	4.4	10	−1	4.4	10	−1	4.4	10	−1	4.4	10	−1	4.4	10	−1	4.4	10
1	6	7	0	0	0	0	3	0	0	9	6	0	0	0	4	0	2	0
2			2			10			0			0			3			0
3	5	0	0	1	1	18	2	0	0	2	2	0	0	0	13	3	3	0
4			1			3			0			0			9			0
5/6	3	0		2	0		2	0		0	0		0	4		5	7	
7/8	0	1		3	3		0	0		1	0		0	10		8	9	
9/10	0	0		6	9		2	0		0	0		0	0		16	9	

[a] Bacterial strains were obtained from meat samples stored at −1°C at weeks 1, 3, 6, 8, and 10; at 4.4°C at weeks 1, 3, 5, 7, and 9; and at 10°C for the first 4 weeks of storage.

TABLE VI

Classification of lactic acid bacteria isolated on Plate Count and Lactobacilli MRS agar from pork samples stored at −1, 4.4 and 10°C [a]

Storage time (weeks)	Number of strains														
	Homofermentative lactobacilli			Heterofermentative lactobacilli			Carnobacteria			Leuconostocs			Pediococci		
	−1	4.4	10	−1	4.4	10	−1	4.4	10	−1	4.4	10	−1	4.4	10
(a) Plate Count agar															
1	4	5	7	0	4	5	4	0	1	0	1	1	1	0	0
2			0			0			12			0			1
3	0	7	4	2	10	0	12	16	9	0	2	0	1	1	1
4			2			0			6			1			0
5/6	3	14		0	2		14	21		1	0		0	0	
7/8	11	9		1	0		19	20		0	0		1	0	
9/10	0	8		0	1		22	12		0	6		0	0	
(b) Lactobacilli MRS agar															
1	0	0	17	0	0	4	0	0	0	1	5	4	0	1	5
2			26			3			0			3			4
3	4	19	24	2	2	2	2	0	0	10	4	3	0	0	5
4			10			2			1			1			0
5/6	11	15		1	6		1	6		0	6		0	0	
7/8	13	13		2	2		2	3		0	1		0	0	
9/10	13	13		4	3		0	1		0	6		0	3	

[a] Bacterial strains were obtained from meat samples stored at −1°C at weeks 1, 3, 6, 8, and 10; at 4.4°C at weeks 1, 3, 5, 7, and 9; and at 10°C for the first 4 weeks of storage.

TABLE VII

Number of strains within each group of lactic acid bacteria that were inhibitory to each of the indicator strains

Indicator strains	Homofermentative lactobacilli ($n = 42$)	Heterofermentative lactobacilli ($n = 15$)	Carnobacteria ($n = 65$)	Leuconostocs ($n = 35$)	Pediococci ($n = 5$)
C. piscicola LV17	8 (19) [a]	11 (73)	18 (23)	6 (17)	0
C. divergens LV13	18 (43)	10 (67)	12 (18)	14 (40)	0
Lactobacilli group II LV69	10 (24)	9 (60)	0	5 (14)	1 (20)
L. plantarum ATCC 4008	3 (7)	1 (7)	1 (2)	0	0
P. parvulus ATCC 19371	10 (24)	10 (67)	6 (9)	9 (26)	0
Other lactic acid bacteria [b]	13 (31)	11 (73)	28 (43)	9 (26)	2 (40)
E. faecalis ATCC 19433	8 (19)	12 (80)	4 (6)	6 (17)	0
E. faecium ATCC 19434	8 (19)	0	0	0	0
B. thermosphacta ATCC 11509	14 (33)	0	0	0	1 (20)
B. thermosphacta UA 41	11 (26)	1 (7)	0	0	0
L. monocytogenes ATCC 15313	16 (38)	12 (80)	2 (3)	15 (43)	0
L. monocytogenes Scott A	11 (26)	7 (47)	2 (3)	10 (26)	0
Listeria sp. UA 42	11 (26)	12 (80)	1 (2)	34 (97)	0
L. innocua ATCC 33090	14 (33)	12 (80)	2 (3)	21 (60)	1 (20)
S. aureus S6	11 (26)	1 (7)	0	0	0
S. aureus ATCC 13565	10 (24)	1 (7)	0	0	0
B. macerans ATCC 7048	9 (21)	0	0	0	0
B. cereus ATCC 14579	13 (31)	1 (7)	0	1 (3)	0
C. bifermentans ATCC 19299	15 (36)	9 (60)	32 (49)	25 (71)	0

[a] Numbers in parentheses represent percentages.

