# SHELF LIFE, MICROBIAL ECOLOGY, AND SAFETY OF A COOKED MODIFIED ATMOSPHERE PACKAGED REFRIGERATED POULTRY PRODUCT

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

REEM K. BARAKAT

In partial fulfilment of requirements

for the degree of

Doctor of Philosophy

April, 1998

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

*Your file Votre référence*

*Our file Notre référence*



0-612-33295-0

Canada

# ABSTRACT

## SHELF LIFE, SAFETY, AND MICROBIAL ECOLOGY OF A COOKED MODIFIED ATMOSPHERE PACKAGED REFRIGERATED POULTRY PRODUCT



Reem Barakat
University of Guelph, 1998

Advisors:
Dr. Linda. J. Harris
Dr. Mansel W. Griffiths



The microbiota of commercially produced oven-roasted poultry cuts, packaged under 40:60 $CO_2$:$N_2$, and stored at 3.5 or 10°C ± 0.5°C, was followed for a period of seven or five weeks, respectively. The product stored at 3.5°C was acceptable to an untrained sensory panel throughout the seven-week storage period and showed no visible signs of deterioration. However, the product stored at 10°C spoiled within four to five weeks of storage and developed an offensive odour.

Bacterial isolates from the highest dilution of aerobes, anaerobes and psychrotrophs were identified by chemotaxonomic methods and were classified as *Lactococcus raffinolactis* (117 isolates), *Carnobacterium divergens* (61 isolates), *C. piscicola* (11 isolates), *L. garvieae* (four isolates), *L. lactis* (one isolate) and *Enterococcus faecalis* (three isolates). All isolates were screened for production of bacteriocins, and only *C. piscicola* strains produced an inhibitory substance active against other lactic acid bacteria and against several *Listeria* spp.

Species-specific polymerase chain reaction (PCR) primers were used for the differentiation of *Carnobacterium*, *L. raffinolactis*, *L. lactis*, and *L. garvieae* spp. One

universal forward primer and seven species-specific reverse primers were used to identify species of carnobacteria and lactococci associated with these modified atmosphere packaged poultry products. No false PCR products were observed with other closely related bacterial species.

The modified atmosphere packaged cooked poultry was evaluated for its ability to support the growth of psychrotrophic pathogens at 3.5, 6.5, and 10°C, when formulated with sodium lactate and shelf life extender ALTA™ 2341, and in the presence or absence of a competitive lactic acid microbiota. Following cooking and cooling, cuts were inoculated (1000 CFU/150-g piece) with either *Listeria monocytogenes* or *Yersinia enterocolitica* and were packaged in 56:44 $CO_2$:$N_2$. Both pathogens grew under all test conditions. Temperature had the greatest effect on pathogen growth. Addition of lactate and ALTA™ 2341 extended the lag phase of the pathogens but did not prevent growth. The presence of background microbiota did not influence the growth of either pathogen. Growth of lactic acid bacteria and/or addition of lactate and ALTA™ 2341 cannot replace prevention of contamination and strict temperature control for the safety of ready-to-eat MAP poultry products.

## ACKNOWLEDGEMENTS

This research was carried out with the advising of Dr. L. J. Harris and Dr. M. W. Griffiths, with thanks. I would also like to thank Dr. D. L. Collins-Thompson who offered me the opportunity to start graduate work, and the members of my advisory committee Dr. S. Barbut and Dr. A. Lammerding.

I am grateful to Dr. H. Lee for the use of his computer facilities and his lab equipment, to Ms. Marina Steele for the fatty acid analyses, and to all those from whom I borrowed space, equipment, and reagents. I would also like to thank Jim Gordon and other employees of J. M. Schneider, Inc. (Kitchener, ON) who have assisted in this project.

Thanks to fellow graduate students, technicians, and post-docs in the Departments of Environmental Biology and Food Science for their input, support, and friendship. Finally, special thanks go to my mother and my family for their love and support throughout this endeavour.

This work was partially supported by an Ontario Graduate Scholarship and by a research grant from J. M. Schneider, Inc.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDIX FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| **bp** | basepair |
| **CFU** | colony forming units |
| **DNA** | deoxyribonucleic acid |
| **dNTP** | deoxynucleoside triphosphate |
| ***L.*** | *Lactococcus* |
| **LAB** | lactic acid bacteria |
| ***Lb.*** | *Lactobacillus* |
| ***Lc.*** | *Leuconostoc* |
| ***Li.*** | *Listeria* |
| **MAP** | Modified atmosphere packaging |
| **MVTR** | Moisture vapour transmission rate |
| **RH** | Relative humidity |
| **OTR** | Oxygen transmission rate |
| **PCR** | polymerase chain reaction |
| **rRNA** | ribosomal ribonucleic acid |
| **RTE** | ready-to-eat |
| **ssp.** | subspecies |
| **spp.** | species |
| ***Taq*** | *Thermus aquaticus* |
| **VP** | vacuum packaging |

# CHAPTER 1 LITERATURE REVIEW

## 1.1 MODIFIED ATMOSPHERE PACKAGING

### 1.1.1 Introduction

An individual wandering along the refrigerated aisles of a supermarket in the UK or in France cannot miss the large diversity of modified atmosphere packaged (MAP) extended shelf life products on display, which include raw, cooked or cured meats, vegetables, pasta, salads, and ready-to-eat entrées. Food items which rely on modified atmospheres and refrigerated storage for shelf life extension and maximal quality retention, are a growing section of the "convenience" food category. The development of these products came about as an answer to increasing consumer demand for high quality refrigerated "fresh" not frozen, minimally preserved, extended shelf life foods requiring little or no preparation prior to consumption (Farber, 1991).

MAP is not a new technology. In the 1930's it was used for the bulk transport of red meats from New Zealand and Australia to Britain (Brody, 1989). For the next 40 years the use of MAP was restricted to bulk storage and transport of raw red meats, poultry, and fish. The practice of chilled MAP foods for consumer size items was launched in 1979 by Marks and Spencer's in the UK, and has gained popularity in other European countries, mainly France, the Netherlands, Germany, Belgium, and Italy, with the UK and France having the largest market (Brody, 1993). The selection of MAP food products includes processed meats, fresh meats, sous-vide products, pasta, bakery goods, fresh vegetables, and salads.

In North America, the market for MAP, chilled products in consumer size packages has been slower to develop. The shelf life of MAP products (3 to 4 weeks on average) is insufficient for large distribution distances and the infrequent shopping habits of North American consumers. In recent years, however, market share of MAP foods has been steadily increasing, with ready-to-eat meats, salads and entrees occupying a prominent position. MAP fresh cut produce has grown by 70 to 90% annually, with a retail value of US $700 million in 1995 (Brody, 1996). Pastas and sauces follow at over $200 million, ground turkey, prepared poultry, and sandwiches at $100 million each. Lunch kits alone have a $300 million retail value. The estimated annual growth rate for all the classes of chilled foods is approximately 9%, not including the master packaging of raw meats (Brody, 1996).

In Canada, the market for MAP prepared foods is the most rapidly growing food category. Fresh pasta and sandwiches are the most common applications, and shelf life is commonly extended from 5 to 6 days to 3 to 4 weeks for these products (Agriculture Canada, 1990a). Precooked MAP meats include roast beef, retail packaged poultry, pork ribs, chicken wings, sliced deli meats, and a variety of meat-based entrees and dinners. Several of these applications are still restricted to delicatessen services and the hotel-restaurant-institution (HRI) sector, however, interest in consumer-sized packages is increasing (Brody, 1993). Several types of MAP sandwiches are presently available on the market (Agriculture Canada, 1990a). Other recent applications in Canada or in the US include crumpets, sausage and biscuits, precooked hamburgers, filled croissants, parbaked French baguettes, hot-filled pasta sauces, fresh pizza and pizza kits, salads, Chinese foods (chow mein and egg rolls), Mexican tortillas, parfried potatoes, and some

entrees (Brody, 1993). Recently, retail stores in southwestern Ontario have started displaying a variety of MAP refrigerated fresh meats, including poultry, veal, pork, and beef. The main advantages of MAP, which include higher quality products, the extension of shelf life and a concomitant reduction in economic losses, have to be balanced against strict production control, higher packaging costs, higher transport costs, larger retail display space (due to package size), and close temperature monitoring during distribution and retail.

While the technical aspects of MAP are well documented, work on the microbiological safety aspects of ready-to-eat MAP products has not been as definitive. Concern over the safety of chilled products requiring little or no heat treatment prior to consumption was the major deterrent to the use of the technology in North America (Farber, 1991). Recent awareness of the potential health hazards associated with such items have prompted several studies on the subject, and the main objectives of this review is to present an overview of MAP technology, and its effects on the survival of pathogenic microorganisms in ready-to-eat (RTE) foods, as well as to review the control mechanisms that would help ensure a safe product, mostly focusing on the use of antagonistic organisms, or their products, as biological control agents.

### 1.1.2 What is modified atmosphere packaging?

Modified atmosphere packaging (MAP) is the packaging of food products in an environment other than air, for the purpose of extending storage life. MAP will inhibit

the aerobic, mainly gram-negative spoilage organisms and favour the establishment of a microbiota dominated by gram-positive organisms.

### 1.1.3 Types of MAP

MAP encompasses several types of packaging, namely vacuum packaging (VP), gas-flush packaging, controlled atmosphere packaging (CAP) or controlled atmosphere storage (CAS), and sous-vide.

#### 1.1.3.1 Vacuum packaging (VP)

In vacuum-packed products, the air is evacuated and the package sealed. Vacuum packaging is actually a form of gas packaging as the product is not inert and metabolism of residual oxygen present in the package by the resident microbiota produces $CO_2$ and creates a modified atmosphere environment within the package. Gardner *et al.* (1967) packaged raw pork in gas impermeable film, without changing the atmosphere of the pack. Packages were stored at 2 or 16°C. Carbon dioxide concentrations rose to 3-5% after 5 hours at both storage temperatures. By the 5th day, $CO_2$ concentrations had risen to 15 and 24% in packages stored at 2 and 16°C, respectively.