[b] Tested against all inhibitory lactic acid bacteria strains isolated from pork samples ($n = 48$).

bacteria isolated from the pork samples. Deferred antagonism tests detected 162 strains (30%) that produced inhibitory activity against indicator strains used in our study, representing as many as 64 different inhibitory patterns. Antagonistic strains were detected among the prevalent lactic acid bacteria throughout storage at all temperatures. Strains isolated from samples stored at −1°C had narrow spectra and often inhibited only one of the indicator organisms. The percentage of antagonistic strains detected among the homofermentative and heterofermentative lactobacilli, carnobacteria, leuconostocs and pediococci were 18, 27, 37, 66 and 22%, respectively. Of the 162 strains that showed antagonistic activity, only 32 (6% of the total number of lactic acid bacteria tested) were active against at least 50% of the indicator strains used for screening.

Within each group of lactic acid bacteria tested, the activity spectra were diverse. As a group, the antagonistic spectrum of the homofermentative lactobacilli included all of the indicator strains tested (Table VII), but individually their activity spectrum was limited and only 12 strains (28%) were antagonistic to greater than 50% of the indicator strains tested. The activity spectra for the heterofermentative group of lactic acid bacteria was narrower than that for the homofermentative group. None of the heterofermentative lactobacilli was antago-

nistic to *E. faecium, B. thermosphacta* ATCC 11509 or *Bac. macerans*. However, the activity spectra were not as diverse and a greater proportion of the strains were antagonistic to the same indicator organisms. The carnobacteria also had narrow activity spectra. None of the carnobacteria was antagonistic to aciduric *Lactobacillus* strain LV69, *E. faecium, B. thermosphacta* strains, *Staphylococcus aureus* and *Bac. macerans* and very few inhibited the growth of *Listeria* spp. Although the activity spectra for the leuconostoc strains were similar to those for the carnobacteria, a greater proportion of the leuconostocs was inhibitory to *Listeria*. The activity spectrum for each of the *Pediococcus* sp. was limited to one indicator organism.

Of the 162 antagonistic strains, 48 were selected for further characterization of their inhibitory activity. No antimicrobial activity was detected in the supernatant fluids of 14 (29%) of the selected strains. When deferred inhibition tests were repeated for these strains, no antagonism was detected. One strain had activity only in the unheated supernatant. The remaining 33 strains (69%) were sensitive to pronase E, insensitive to catalase and active at pH 6.5, indicating that the activity is due to the production of antagonistic proteinaceous substances (bacteriocins). The activity of the antagonistic strains measured against *C. divergens* LV13 varied between strains within each group of lactic acid bacteria and ranged from 100 to 25 600 AU/ml.

Discussion

The development of a lactic acid bacteria microflora is well established for MAP fresh meats stored below 5°C. Previous studies on the composition of the prevalent microflora of MAP meats have not detailed the specific lactic acid bacteria nor the incidence of bacteriocin-producing lactic acid bacteria throughout storage at different temperatures. When storage temperatures rise above 7 to 10°C, temperature has a significant effect on the composition of the prevalent microbial population. At higher temperatures, lactic acid bacteria do not prevail (McMullen and Stiles, 1991), and Enterobacteriaceae and aeromonads are part of the prevalent microflora of MAP fresh meats stored at temperatures greater than 7°C (Gardner et al., 1967; Lee et al., 1985; Simard et al., 1985). In our study, the prevalent microbial population of fresh pork stored at 10°C included lactic acid bacteria, Enterobacteriaceae and aeromonads in almost equal numbers.

Aeromonads were previously isolated from MAP pork by Blickstad and Molin (1983); however, they were isolated from the fat surfaces and not from the lean. In the present study, aeromonads were isolated as part of the prevailing microflora of lean pork loin cuts packaged in a MA and stored at 10°C, and with prolonged storage at −1 and 4.4°C. Storage temperature was not a factor influencing the presence of aeromonads. In contrast, Gill and Reichel (1989) reported that the growth of *A. hydrophila* inoculated onto high pH beef stored under 100% CO_2 was temperature dependent.

Listeriae have also been isolated from fresh meats (Johnson et al., 1990) but there have been no previous reports of their isolation from MAP fresh meats

stored for extended periods of time. Listeriae were detected among the prevalent microflora of samples stored at −1°C. The slow development of a lactic acid bacteria microflora on samples stored at −1°C (McMullen and Stiles, 1991) may have allowed listeriae to grow as part of the prevalent microflora. The *Listeria* strains were not speciated, thus the implication of their presence for food safety is not known.

Viable counts for *B. thermosphacta* determined on STAA indicated that this organism is a significant part of the prevalent microbial population of MAP pork cuts stored at −1 and 4.4°C in a plastic film that allowed oxygen levels to increase to 2–3% over 9–10 weeks (McMullen and Stiles, 1991). This was confirmed by the identification of isolates in this study. *B. thermosphacta* can be a major spoilage organism of MAP meats when O_2 is present in the pack (Blickstad and Molin, 1983; Gill and Harrison, 1989) because its metabolic endproducts are organoleptically unpleasant (Gibbs et al., 1979).