#### 1.1.3.2 Gas-flush (MAP)

In gas-flush, a gas or mixture of gases is back-flushed into the package prior to sealing, either directly, or following evacuation of the product environment. The term MAP is often used synonymously with gas-flush.

#### 1.1.3.3 Controlled atmosphere packaging/Controlled atmosphere storage

Controlled atmosphere packaging is a misnomer because it is impossible to control the composition of an atmosphere inside a gas-impermeable package once it is sealed. Controlled atmosphere-stored products are exposed to a constant gas mixture and this storage method is often used for the transport and storage of fruits and vegetables.

#### 1.1.3.4 Sous-vide

A relatively recent form of VP, sous-vide, was initiated in France during the late 1970's and early 1980's (Light *et al.*, 1988). Raw or partially cooked food products are vacuum-packed and subjected to a heat treatment (cooking or pasteurization) prior to chilled storage. This system is popular in France and was originally used for hotel-restaurant-institution distributions, although the last decade has brought sous-vide products to the retail level (Brody, 1993). Products have a shelf life of 21 days at 0-3°C. Sous-vide is a preservation method dependent on packaging and cooking of the food. Although a distinct preservation method, it is often included in discussions of modified atmosphere packaging since the shelf life of the food product is partially achieved by the modified atmosphere.

### 1.1.4 Gases used in MAP

Gases used in MAP are nitrogen, oxygen, and carbon dioxide. The relative proportion of each gas in a MAP product is a function of the product and is dependent on several factors. The focus of this literature review is meat products and the discussion will be restricted to the effects of the different gases on raw and cooked meats.

#### 1.1.4.1 Nitrogen

Nitrogen is an inert gas and has little or no antimicrobial activity (Seideman, 1984a). It is usually included in the package to displace oxygen, and as a filler preventing package collapse as oxygen and carbon dioxide are used up. It reduces the possibility of package puncture and allows easier separation of sliced meats (Church, 1993).

#### 1.1.4.2 Oxygen

Oxygen plays a major role in red meat products in maintaining the desirable cherry red colour of raw meats, perceived as an indicator of quality and freshness, and to control the growth of some pathogenic organisms. It is, however, undesirable in partially cooked, cured, or ready-to-eat meat products, as it is a major cause of sensory defects. The amount of oxygen to be used with red meat has to be carefully evaluated; too much oxygen would encourage growth of aerobic spoilage bacteria.

### 1.1.4.3 Carbon dioxide

$CO_2$ is the most important gas used in MAP as it inhibits the development of aerobic, gram-negative spoilage microbiota, thus favouring the establishment of a gram-positive, facultative anaerobic population dominated by lactic acid bacteria (Enfors *et al.*, 1979). Growth inhibition of *Pseudomonas aeruginosa* by $CO_2$ was reported by King and Nagel (1967). Studies on the effect of $CO_2$ on the respiration and aerobic growth of meat organisms such as *Pseudomonas* spp., *Acinetobacter* spp., *Alteromonas putrefaciens*, *Yersinia enterocolitica*, *Enterobacter* spp., and *Microbacterium thermosphactum* (*Brochothrix thermosphacta*) indicated that *Enterobacter* spp. and *M. thermosphactum* were not affected by $CO_2$ while aerobic growth and respiration of other genera were inhibited (Gill and Tan, 1980). In the absence of $O_2$, growth of facultative anaerobes *M. thermosphactum*, *A. putrefaciens*, *Y. enterocolitica*, and *Enterobacter* spp. was not affected by $CO_2$.

The bacteriostatic activity of $CO_2$ is dependent on several factors, the most important being the maintenance of proper refrigeration temperatures. Because $CO_2$ is a gas and acts by dissolving in the tissues during refrigerated storage, increases in temperature will void any beneficial effects of the gas. Growth rate of microorganisms increases, and the dominant spoilage microbiota will vary depending on the storage temperature. Increased growth inhibition of *P. fragi* and *Bacillus cereus* by $CO_2$ as incubation temperature decreased reflects increased solubility of the gas at lower temperatures (Enfors and Molin, 1981a).

Other factors which influence the inhibitory effect of $CO_2$ include the initial microbial load, the growth phase of the resident microbiota, $CO_2$ concentration, type of organisms present on the food, and volume of head space gas in the package (Farber, 1991).

There is no agreement among researchers as to the optimal $CO_2$ concentration to be applied. $CO_2$ concentrations have to be tailored for different products, but there is evidence that bacterial inhibition increases with increasing $CO_2$ concentration (Brody, 1989).

### 1.1.5 Mechanisms of $CO_2$ inhibition

The effects of carbon dioxide on microbial growth have been reviewed by Enfors and Molin (1980), Daniels *et al.* (1985) and Dixon *et al.* (1989). Several hypotheses have been suggested in order to explain the mechanism of $CO_2$ inhibition, however, none is conclusive. The specific inhibitory mechanism is not known but appears to be bacteriostatic in nature and acts by increasing both the lag phase and the generation time of microorganisms (Daniels *et al.*, 1985). Carbon dioxide is solubilized in the liquid phase of the food as soluble gas and carbonic acid. The acid further dissociates into bicarbonate and hydrogen ions in high pH foods causing a minimal drop in pH (< 0.1 pH unit) which does not account by itself for the bacteriostatic activity observed. Possible inhibition mechanisms include interferences with various metabolic and biochemical pathways, inhibition of cell division, alteration in cell membrane fluidity thus affecting nutrient uptake, intracellular pH changes, or inhibition of enzyme activity.

### 1.1.6 Gas mixtures used for MAP of meat products

Proportions of the different gases to be used for MAP will depend on the particular food and have to be tailored for different meat items. Shelf life evaluations should be conducted to determine the optimal gas composition for each individual product. Church (1993) and Farber (1991) compiled lists of gas compositions commonly used for various meat or meat-containing products. Often, different optimal gas compositions are given for the same type of product. Ready-to-eat products, including meat, cheese and egg sandwiches, pizza sub, cheeseburgers, cretons, and beef jerky are packaged under a mixture of $CO_2$ and $N_2$, with one exception where a pizza sub was packaged under 50% $CO_2$, with the balance air (Farber, 1991). Expected shelf life varies from 17 days to 7 weeks depending on the product. Gas mixtures used for fresh meats (beef, poultry, pork and fish) may or may not include oxygen in the package (Farber, 1991). Gas compositions reported by Church (1993) for various cured, processed, or cooked meat product seldom include oxygen.

### 1.1.7 Factors affecting shelf life of MAP products

The successful application of MAP to extend the shelf life of refrigerated meat and meat products depends on several factors: (a) fresh, high quality raw material with low numbers of background microbiota; (b) sanitary conditions during processing and packaging, particularly important for cooked, RTE items; (c) composition of the gas used; (d) barrier properties of the packaging material; (e) the amount of headspace gas in the package and (f) strict temperature control during production, packaging, storage,

distribution, retail display, and consumer handling. It is likely that the last link in the chain, the consumer, is where the highest opportunity for temperature abuse occurs, either during shopping or during storage in home refrigerators.

### 1.1.8 Shelf life and spoilage microbiota of VP and MAP raw or cured meats

Changing the surrounding atmosphere of meat and meat products by VP or MAP will achieve a tremendous extension of a product's shelf life, as long as proper refrigerated storage is maintained from production to the consumer's refrigerator. Effects of VP and MAP on shelf life extension have been the subject of several reviews on fresh and processed meats and meat products (Seideman and Durland, 1984a, 1984b; Gill and Harrison, 1989; Lambert *et al.*, 1991; Stiles, 1991; Church, 1993; Hood and Mead, 1993; Borch *et al.* 1996) and fresh fish and seafood products (Stammen *et al.*, 1990; Skura, 1991; Reddy *et al.*, 1992; Davis, 1993).

The inhibitory action of carbon dioxide on the growth of psychrotrophic aerobic, mostly Gram-negative, spoilage microbiota on MAP products have been extensively documented for fresh red meats (Clark and Lentz, 1969; 1972; Partman *et al.*, 1975; Silliker *et al.*, 1977; Sutherland *et al.*, 1977; Seideman *et al.*, 1979; Christopher *et al.*, 1979; Blickstad and Molin, 1983a; Gill and Harrison, 1989), cured meats (Blickstad & Molin, 1983a; 1983b), poultry (Gardner *et al.*, 1977; Sander and Soo, 1978; Mead 1983; Baker *et al.*, 1985), and fish (Stier *et al.*, 1981; Fey and Regenstein, 1982; Mokhele *et al.*, 1983; Wang & Brown, 1983; Wang and Ogrydziak, 1986).

By the end of the storage life of fresh lamb chops held at -1°C, *Brochothrix thermosphacta* was present in all packaging atmospheres (air, $N_2$, $H_2$, and various mixtures of air, $CO_2$, $H_2$, and $N_2$) and was dominant in the $O_2$ and $CO_2$ environment. *Enterobacteriaceae* were isolated from low $O_2$ and $O_2$-free atmospheres, while lactobacilli were present in $O_2$-free environments only (Newton *et al.*, 1977). Lactic acid bacteria were dominant on refrigerated, vacuum-packed luncheon meats at 5°C (Kempton and Bobier, 1970). *Lactobacillus plantarum* and heterofermentative LAB were isolated from $CO_2$-packed fresh pork at 4°C, while pseudomonads became the dominant microbiota on samples stored in air or in nitrogen (Enfors *et al.*, 1979). Erichsen and Molin (1981) found lactic acid bacteria to be dominant on normal pH (5.8) and high pH (6.6) beef stored at 4°C in 100% $CO_2$. In the presence of $O_2$, pseudomonads, *B. thermosphacta*, and lactic acid bacteria developed on the high pH beef, while *Enterobacteriaceae* and coryneforms were found on the normal pH beef with the lactic acid bacteria (Erichsen and Molin, 1981). *Leuconostoc* spp. were predominant on steaks prepared from vacuum-packed beef strip loins and stored in a mixture of $O_2$, $CO_2$ and $N_2$ (Savell *et al.*, 1981). The microbiota of smoked pork loin and frankfurter sausage stored at 4°C under vacuum, nitrogen, or $CO_2$ consisted mainly of *Lactobacillus* spp. and some unidentified organisms (Blickstad and Molin, 1983b). Lactobacilli were also dominant on $CO_2$-packed fresh pork stored at 0°C or at 4°C. Shelf life was extended to approximately 2 or 3 months, respectively (Blickstad and Molin, 1983a). By the end of 4 weeks at 2°C, lactobacilli were dominant in ground chicken under atmospheres containing 20 to 100% $CO_2$ (Baker *et al.*, 1985). The microbiota on VP porcine and bovine meat stored at 0, 2, or 5-6°C was dominated by lactic acid bacteria at all storage

temperatures, mainly homofermentative and heterofermentative lactobacilli, leuconostocs, and lactococci. Numbers of *B. thermosphacta* and *Enterobacteriaceae* were lower than the population of LAB by 2 to 4 log units at 0 or 2°C, however, counts of these organisms increased at the higher storage temperatures (Schillinger and Lücke, 1987b). Nissen *et al.* (1996) found that packaging beef under vacuum or various concentrations of $CO_2$ and $N_2$, and subsequent storage at -1 or 2°C inhibited growth of pseudomonads and *B. thermosphacta*. Leuconostocs were dominant in vacuum and $CO_2$-packed beef at both storage temperatures, while carnobacteria were dominant in $N_2$-packed beef kept at -1°C. In poultry meat, VP or MAP in 100% $CO_2$, 100% $N_2$ or 20:80 $CO_2$:$O_2$ and stored at 3 or 10°C, lactic acid bacteria and *B. thermosphacta* were the dominant microbiota in $O_2$-free atmospheres.

### 1.1.9 Shelf life and microbiota of cooked, ready-to-eat MAP meat products

Ready-to-eat (RTE) minimally preserved MAP foods have not been available on the market as long as raw and cured meats. Information about shelf life and spoilage microbiota is not as extensive as it is for raw and cured meat products.

Under aerobic storage, refrigerated sandwiches have an approximate storage life of 5 days before sensory deterioration occurs (Kraft, 1986). McMullen and Stiles (1989) reported that MAP sandwiches (50% $CO_2$, 50% air) stored at 4°C had a shelf life of 35 days for processed meats, 28 to 35 days for roast beef, and 14 days for hamburger. Exclusion of oxygen from the package extended refrigerated shelf life of hamburger sandwiches to 35 days. Sensory characteristics of MAP sandwiches were acceptable after achievement of

maximal microbial load (approximately $10^8$ CFU/g). Shelf life of most sandwiches packaged in 30, 50, or 70% $CO_2$ with a balance of air or nitrogen, was influenced more by initial product quality than by variations in the concentration of $CO_2$.

When precooked beef roasts were subjected to one of three packaging treatments, VP, 100% $CO_2$, or 15:30:55 $CO_2$:$O_2$:$N_2$, the 100% $CO_2$ atmosphere was the most inhibitory to the growth of mesophiles and psychrotrophs; however, VP roasts exhibited the least sensory deterioration after 21 days of storage at 4°C (McDaniel *et al.*, 1984). Sliced roast beef packaged in an atmosphere of 75:15:10 $CO_2$:$O_2$:$N_2$ had a microbial shelf life of 42 days at 4.4°C. However, sensory spoilage was detected within the first week of storage (Hintlian and Hotchkiss, 1987b). The early development of warmed-over flavour was possibly due to the inclusion of oxygen in the package. Carr and Marchello (1986) investigated the effectiveness of VP or MAP (15:45:40 $CO_2$:$O_2$:$N_2$) for cooked sliced beef at 2, 6 or 10°C. Off-colours and flavours were detected earlier in MAP beef slices. Psychrotrophs were not inhibited by MAP at 6 or at 10°C. When cooked slices were packaged under 15:45:40, 15:65:20, or 15:75:10 $CO_2$:$N_2$:$O_2$, or VP, and stored at 4.5°C, aerobic psychrotrophic microbiota were least inhibited in the presence of 20% $O_2$ and in MAP packages. Off odours were detected earlier in MAP packages (Carr and Marchello, 1987). Commercially produced sliced roast beef in oxygen-free saturated $CO_2$ atmosphere packs, stored at +10, +3, and -1.5°C, was acceptable after 4 days, 10 weeks, and 16 weeks, respectively (Penney *et al.*, 1993). These results suggest that inclusion of oxygen in MAP cooked meat product may negatively affect sensory and microbiological shelf life of the product.

Young *et al.* (1987) reported that storage of two MAP precooked chicken items, fried chicken drumsticks and chicken a la king, at 0 and 4°C inhibited bacterial growth to levels below $10^5$ CFU/g for up to 17 days. *Pseudomonas* spp. were dominant in the air controls, while lactobacilli constituted the microbiota of samples stored in 70:30 $CO_2$:$N_2$. The dominant microbiota of VP samples was not identified. In a subsequent study, the sensory attributes of these two products were evaluated (Young *et al.*, 1989). Spoilage was delayed by 1 to 2 weeks in the MAP products compared to the air controls which spoiled after 4 days at 0-3°C. No differences were observed between VP or MAP. Warmed-over flavour was more pronounced in the chicken drumsticks than in the chicken a la king. Spices, herbs, and vegetables present in the latter may have masked a rancid flavour. Vacuum-packed turkey breast rolls heated to an internal temperature of 71°C were stable for 87 days at 4°C (Smith and Alvarez, 1988). Aerobic psychrotrophs were not detected during storage and anaerobic mesophiles remained at levels below 200 MPN/g. No sensory tests were done on the product (Smith and Alvarez, 1988).

A sous-vide spaghetti and meat sauce product was stored up to 35 days at 5 or 15°C following processing at 65 or 75°C. At proper refrigeration temperatures (5°C), microbiological and sensory properties were acceptable for a period of 35 days. Storage at 15°C decreased the shelf life to 14 days in products heated at 65°C and to 21 days for the 75°C heat treatment (Simpson *et al.*, 1994). Although the initial microbiota consisted of *Bacillus* spp., lactic acid bacteria were dominant by the end of the shelf life at both storage temperatures.

These studies demonstrate RTE, refrigerated, MAP meat products offer an attractive alternative to frozen entrees. The removal of even traces of oxygen from the environment is desirable from a sensory standpoint since cooked meat products are prone to oxidative rancidity.

The effectiveness of MAP and VP in extending the shelf life of raw and cooked food items, while maintaining freshness and quality has been well established in the scientific literature. Lactic acid bacteria and *B. thermosphacta* are most often isolated from MAP and VP meats. LAB have been reported to reach maximum population numbers ($10^8$ CFU/g) before sensory spoilage is detected ( Egan *et al.*, 1980; 1989; McMullen and Stiles, 1989), while spoilage is evident by the time numbers of *B. thermosphacta* reach this level (Egan *et al.*, 1980).

## 1.2 SAFETY CONCERNS OF RTE MAP PRODUCTS

### 1.2.1 Introduction

Two main types of MAP products are available on the market, those requiring complete cooking prior to consumption, and low-acid RTE products which do not need a heat treatment. The safety of the latter group is of particular concern and has elicited several reviews on the potential heath hazards associated with such items (Conner, 1989; Brackett, 1992; Schellekens, 1996; Peck, 1997). While extensive technical information is available regarding packaging machinery and high barrier polymers, and comprehensive material exists pertaining to MAP raw meats and fish, little data are available concerning the safety aspect of precooked, minimally preserved refrigerated MAP foods although

they may prove to be very hazardous (Farber, 1991). Raw meat products will be subjected to a heat treatment prior to consumption, however, RTE meat products do not necessarily require a heat treatment. The aerobic spoilage microbiota that normally grows and warns consumers of spoilage are inhibited by the combined effects of carbon dioxide and refrigeration. The microbiota is dominated by lactic acid bacteria which may grow to high numbers without any deleterious effects, thus potentially allowing the undetected growth of pathogens in the product.

In the light of the emerging "new" era of foodborne psychrotrophic pathogens such as non-proteolytic *Clostridium* spp., *Listeria monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, and *Bacillus* spp., there are potential health hazards within the ready-to-eat MAP chilled food line (Farber, 1991; Schofield, 1992). The extended shelf life may allow psychrotrophic, facultative anaerobic pathogens to grow and produce toxin (in the case of *C. botulinum* and *B. cereus* spp.) particularly if the aerobic spoilage flora is inhibited by the carbon dioxide, thus providing no warning to the consumer about the potential hazard associated with consumption of the product. Modified atmospheres will extend shelf life of a product but do not represent a safety barrier. A major concern is the temperature abuse that may occur in the distribution and retail chain. These may include temperature abuse by the consumer during shopping, temperature fluctuations that occur in home refrigerators in the course of a day, and the often improper chill display of packages in retail stores, for example in open overstacked chill cabinets. Such temperature abuse could drastically increase the generation rates of psychrotrophic organisms, and allow mesophilic pathogens to grow, in addition to obliterating any inhibitory effects of $CO_2$.

The acceptable temperature range for all MAP applications is between 0 and 4°C, excluding baked goods (Agriculture Canada, 1990a). There is, however, a high incidence of inadequate temperature control in the food service sector. A survey of refrigerated products from 20 convenience stores showed that 50% of the products were stored at temperatures ranging between 3.9 and 9°C, and 15% of the products were stored at temperatures > 9°C. Tolstoy (1991) and Van Garde and Woodburn (1987) reported that refrigerator food product temperatures often exceed 10°C. The temperatures of seven out of ten retail meat-holding cases surveyed by Wyatt and Guy (1980) were > 7.2°C. Davidson (1987) reported that temperatures of 7 to 10°C in retail display cases were not uncommon. A national audit report in the US showed that average product temperatures were over 10°C in approximately 16% of retail dairy coolers (Harris, 1989).

### 1.2.2 Psychrotrophic foodborne pathogens

Several studies have shown the potential for psychrotrophic and mesophilic foodborne pathogens to grow or survive on MAP raw (Table 1.1) or cooked meat products (Table 1.2).