The relatively high proportion of yeast isolates from both PCA and MRS agars were associated with meat samples packaged in the relatively gas permeable film. The increase in O_2 to 2 to 3% during storage (McMullen and Stiles, 1991) may have allowed yeasts to compete in the prevalent microbial population. Yeasts were reported to increase in numbers during storage of minced meat in 100% CO_2 at 3°C (Nychas and Arkoudelos, 1990); however, yeasts were not considered to be a major part of the prevalent microflora based on enumeration on selective agar. Sutherland et al. (1975) recovered yeast from vacuum-packaged beef throughout a 9-week storage period at 0–2°C. Other studies on the microflora that develops on MAP fresh meats did not report the presence or growth of yeasts (Enfors et al., 1979; Blickstad and Molin, 1983).

At all three storage temperatures, the population of lactic acid bacteria obtained from PCA agar was principally carnobacteria, whereas the prevalent population based on isolates from MRS agar was homofermentative lactic acid bacteria. Very few carnobacteria were isolated. Differences in growth medium may account for conflicting reports in the literature on the principal types of lactic acid bacteria on MAP fresh meat. No reports on the isolation of lactic acid bacteria from PCA are available; however, when isolating lactic acid bacteria from meat using tryptone glucose extract agar or trypic soy agar, Enfors et al. (1979) and Vanderzant et al. (1982) reported a change from a predominantly homofermentative population to a predominantly heterofermentative population during storage. In contrast, Schillinger and Lücke (1987) reported that homofermentative lactic acid bacteria prevailed when MRS agar (pH not stated) was used as the medium for isolation. Hitchener et al. (1982) suggested that MRS agar may be selective for certain strains of lactic acid bacteria. The presence of acetate in MRS agar and the relatively low pH could account for the difference in the types of lactic acid bacteria isolated from the two media. Carnobacteria do not grow well in the presence of acetate (Schillinger and Holzapfel, 1990).

Leuconostocs made up a relatively small proportion of the prevailing microflora from the pork samples at all three storage temperatures. The majority of these strains were isolated from MRS agar, indicating the possibility of selection due to

growth medium. Almost all of the leuconostocs were identified as *Leuc. gelidum*, a species that has been isolated from meats by other researchers (Shaw and Harding, 1989; Hastings and Stiles, 1991).

Antibacterial activity was detected in 30% of the lactic acid strains isolated; however, only 6% of the total number of strains screened for activity were inhibitory to a relatively broad spectrum of indicator strains. Few reports are available on the proportion of antagonistic or bacteriocin-producing lactic acid bacteria isolated from foods. Sobrino et al. (1991) found that 16% of the lactic organisms isolated from dry fermented sausages exhibited activity against a single strain of *L. farciminis*, whereas Harding and Shaw (1990) found that only 4% of the lactic acid bacteria that they isolated from a variety of foods produced bacteriocins.

The incidence of bacteriocin-producing lactic acid bacteria in foods seems to be low. However, the relatively infrequent isolation of bacteriocin-producing lactic acid bacteria may be due to the use of insensitive indicator organisms or possible differences in microbial metabolism between in vitro and in vivo environments. Tagg et al. (1976) suggested that all bacteria produce bacteriocin. It is possible that we are not detecting all bacteriocin-producing lactic acid bacteria during screening. No further characterization of the nature of the inhibitory activity was done on the 14 strains that produced deferred antagonism in initial screening tests, but antagonistic activity could not be demonstrated in the culture supernatant or when deferred tests were repeated. The unexpected loss of antagonism has been noted in other strains of lactic acid bacteria tested in our laboratory. The apparent loss of antimicrobial activity may also contribute to the low frequency of recovery of bacteriocin-producing lactic acid bacteria from foods.

The antimicrobial spectrum of the majority of the bacteriocin-producing strains was limited to other lactic acid bacteria, *Enterococcus faecium* and *Listeria* species. The large number of bacteriocin-producing lactic acid bacteria active against listeriae is not surprising because they are closely related to lactics (Wilkinson and Jones, 1977). The relatively high sensitivity of listeriae to lactic acid bacteria has been reported by several workers (Harris et al., 1989; Lewus et al., 1991; Schillinger and Lücke, 1991). The sensitivity of listeriae to the antibacterial activity of lactic acid bacteria should preclude their domination of the microflora of MAP meats; however, in this study, listeriae were isolated as part of the prevalent microflora of samples stored at -1°C. This could possibly be due to a lack of growth of bacteriocin-producing lactic acid bacteria at such a low temperature. The production of inhibitory substances in meat systems stored at low temperatures should be examined because the majority of the isolates with a broad spectrum of activity were isolated from samples stored at 4.4 or 10°C.

Knowledge of the composition of the microflora present on modified atmosphere packaged fresh meats is important to understand spoilage and safety of the products. It may be possible to extend further the storage life and enhance the safety of MAP meats by controlling the fermentation that occurs by inoculation of the meats with bacteriocin-producing lactic acid bacteria to regulate the growth of spoilage organisms and potential pathogens.

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