#### 1.2.2.1 *Listeria monocytogenes*

*Overview*

*L. monocytogenes* grows actively at chill temperatures (Lovett, 1989), is ubiquitous in nature, and has been isolated from the food processing plant environment (Cox, 1989;

Cox et al., 1989; Jemmi and Keusch, 1994) and from hands of food workers (Kerr *et al.*, 1993). The case fatality rate is near 30% among susceptible individuals which include the immunosupressed, pregnant women, elderly people, and newborn infants (Farber and Peterkin, 1991). Food products linked to outbreaks of human listeriosis include coleslaw in Nova Scotia (Schlech *et al.*, 1983), pasteurized milk in Boston (Fleming *et al.*, 1985), Mexican-style cheese in Los Angeles (Linnan *et al.*, 1988), Vacherin cheese in Switzerland (Bula *et al.*, 1995), jellied pork tongue in France (Jacquet *et al.*, 1995), and chocolate milk in the US (Shank *et al.*, 1996). Undercooked hotdogs and chicken have been epidemiologically linked to sporadic occurrences of listeriosis in the US (Schwartz *et al.*, 1988; Gellin *et al.*, 1991). Jay (1996a) summarizes the prevalence of the pathogen in meat and poultry products and lists several confirmed and suspected cases of listeriosis linked to consumption of different meat products.

Gilbert *et al.* (1989) reported the detection of *Li. monocytogenes* in 12% of RTE poultry. The organism was present in 26.5% (of 102 samples) of pre-cooked chilled chicken products in the UK (Kerr *et al.*, 1990). It was isolated from 2.8% of a variety of RTE meat products from 4,105 plants in the US. (Green, 1990). Farber *et al.* (1990) surveyed an array of MAP chilled sandwiches and meats in Canada, and reported the occurrence of *Li. monocytogenes* in 8.5% of samples examined. The organism was present in turkey, submarine, and ham and cheese sandwiches. Other *Listeria* spp. were present in 5.3% of the products. A survey of 175 samples of vacuum-packed processed meats from retail stores revealed the presence of *Li. monocytogenes* in 52.9% of the products (Grau and Vanderlinde, 1992). The organism occurs naturally in lettuce, cabbage, and other vegetables (Weiss and Seeliger, 1975), and may persist as a contaminant of MA prepared

salads. It was present in 4 out of 60 prepared salads (Sizmur and Walker, 1988). Several authors have recently reviewed the occurrence of *Li. monocytogenes* in dairy products (Kozak *et al.*, 1996), vegetables (Beuchat, 1996), and in meat and poultry products (Johnson, 1990; Jay, 1996a).

***Regulatory policies or guidelines for Li. monocytogenes***

**USA and Canada**

Presently, a zero tolerance (absence of the organism from 25-g samples) for *Li. monocytogenes* in cooked RTE foods has been established by the U.S. Department of Agriculture Food Safety and Inspection Service (Shank *et al.*, 1996). In Canada, the regulatory policy on *Listeria* was formulated based on a health risk assessment approach (Farber *et al.*, 1996). RTE foods with extended refrigerated shelf life (> 10 days) are divided into two categories, foods causally linked to listeriosis (e.g. soft cheese, liver pâté, coleslaw mix) and foods capable of supporting growth of *Li. monocytogenes* (vacuum and modified atmosphere packaged RTE products). The presence of more than 0 CFU/50 g in the first category and more than 0 CFU/25 g in the second category would trigger a Class I recall or Class II recall, respectively, with potential public alert and follow-up at the plant level (Farber and Harwig, 1996). Guidelines and sampling schemes have been established for RTE foods to be analyzed for *Li. monocytogenes* (Farber and Harwig, 1996).

**European and Australian perspectives**

The European community has set a zero tolerance for soft cheeses, and absence of the pathogen from 1 g of other milk and milk-based products (Jay, 1996b). Provisional guidelines for RTE foods in the UK indicate that absence of the pathogen from 25-g is satisfactory; < 100 CFU/g in 25 g fairly satisfactory; $10^2$-$10^3$ CFU/g unsatisfactory; and at $>10^3$ CFU/g, the product is unacceptable and potentially hazardous (Jay, 1996b). German authorities set four risk levels for *Li. monocytogenes* applied to different food categories (Teufel, 1994). Failure to meet the criteria would lead to public warning and product recall. A zero tolerance has been set in France for foods that may be consumed by at-risk individuals (Jay, 1996b).

Australia requires the absence of the pathogen from 5 x 25 g samples of some cheeses, manufactured meats and smoked seafood products that may support the growth of *Li. monocytogenes* (Eyles, 1994).

***Growth of Li. monocytogenes in MAP products***

*Li. monocytogenes* has been shown to grow under modified atmosphere in raw (Table 1.1) and processed meat products (Table 1.2), although some reports on the effect of carbon dioxide on *Li. monocytogenes* are contradictory. Growth was observed on high pH (>6.0) beef stored under 100% $CO_2$ at 10°C but not at 5°C, and in VP meat stored at 0, 2, 5, and 10°C (Gill and Reichel, 1989). Avery *et al.* (1994) showed that *Li. monocytogenes* was inhibited by saturated $CO_2$ packaging, but not by VP at 5 and 10°C. Additionally, *Li. monocytogenes* did not grow on steaks during abusive aerobic retail

display (12.5°C) when the beef steaks, inoculated with *Li. monocytogenes*, were previously packaged in saturated $CO_2$ atmospheres for 5 or 7 weeks at -1.5°C (Avery *et al.*, 1995). When the steaks were packaged in saturated $CO_2$ atmospheres for < 3 hours, or were VP prior to abusive aerobic retail display, the pathogen could grow on the meat.

*Li. monocytogenes* was inhibited by 70:30 $CO_2$:$N_2$ in turkey roll slices held at 4 or 10°C. Carbon dioxide levels of 20 and 50% did not inhibit growth, although growth was slower in the modified atmospheres than in air-stored turkey slices (Farber and Daley, 1994). Wimpfheimer and Hotchkiss (1990) reported similar inhibition of *Li. monocytogenes* in raw minced chicken stored under elevated $CO_2$ (72.5:22.5:5 $CO_2$:$N_2$:$O_2$) or in air at 4, 10, or 27°C. The organism was severely inhibited in the absence of oxygen (75:25 $CO_2$:$N_2$). These results are in contrast to the work of Marshall *et al.* (1991) who found than *Li. monocytogenes* grew well in air and in modified atmospheres containing 70-80% $CO_2$ (Marshall *et al.*, 1991). Growth on fresh pork chops was followed for 35 days (Manu-Tawiah *et al.*, 1993). Samples were stored at 4°C under different gas mixtures (20:0:80, 40:0:60, and 40:10:50, $CO_2$:$O_2$:$N_2$), air and under vacuum. Counts of *Li. monocytogenes* did not differ significantly between packaging treatments, and growth was slower than the psychrotrophic spoilage microbiota under all conditions.

Table 1.1 Survival and/or growth of pathogens in raw meat products stored under modified atmospheres

| Pathogen, product, and packaging | Reference |
|---|---|
| ***Clostridium botulinum*** | |
| Cod, whiting, and flounder fillets, VP or MAP in 100% $CO_2$, $N_2$, or 90:8:2, 65:31:4 $CO_2$:$N_2$:$O_2$ | Post *et al.*, 1985 |
| Red snapper homogenate, VP or MAP in 100% $CO_2$, or 70% $CO_2$, balance air | Lindroth and Genigeorgis, 1986 |
| Fresh fish, VP, or MAP in 100% $CO_2$ or 70:30 $CO_2$:$N_2$ | Baker and Genigeorgis, 1990 |
| ***C. perfringens*** | |
| Minced pork, inoculated, VP or MAP in 75:25 $CO_2$:$N_2$, and irradiated | Grant and Patterson, 1991a |
| ***Listeria monocytogenes*** | |
| Ground beef, VP | Johnson *et al.*, 1988 |
| High-pH beef, VP or MAP in 100% $CO_2$ | Gill and Reichel, 1989 |
| Minced chicken MAP in 75:25:0 or 72.5:22.5:5 $CO_2$:$N_2$:$O_2$ | Wimpfheimer *et al.*, 1990 |
| Minced pork, inoculated, VP or MAP in 75:25 $CO_2$:$N_2$, and irradiated | Grant and Patterson, 1991a |
| Skinless chicken breast, MAP in 30% $CO_2$ + air, 30:70 $CO_2$:$N_2$ or 100% $CO_2$ | Hart *et al.*, 1991 |
| Chicken legs, in 90:10 $CO_2$:$O_2$ | Zeitoun and Debevere, 1991 |
| Pork chops, VP, or MAP in 20:80:0, 40:60:0, or 40:50:10 $CO_2$:$N_2$:$O_2$ | Manu-Tawiah *et al.*, 1993 |
| Beef steaks, VP or saturated $CO_2$ packed | Avery *et al.*, 1994 |
| Raw pork and turkey slices, MAP in 100% $N_2$, 20:80 or 40:60 $CO_2$:$O_2$ | Mano *et al.*, 1995 |
| ***Staphylococcus aureus*** | |
| Ground chicken meat, MAP in 80% $CO_2$, balance air | Baker *et al.*, 1986 |
| ***Aeromonas hydrophila*** | |
| High-pH beef, VP or MAP in 100% $CO_2$ | Gill and Reichel, 1989 |

Table 1.1 *(continued)*

| Pathogen, product, and packaging | Reference |
|---|---|
| ***Escherichia coli*** | |
| Minced pork, inoculated, VP or MAP in 75:25 $CO_2$:$N_2$, and irradiated | Grant and Patterson, 1991a |
| ***Salmonella typhimurium*** | |
| Ground chicken meat, MAP in 80% $CO_2$, balance air | Baker *et al.*, 1986 |
| Minced pork, inoculated, VP or MAP in 75:25 $CO_2$:$N_2$, and irradiated | Grant and Patterson, 1991a |
| ***Yersinia enterocolitica*** | |
| High-pH beef, VP or MAP in 100% $CO_2$ | Gill and Reichel, 1989 |
| Minced beef meat, MAP in 20:80 $CO_2$:$O_2$ | Kleinlein and Untermann, 1990 |
| Minced pork, inoculated, VP or MAP in 75:25 $CO_2$:$N_2$, and irradiated | Grant and Patterson, 1991a |
| Pork chops, VP, or MAP in 20:80:0, 40:60:0, or 40:50:10 $CO_2$:$N_2$:$O_2$ | Manu-Tawiah *et al.*, 1993 |

Table 1.2 Growth and/or survival of pathogenic microorganisms on ready-to-eat MAP food products

| Pathogen, food product, and packaging method | Reference |
|---|---|
| ***Bacillus cereus*** | |
| Sliced ham, VP | Stiles and Ng, 1979 |
| Cooked, emulsion-style sausage, VP | Nielsen and Zeuthen, 1985 |
| Chicken breast, inoculated, VP, cooked (cook-in-bag) | Turner *et al.*, 1996 |
| ***Clostridium botulinum*** | |
| Comminuted turkey, inoculated, VP and cooked (cook-in-bag) | Maas *et al.*, 1989 |
| Shredded cabbage in 70% $CO_2$ and 30% $N_2$ | Solomon *et al.*, 1990 |
| Cooked turkey, VP | Genigeorgis *et al.*, 1991 |
| Cooked turkey and chicken, VP | Meng and Genigeorgis, 1993 |
| ***C. perfringens*** | |
| Sliced ham, VP | Stiles and Ng, 1979 |
| Frankfurters, VP | Nielsen and Zeuthen, 1985 |
| Cooked beef, MAP in 75% $CO_2$ and 0, 5 or 10% $O_2$ (balance $N_2$) | Hintlian and Hotchkiss, 1987a |
| Sliced cooked roast beef, in 75:15:10 $CO_2$:$N_2$:$O_2$ | Hintlian and Hotchkiss, 1987b |
| Cooked roast beef slices, VP | Michel *et al.*, 1991 |
| Precooked beef loins, inoculated, VP and pasteurized | Cooksey *et al.*, 1993a |
| Cooked turkey, VP | Jujena *et al.*, 1994a |
| Cooked ground beef, VP | Jujena *et al.*, 1994b |
| Cooked, sliced beef, VP | Miller and Acuff, 1994 |
| Ground beef inoculated, VP, and cooked (cook-in-bag) | Jujena and Majka, 1995 |
| Cook-in-bag ground turkey (inoculated, VP, then cooked) | Jujena and Marmer, 1996 |
| Cooked turkey packed in 75:5:20, 75:10:15, 75:20:5; 25:20:55, or 50:20:30 $CO_2$:$O_2$:$N_2$ | Jujena *et al.*, 1996 |
| Chicken breasts, inoculated ($10^2$, $10^3$, or $10^4$ CFU/g), cooked, and VP | Carpenter and Harrison, 1989b |

Table 1.2 *(continued)*

| Pathogen, food product, and packaging method | Reference |
|---|---|
| ***Listeria monocytogenes*** | |
| Chicken breasts, inoculated ($10^6$-$10^7$ CFU/g), cooked to five different temperatures, and VP | Harrison and Carpenter, 1989a |
| Processed meats: ham, bologna, wieners, sliced chicken or turkey, fermented sausage, bratwurst or cooked roast beef, VP | Glass and Doyle, 1989 |
| Chicken breasts, inoculated, microwaved, and VP | Harrison and Carpenter, 1989a |
| Cooked chicken loaf, in 50:10 or 80:0 $CO_2$:$O_2$, balance proprietary; co-inoculated with *P. fragi* | Ingham *et al.*, 1990a |
| Frankfurters, VP | Buncic *et al.*, 1991 |
| Cooked uncured turkey loaf, VP | Ingham and Tautorus, 1991 |
| Cooked chicken nuggets, MAP in 76:13.3:10.7 or 80:20:0 $CO_2$:$N_2$:$O_2$ | Marshall *et al.*, 1991 |
| Cooked chicken nuggets, MAP in 76:13.3:10.7 or 80:20:0 $CO_2$:$N_2$:$O_2$; co-inoculation with *P. fluorescens* | Marshall *et al.*, 1991 |
| Cooked roast beef slices, VP | Michel *et al.*, 1991 |
| Beef roast, inoculated, VP, cooked (cook-in-bag) | Unda *et al.*, 1991 |
| Wieners, VP | Degnan *et al.*, 1992 |
| Dry fermented sausages, VP | Foegeding *et al.*, 1992 |
| Sliced-corned beef and ham, VP | Grau and Vanderlinde, 1992 |
| Turkey rolls and battered chicken nuggets, inoculated ,VP, and cooked | Line and Harrison, 1992 |
| Hot-smoked trout, VP; inoculated before or after hot-smoking | Jemmi and Keusch, 1992 |
| Turkey summer sausage, VP | Luchansky *et al.*, 1992 |
| Inoculated, VP, sous-vide cooked chicken breast meat | Shamsuzzaman *et al.*, 1992 |
| Precooked beef loins, inoculated, VP and pasteurized | Cooksey *et al.*, 1993b |
| Precooked beef roasts, inoculated, VP and pasteurized | Hardin *et al.*, 1993 |
| Cold-smoked salmon, VP | Hudson and Mott, 1993a |

Table 1.2 (*continued*)

| Pathogen, food product, and packaging method | Reference |
|---|---|
| ***Listeria monocytogenes (continued)*** | |
| Cooked beef, VP | Hudson and Mott, 1993b |
| Beef roasts, inoculated, VP and cooked (cook-in-bag) | Stillmunkes *et al.*, 1993 |
| Cooked beef systems, VP | Winkowski *et al.*, 1993 |
| Cooked sliced beef, pork, turkey or chicken meat, VP | Duffy *et al.*, 1994 |
| Cooked pork, MAP in 100% $CO_2$ or 80% $CO_2$ balance air | Fang and Lin, 1994 |
| Turkey roll slices MAP in 30:70, 50:50, or 70:30 $CO_2$:$N_2$ | Farber and Daley, 1994 |
| Cooked mussel tissue, VP | Hudson and Avery, 1994 |
| Cooked sliced roast beef, VP or packaged in saturated $CO_2$ atmosphere | Hudson *et al.*, 1994 |
| Ground pork, VP | Kim *et al.*, 1994 |
| Beef, poultry, or beef/pork retail wieners, VP | McKellar *et al.*, 1994 |
| Cooked, sliced beef, VP | Miller and Acuff, 1994 |
| Cold-process (smoked) salmon, VP | Pelroy *et al.*, 1994 |
| Sliced bologna sausages, VP | Qvist *et al.*, 1994 |
| Cooked, sliced turkey bologna, VP | Wederquist *et al.*, 1994 |
| Smoked blue cod, VP or MAP in 100% $CO_2$ | Bell *et al.*, 1995 |
| Sterile crawfish tail meat homogenate, VP, or MAP (74.8% $CO_2$, 15.2% $N_2$, 10% $O_2$) | Oh and Marshall, 1995 |
| Sliced cooked meats: luncheon meat, ham, chicken breast, VP or MAP in 30:70 $CO_2$:$N_2$ | Beumer *et al.*, 1996 |
| Cold-smoked salmon, sliced and VP | Wessels and Huss, 1996 |
| Vegetarian foods, VP | Fang and Chen, 1997 |
| ***Aeromonas hydrophila*** | |
| Cooked mince surimi, packed in 51:13:36 $N_2$:$O_2$: $CO_2$, co-inoculated with *P. fragi* | Ingham and Potter, 1988 |

Table 1.2 (*continued*)

| Pathogen, food product, and packaging method | Reference |
|---|---|
| ***Aeromonas hydrophila*** *(continued)* | |
| Sterilized crayfish tails, VP, or MAP under 80% $CO_2$, 0% $O_2$, balance proprietary | Ingham, 1990 |
| Cold-smoked salmon | Hudson and Mott, 1993a |
| Cooked beef, VP | Hudson and Mott, 1993b |
| Cooked mussel tissue, VP | Hudson and Avery, 1994 |
| Cooked sliced roast beef, VP or packaged in saturated $CO_2$ atmosphere | Hudson *et al.*, 1994 |
| Smoked blue cod, VP or MAP in 100% $CO_2$ | Bell *et al.*, 1995 |
| ***Escherichia coli*** | |
| Sliced ham, VP | Stiles and Ng, 1979 |
| Cooked roast beef slices, VP | Michel *et al.*, 1991 |
| ***E. coli*** O157:H7 | |
| Cooked, sliced beef, VP | Miller and Acuff, 1994 |
| ***Salmonella typhimurium*** | |
| Sliced ham, VP | Stiles and Ng, 1979 |
| Cooked, emulsion-style sausage, VP (or *S. enteritidis*) | Nielsen and Zeuthen, 1985 |
| Cooked beef, MAP in 75% $CO_2$ and 0, 5 or 10% $O_2$ (balance $N_2$) | Hintlian and Hotchkiss, 1987a |
| Sliced cooked roast beef, in 75:15:10 $CO_2$:$N_2$:$O_2$ | Hintlian and Hotchkiss, 1987b |
| Cooked crab meat, MAP in 50:10 $CO_2$:$O_2$, balance proprietary; co-inoculated with *P. fragi* | Ingham *et al.*, 1990b |
| Cooked uncured turkey loaf, VP | Ingham and Tautorus, 1991 |
| Cooked roast beef slices, VP | Michel *et al.*, 1991 |
| Cooked, sliced beef, VP | Miller and Acuff, 1994 |

Table 1.2 *(continued)*

| Pathogen, food product, and packaging method | Reference |
|---|---|
| ***Staphylococcus aureus*** | |
| Sliced ham, vacuum-packed | Stiles and Ng, 1979 |
| Cooked, emulsion-style sausage, VP | Nielsen and Zeuthen, 1985 |
| Cooked beef, MAP in 75% $CO_2$ and 0, 5 or 10% $O_2$ (balance $N_2$) | Hintlian and Hotchkiss, 1987a |
| Sliced cooked roast beef, in 75:15:10 $CO_2$:$N_2$:$O_2$ | Hintlian and Hotchkiss, 1987b |
| Cooked roast beef slices, VP | Michel *et al.*, 1991 |
| ***Yersinia enterocolitica*** | |
| Cooked, emulsion-style sausage, VP | Nielsen and Zeuthen, 1985 |
| Cold-smoked salmon, VP | Hudson and Mott, 1993a |
| Cooked beef, VP | Hudson and Mott, 1993b |
| Cooked mussel tissue, VP | Hudson and Avery, 1994 |
| Cooked sliced roast beef, VP or packaged in saturated $CO_2$ atmosphere | Hudson *et al.*, 1994 |
| Smoked blue cod, VP or MAP in 100% $CO_2$ | Bell *et al.*, 1995 |

**Growth in processed meat products**

When precooked sterilized chicken nuggets, inoculated with either *Li. monocytogenes* or *Pseudomonas fluorescens* were stored under modified atmospheres ($MA_1$: 76:13.3:10.7 $CO_2$:$N_2$:$O_2$; $MA_2$: 80:20 $CO_2$:$N_2$) at 3, 7, and 11°C, growth of *L. monocytogenes* was reduced but was not inhibited under MAP. The effectiveness of the modified atmospheres decreased as the storage temperature increased. Growth of *P. fluorescens* was substantially reduced throughout the duration of the experiment, more so at the lower storage temperatures (Marshall *et al.*, 1991). When both organisms were co-inoculated on the chicken nuggets and stored under the same conditions, *P. fluorescens* stimulated the growth of *Li. monocytogenes* at 3°C in air and $MA_1$, but not $MA_2$. At higher temperatures, the stimulatory effect was absent, and *Li. monocytogenes* inhibited the growth of *P. fluorescens* (Marshall *et al.*, 1992). *Pseudomonas* spp. have been shown stimulate growth of *Li. monocytogenes* in milk at 10°C (Marshall and Schmidt, 1988).

A major target for scientists attempting to assess safety of MAP foods is to understand the growth interactions of pathogenic and spoilage organisms (Hotchkiss, 1988). Growth of *Li. monocytogenes* was not inhibited on cooked chicken loaf stored under two MA. The growth rates of *Li. monocytogenes* and *P. fragi* were followed after co-inoculation on cooked chicken loaf samples which were stored under two MA ($MA_1$: 50:10 $CO_2$:$O_2$; $MA_2$: 80:0 $CO_2$:$O_2$, balance proprietary) at 3, 7, and 11°C. The organisms grew well at the three storage temperatures in the air controls, with *P. fragi* having a slight advantage. Under $MA_1$, growth was slowed at all three temperatures, although less severely for *Li.*

*monocytogenes* at 7 and 11°C. $MA_2$ reduced growth of both species, but the inhibition was more obvious for *P. fragi* (Ingham *et al.*, 1990a).

### 1.2.2.2 *Clostridium botulinum*

Psychrotrophic *Clostridium botulinum* strains are another major concern in extended shelf life chilled products (Conner *et al.*, 1989; Peck, 1997). These strains have a minimal growth temperature of 3.3°C and are non-proteolytic, thus toxin could be present in a product presenting no detectable signs of spoilage (Hauschild, 1989). The elevated $CO_2$ in MAP products is not a hurdle, quite the opposite, as it stimulates the germination and growth of non-proteolytic *Clostridium botulinum* spp. types B, E, and F (Foegeding and Busta, 1983; Hotchkiss, 1988). *C. botulinum* spores might be present in the raw material, or might be added due to poor handling during production. The heat treatment applied to MAP foods is often insufficient to destroy the spores that might germinate and produce toxin in the final product constituting a health hazard in RTE foods that do not require a heat treatment prior to consumption. The normal spoilage microbiota has been inhibited, possibly eliminating a potential competitor to *C. botulinum.*

Toxin formation by *C. botulinum* is time and temperature dependent. At refrigeration temperatures there is a potential for growth of the psychrotrophic, non-proteolytic strains of *C. botulinum* and any temperature abuse would shorten the time for toxin production. At temperatures greater or equal to 10°C, non-proteolytic strains of *C. botulinum* were able to produce toxin in 6 days (Solomon *et al.*, 1982). Such elevated temperatures will furthermore allow growth and toxin production by the proteolytic strains. Toxigenesis by

proteolytic strains of *C. botulinum* inoculated into a sous-vide spaghetti and meat sauce was detected after 14 days at 15°C at pH 5.5 and above (Simpson *et al.*, 1995). Spoilage preceded toxigenesis in samples with a pH of 5.75 and 6.00, however, toxigenesis preceded spoilage at pH 5.25 and 5.50.

Notermans *et al.* (1981) reported that proteolytic and non-proteolytic strains of *C. botulinum* could survive a heat treatment of 95°C for 40 min, when applied to potatoes which were subsequently VP and stored at 4 and 10°C. No toxin was detected after a 6-week shelf life in the samples stored at 4°C; however, the 10°C samples exhibited growth of *C. botulinum* and toxin production, with no visible spoilage of the product, within the 6 weeks storage period. Ikawa (1991) detected botulinal toxin in shelf-stable, anaerobically stored noodles with initial pH less than 4.5, after the pH was increased by microbial growth. Carlin *et al.* (1995, 1996) have shown that cooked vegetables can support growth and toxin formation by non-proteolytic strains of *C. botulinum*. Toxin formation in cooked vegetables stored anaerobically was detected after 3-5 days at 16°C, 13 days at 10°C, 10-34 days at 8°C, and 17-20 days at 5°C.

Nitrogen-packed hamburger sandwiches, inoculated with *C. botulinum* type E, became toxic within 30 days at 12°C, but not at 8°C (Kautter *et al.*, 1981). The toxic sandwiches had an acceptable appearance and odour. Type E toxin was not detected in turkey or sausage sandwiches. *C. botulinum* types A and B did not produce toxin in hamburger, turkey, or sausage sandwiches stored under the same conditions. Hamburger sandwiches inoculated with *C. botulinum* types A and B were toxic after 4 days of storage at room temperature, whereas signs of spoilage did not appear until one day later. Sausage

sandwiches became toxic by day 7, while still marginally acceptable. Vacuum-packed bacon was able to support growth and toxin production by *C. botulinum* strains without any detectable off-odours (Thatcher *et al.*, 1962). Lag phase and probability of toxin production by strains of *C. botulinum* was modeled in cooked, homogenized vacuum-packed turkey at temperatures ranging from 4 to 30°C (Genigeorgis *et al.*, 1991). Lag phase to toxin production was 180 days for samples stored at 4°C, and decreased to 8 days in samples stored at 8°C. In a subsequent study, lag phase of *C. botulinum* toxigenesis was modeled at different temperatures, and levels of sodium chloride, sodium lactate, and spore inoculum (Meng and Genigeorgis, 1994).

*Regulatory policies or guidelines*

To avoid or minimize hazards associated with RTE MAP chilled foods, several guidelines and recommendations have been drafted (Peck, 1997). The Advisory Committee on the Microbiological Safety of Food (ACMSF) in the UK recommended that heat treatment or combination processes applied should achieve a six-decimal reduction in numbers of viable spores of non-proteolytic *C. botulinum* in the food. In addition to chilled storage, the following control factors should be used singly or in combination to ensure the safety of extended shelf life RTE foods (adapted from Peck, 1997):

- Heat treatment at 90°C for 10 min or alternative equivalent heat treatments
- A pH of 5.0 or lower throughout the food
- A salt concentration of 3.5% or higher throughout the food

- A water activity of 0.97 or lower throughout the food
- Combinations of heat treatments and other preservative factors consistently shown to prevent toxigenesis by *C. botulinum.*

### 1.2.2.3 *Aeromonas hydrophila*

*Aeromonas* spp. have been implicated as causative agents of human disease (Gracey *et al.*, 1982; Burke *et al.*, 1983; Abeyta *et al.*, 1896). These gram-negative rods are facultative anaerobes, and are ubiquitous in nature. Buchanan and Palumbo (1985) suggested that these organisms were associated with spoilage of refrigerated foods, and thus would be of concern in RTE MAP products. Resistance of *Aeromonas* spp. to $CO_2$ is similar to *Lactobacillus*, and a small decrease in $CO_2$ concentrations below 100% may favour the growth of aeromonads (Blickstad and Molin, 1983b).

*Aeromonas* spp. have been isolated from various foods, particularly of animal origin (Palumbo *et al.*, 1985a; 1989; Abeyta *et al.*, 1986; Okrend *et al.*, 1987; Ternström and Molin, 1987; Barnhart *et al.*, 1989; Fricker and Tompsett, 1989). A survey of several types of foods sold in consumer packages at retail markets revealed presence of *A. hydrophila* in all types of food tested (Palumbo *et al.* 1985a). Fricker and Tompsett (1989) reported the presence of *Aeromonas* spp. in 37.5% of cooked meats, and 21.6% of pre-prepared salads. *A. hydrophila* was also isolated from VP pork (Myers *et al.*, 1982).

A large proportion of the *Aeromonas* spp. are psychrotrophic (Palumbo *et al.*, 1985b; Krovacek *et al.*, 1991), and are able to grow competitively at 5°C (Callister and Agger, 1987). *A. hydrophila* was able to grow on nitrogen-packaged raw pork at 4°C, but poor

growth was observed in $CO_2$ packed pork (Enfors *et al.*, 1979). Ingham and Potter (1988) reported the ability of *A. hydrophila* to compete with *P. fragi* on mince, salt-added surimi, and low-salt surimi stored under air or MA (51:13:36 $N_2$:$O_2$:$CO_2$) at 5 and 13°C. Several strains of enterotoxigenic *A. hydrophila* and *A. sobria* were able to produce exotoxin in a meat extract after 5 days at 12°C, and after 8 days at 8°C (Ingham and Potter, 1988). The organism well on high pH (>6.0) beef packaged under $CO_2$ and stored at 10°C, but did not grow at the lower storage temperatures (Gill and Reichel, 1989). Several studies investigated the growth of *A. hydrophila* in vacuum-packed flesh foods stored at 5 or 10°C. In cold-smoked salmon, *A. hydrophila* could grow at 10°C (Hudson and Mott, 1993a); however, growth was observed at 5°C but not at 10°C in beef (Hudson and Mott, 1993b). The organism grew at 5 and 10°C in cooked mussel tissue (Hudson and Avery, 1994). In VP or $CO_2$-packed sliced roast beef, the pathogen grew at 3°C in both environments (Hudson *et al.*, 1994). When the storage temperature was decreased to -1.5°C, growth was present in vacuum-packs only. Similar results were obtained by Bell *et al.* (1995) in VP or $CO_2$-packed blue cod. *A. hydrophila* could grow in vacuum-packs at 3 and -1.5°C, although growth was delayed at the lower storage temperature. In $CO_2$ atmospheres, *A. hydrophila* was inhibited at -1.5°C, while the lag phase was extended to 21 days at 3°C (Bell *et al.*, 1995).

#### 1.2.2.4 *Yersinia enterocolitica*

*Yersinia enterocolitica* is a gram-negative facultative anaerobe which can grow at refrigeration temperatures and is commonly associated with swine (Schiemann, 1989). It

has been isolated from meat and dairy sources (Hanna, 1976; Harmon *et al.*, 1984; Ibrahim and Mac Rae, 1991; Toora *et al.*, 1994). Storage in an atmosphere of carbon dioxide was inhibitory to the growth of *Y. enterocolitica* in tryptone soya broth (Eklund and Jarmund, 1983). The extent of inhibition by $CO_2$ was dependent on the storage temperature. The organism did not grow at 2°C, while a 98% or 43% inhibition (compared to growth in air) was observed at 6 or 20°C, respectively. A slight inhibitory effect was observed in vacuum packs or in nitrogen at 2 and 6°C. Bennik *et al.* (1995) studied the effect of $CO_2$ atmospheres (0, 5, 20, or 50%) combined with $O_2$ (1.5 or 21%) on the growth of several psychrotrophic pathogens which were incubated on the surface of buffered brain heart infusion agar at 8°C. Oxygen levels did not affect growth. Growth rate of *Y. enterocolitica* decreased with increasing concentrations of $CO_2$; however, maximum populations densities were not affected within the $CO_2$ range tested. The lag phase of the pathogen was increased only under 50% $CO_2$ and 21 % $O_2$.

When inoculated in foodstuff, *Y. enterocolitica* could grow on high-pH beef (>6.0) packaged under 100% $CO_2$ at both 5 and 10°C (Gill and Reichel, 1989). *Li. monocytogenes* and *A. hydrophila* could grow at 10°C but not at 5°C under the same conditions. *Y. enterocolitica* did not grow when the meat was stored at -2, 0, and 2°C, which is in agreement with the results of Eklund and Jarmund (1983). In VP beef, *Y. enterocolitica* grew at all storage temperatures (-2, 0, 2, 5, and 10°C). Growth was inhibited in MAP minced beef (20:80 $CO_2$:$O_2$) stored at 4°C (Kleinlein and Unterman, 1990), but slightly delayed at 10°C. At 15°C, growth of the pathogen was similar in air and in the modified atmosphere. When inoculated on fresh pork chops packaged under different gas mixtures (20:0:80; 40:0:60; and 40:10:50, $CO_2$:$O_2$:$N_2$), vacuum, and air, and

stored at 4°C, *Y. enterocolitica* grew more slowly than the psychrotrophic spoilage microbiota under aerobic conditions, while similar rates of growth were observed under modified atmospheres, and VP (Manu-Tawiah *et al.*, 1993). *Y. enterocolitica* could grow on sliced roast beef in $CO_2$-saturated environments at 3°C but not at -1.5°C (Hudson *et al.*, 1994). A reduction in numbers of the pathogen was observed at the lower storage temperature. In vacuum packs, growth was observed at -1.5 and 3°C (Hudson *et al.*, 1994). The same research lab in New Zealand investigated the growth of *Y. enterocolitica*, *Li. monocytogenes*, and *A. hydrophila* in different food products stored in air or VP, at 5 or 10°C. Growth of *Y. enterocolitica* was observed in VP cold-smoked salmon (Hudson and Mott, 1993a), cooked mussel tissue (Hudson and Avery, 1994), and cooked beef (Hudson and Mott, 1993b).

### 1.2.3 Mesophilic foodborne pathogens

Psychrotrophic organisms are not the only safety hazard present in MAP foods. Organisms able to grow between 5 and 12°C are an ever-present concern in view of the temperature abuse liable to occur to any refrigerated product. The organisms of interest are *Salmonella* spp., *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus*, and *Campylobacter jejuni*.

Over a 10-day period, *Salmonella* counts increased by 0.5 log cycles in ground beef packaged under MA (60:25:15 $CO_2$:$O_2$:$N_2$) and stored at 10°C, while an increase of over 3 log cycles was seen in air-stored samples (Silliker and Wolfe, 1980). When the storage temperature was increased to 20°C, minimal differences were observed between storage

environments. A decrease of 1.5 log cycles was observed for *S. aureus* populations in MAP ground beef (60:25:15 $CO_2$:$O_2$:$N_2$), kept at 10°C. Storage at 20°C eliminated the inhibitory effects of MAP (Silliker and Wolfe, 1980). *S. typhimurium* and *S. aureus* did not grow on beef steaks packaged in 60:40 $CO_2$:$O_2$, and stored at 10°C for 9 days (Luiten *et al.*, 1982a; Luiten *et al.*, 1982b). They were also inhibited in broth or in ground chicken meat stored under 80:20 $CO_2$:air at 2, 7, and 13°C (Baker *et al.*, 1986). The extent of inhibition decreased as the storage temperature increased.

Hintlian and Hotchkiss (1987a) reported that MAs (75% $CO_2$, and 0, 2, 5, 10, or 25% $O_2$, balance $N_2$) inhibited growth of *S. aureus* on sliced cooked roast beef during abusive storage (12.8°C and 26.7°C), but were less effective for *S. typhi*. *C. perfringens* grew well in the air controls at 12.8 and 26.7°C, and in MAs at 26.7°C; however, it was inhibited in MAs containing oxygen at 12.8°C. At 4.4°C, a MA of 75:10:15 $CO_2$:$O_2$:$N_2$ was the most effective in the simultaneous inhibition of *P. fragi*, *S. typhi*, *S. aureus*, and *C. perfringens*, co-inoculated on cooked sliced roast beef. The authors concluded that at lower storage temperatures, *S. aureus* and *S. typhi* were inhibited by the elevated $CO_2$ and reduced $O_2$ levels, and *C. perfringens* by the presence of $O_2$. High concentrations of $CO_2$ may act synergistically with chill temperatures to inhibit growth of pathogens, and the degree of inhibition increases as temperature decreases.

A subsequent study by the same authors (Hintlian and Hotchkiss, 1987b) reported the effectiveness of MAP (75:15:10 $CO_2$:$N_2$:$O_2$) in controlling the outgrowth of *P. fragi*, *C. perfringens*, *S. aureus*, and *S. typhi* in cooked roast beef stored at 4.4°C for 42 days.

Populations of *S. aureus* did not decrease under MA or in air. Sensory deterioration occurred within the first 7 days of storage.

Sandwiches and sausages inoculated with *S. aureus* and held in $N_2$ became toxic prior to any visible sign of sensory spoilage (Bennett and Amos, 1982). Effects of carbon dioxide were not tested. *S. typhimurium*, *S. aureus*, *C. perfringens*, and *E. coli* could survive but did not grow on vacuum-packed, precooked, roast beef slices stored at 3°C for 70 days (Michel *et al.*, 1991). Gill and DeLacy (1991) reported the growth *of E. coli* and *S. typhimurium* in VP high-pH beef (>6.0) stored at 8, 10, 12, 15, 20, or 30°C. In $CO_2$-packaged beef, growth of both pathogens was inhibited at 8°C, while only *S. typhimurium* was inhibited at 10°C. The lag phase was considerably extended at 12°C in $CO_2$ packs.

*Bacillus cereus* might not constitute as great a hazard as other pathogens in MAP foods since several studies have shown its sensitivity to $CO_2$ (Enfors and Molin, 1980; 1981b; Bennik *et al.*, 1995). Incubation at 25°C in 100% $CO_2$ reduced growth of the organism by 83 and 67%, as compared to aerobic and anaerobic growth (5:95 $CO_2$:$N_2$) (Molin, 1983). An atmosphere of 100% $CO_2$ inhibited germination of *B. cereus* spores by 70-90% as compared to approximately complete germination under 100% $N_2$ (Enfors and Molin, 1978). Although MAP seems to inhibit growth of *B. cereus* in pure culture studies, the behavior of this facultative anaerobe might differ in actual foodstuff. *B. cereus* was responsible for several food poisoning outbreaks implicating poultry products (Todd *et al.*, 1992). The organism was present in 6.9% of retail samples of poultry meat products surveyed by Sooltan *et al.* (1987). The products tested included both raw and cooked

meats, either frozen or refrigerated, however, *B. cereus* occurred only in the raw samples and in the pâtés.

### 1.2.4 Enhancing the safety of MAP RTE foods: Multiple hurdles concept

Ready-to-eat, MAP products can be considered potentially hazardous if no steps are taken to ensure their safety. Intrinsic or extrinsic barriers could be incorporated into extended shelf life refrigerated foods, such as preservatives (lactates, bacteriocins, organic acids, spices, etc.), competitive microbiota, or post-packaging heat treatments. While one treatment may not be effective enough to inhibit spoilage or pathogen growth, a combination of factors may act synergistically to enhance shelf life and safety. Leistner and Rodel (1976), and Mossel (1983) introduced the "hurdles" concept i.e., combining several inhibitory factors at subinhibitory levels to achieve a safe product without substantially altering the character of the product (Conner *et al.*, 1989). These principles were recently reviewed (Scott, 1989, Leistner, 1994; 1995).

As demand increases for convenience foods with minimal preparation time and minimal preservatives, the industry faces a challenge to ensure that their products are safe and wholesome. Foodborne poisoning outbreaks are highly publicized and the economic welfare of food industries is often at stake. An outbreak means large economic losses due to financial compensations, loss of business due to the bad publicity an outbreak generates, and heavy fines indicted by regulatory agencies. With recent consumer demand for 'natural' foods containing minimal or no chemical preservatives, alternative safeguards are required to minimize risks associated with RTE, MAP, chilled foods.

With this in mind, natural biological control agents are being investigated as biopreservatives. With their safe history record, lactic acid bacteria and/or their by-products may be the agents of choice for biological control of food-associated hazards.

## 1.3 BIOPRESERVATION BY LACTIC ACID BACTERIA

### 1.3.1 Introduction

Lactic acid bacteria (LAB) have been used in food fermentations for centuries for the safe preservation of dairy and meat products. Traditional processes rely on the natural establishment of a lactic acid microbiota and these organisms have a well established safety record. LAB produce a variety of antimicrobial compounds as by-products of their metabolic activity including organic acids, bacteriocins, $H_2O_2$, diacetyl, alcohols, carbon dioxide, and reuterin (Davidson and Hoover, 1993).

The inhibitory action of LAB against other organisms may be due to a drop in pH through the production of organic acids, by competing for nutrients, and by synthesizing proteinaceous antibacterial agents known as bacteriocins. These substances have been reported to be mainly active against closely-related gram-positive organisms. Antilisterial bacteriocins produced by LAB isolated from food have been recently reviewed by Muriana (1996). Producer organisms include *Carnobacterium* spp., *Enterococcus* spp., *Lactobacillus* (*Lb.*) spp., *Leuconostoc* (*Lc.*) spp., and *Pediococcus* spp. isolated from dairy, meat, and vegetable sources. Okereke and Montville (1991a; 1991b) reported bacteriocin-mediated inhibition of *C. botulinum* spores by *L. lactis*, *Lb. plantarum*, and *P. pentosaceus* strains. Bacteriocin-producing LAB active against *Li. monocytogenes*, *S.*

*aureus*, and *A. hydrophila* have been isolated from retail cuts of meat (Lewus *et al.*, 1991).

Bacteriocin-producing LAB occur commonly in food products as attested by the frequency of their isolation (Uhlman *et al.*, 1992; Garriga *et al.*, 1993; Garver and Muriana, 1993; Vaughan *et al.*, 1994; Kelly *et al.*, 1996; Coventry *et al.*, 1997). Several articles are published yearly about the purification and characterization of newly discovered bacteriocins produced by enterococci, lactobacilli, carnobacteria, leuconostocs, or pediococci isolated from foods.

The use of lactic acid bacteria or their byproducts to delay food spoilage or to enhance safety is not a recent concept. The use of nisin to inhibit pathogens in foods dates back to the 1960's. Attempts to control spoilage of meat by the introduction of a competitive microbiota were made in the 1970's and early 80's. Interest in the use of LAB or their byproducts as biopreservatives has gained momentum in the past decade. Numerous articles and reviews revolving around biopreservation by LAB have been published during the past 8 years (Table 1.3).

Table 1.3 Reviews and general interest articles published since 1989 on the subject of biopreservation by lactic acid bacteria

| Title | Reference |
|---|---|
| Antimicrobial substances from lactic acid bacteria for use as food preservatives | Daeschel, 1989 |
| Biological competition as a preserving mechanism | Gombas, 1989 |
| Use of lactic acid bacteria as protective cultures in meat products | Schillinger and Lücke, 1989b |
| Lactic acid bacteria in meat fermentation | Hammes *et al.*, 1990 |
| Antagonistic activities of lactic acid bacteria in food and feed fermentations | Lindgren and Dobrogosz, 1990 |
| Bacteriocin production by lactic acid bacteria: potential for use in meat preservation | Stiles and Hastings, 1991 |
| Applications and interactions of bacteriocins from lactic acid bacteria in foods and beverages | Daeschel, 1993 |
| Potential for biological control of agents of foodborne disease | Stiles, 1994 |
| Bacteriocins of lactic acid bacteria: their potential as food biopreservatives | Kim, 1993 |
| The potential of lactic acid bacteria for the production of safe and wholesome food | Hammes and Tichaczek, 1994 |
| Bacteriocins: mode of action and potentials in food preservation and control of food poisoning | Abee *et al.*, 1995 |
| Enterococcal bacteriocins: their potential as anti-*Listeria* factors in dairy technology | Giraffa, 1995 |
| Biological preservation of foods with reference to protective cultures, bacteriocins and food grade-enzymes | Holzapfel *et al.*, 1995 |
| Biopreservation of fish products - a review of recent approaches and results | Huss *et al.*, 1995 |
| Biological control of pathogens in food: option or fiction? | Scherer, 1995 |
| Lactic and non-lactic acid bacteria as a tool for improving the safety of dairy products | Giraffa, 1996 |
| Biopreservation for ready-to-eat salads | Gorris and Abee, 1996 |
| Potential for use of bacteriocin-producing lactic acid bacteria in the preservation of meats | McMullen and Stiles, 1996 |

Table 1.3 *(continued)*

| Title | Reference |
|---|---|
| Bacteriocins for control of *Listeria* spp. in food | Muriana, 1996 |
| Utilisation de bactériocines pour la production d'aliments plus sûrs: mythe ou réalité?[a] | Richard, 1996 |
| Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods | Schillinger *et al.*, 1996 |
| Biopreservation by lactic acid bacteria | Stiles, 1996 |
| Using lactic acid bacteria to improve the safety of minimally processed fruits and vegetables | Breidt and Fleming, 1997 |
| Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria | Helander *et al.*, 1997 |

[a]Use of bacteriocins to enhance the safety of food: myth or reality?

### 1.3.2 Characteristics of a competitive microbiota

For LAB to be used successfully in RTE chilled foods as biocontrol agents, whether to defer spoilage or to inhibit pathogenic microorganisms, they should satisfy several criteria (McMullen and Stiles, 1996):

- Able to grow in MAP, chilled meats under refrigerated storage
- Reliable bacteriocin production early in the growth cycle
- No loss of bacteriocin activity in meat products
- Should not alter the nature of the product
- Should not spoil the product faster than a naturally occurring microbiota
- Should grow faster than pathogens in the product

These requirements for a competitive microbiota may be difficult to meet in one single organism. Some strains of *Lactococcus lactis* are good producers of nisin, however, they are mesophilic organisms. Carnobacteria, which are psychrotrophic, non-aciduric, and commonly associated with meat products, produce only narrow-spectrum bacteriocins. Few organisms, if any, will possess all the desirable properties of a successful competitor. Additionally, effective competitive ability of one organism in one product does not imply the same will be achieved in another product.

Hammes and Hertel (1996) proposed several criteria and examples for the improvement of LAB acid bacteria used in meat fermentations. Highly competitive strains with desirable traits could be genetically modified to improve expression of those traits or acquire additional advantageous properties (Hammes and Hertel, 1996).

### 1.3.3 Biopreservation against spoilage organisms

Introduction of LAB in food products in an attempt to control the adventitious spoilage microbiota by competitive exclusion or through bacteriocin production have produced mixed results. Inoculation of fresh beef steaks, VP, with lactobacilli, did not improve shelf life or sensory properties of the meat (Hanna *et al.*, 1980; Smith *et al.*, 1980). Vacuum-packed beef samples inoculated with different meat isolates (lactobacilli, carnobacteria and lactococci) were not different from the control sample (Schillinger and Lücke, 1987b). Growth of *B. thermosphacta*, a spoilage organism common in MAP meat products, was inhibited in the presence of *Lb. brevis* or *Lb. plantarum* in vacuum-packed bologna (Collins-Thompson and Lopez, 1982).

In the past decade, knowledge of bacteriocins and producer cultures has expanded considerably. The wide array of isolated LAB that produce bacteriocins helps to ensure that appropriate cultures for a food matrix are selected for biopreservation studies. Bacteriocin-producing *Lc. gelidum* UAL187 co-inoculated with spoilage organism *Lb. sake* on VP beef delayed spoilage by 6 weeks. Beef co-inoculated with *Lb. sake* and the bacteriocin-negative variant of UAL187 or a bacteriocin-producing, but slow growing variant spoiled within 2 weeks at 2°C (Leisner, 1996). *Lc. gelidum* 187, originally isolated from VP chilled meat (Shaw and Harding, 1989), produces a bacteriocin (leucocin A) and grows well at refrigerated temperatures. Experimental conditions have shown that it is effective in the preservation of MAP fresh meats. However, that same organism will produce dextran in the presence of sucrose and, as such, cannot be used in

sucrose-containing products such as processed or cured meats (McMullen and Stiles, 1996).

Control of spoilage bacteria was also attempted using purified or semi-purified bacteriocins of LAB. Addition of nisin, produced by *L. lactis*, or pediocin $PO_2$ (produced by *P. acidilactici* $PO_2$), to VP cooked corned beef did not inhibit growth of inoculated spoilage organism *Lb. curvatus* (Coventry *et al.*, 1995). Bacteriocin activity was minimal or completely lost during storage at 5 or 10°C. Shelf life of brined shrimp was extended by addition of nisin or bavaricin (*Lb. bavaricus*) although not to the extent achieved by addition of a benzoate-sorbate solution (Einarsson and Lauzon, 1995). Addition of nisin to vacuum-packaged cooked beef inoculated with spores of *C. sporogenes* and stored at 4 or 10°C for 21 days and 70 days achieved a significant reduction in numbers (3-log units) of vegetative cells recovered, except for one treatment (nisin-treated beef at 4°C after 21 days) where no significant difference was present among controls and nisin-treated samples (Hague *et al.*, 1997). Table 1.4 summarizes some of the studies using lactic acid bacteria or bacteriocins to control food spoilage.

Table 1.4 Biopreservation of meat products to improve shelf life

| Competitor and/or bacteriocin used | Product | Summary | Reference |
|---|---|---|---|
| *Pediococcus cerevisiae* or *Lactobacillus plantarum* | Ground and mechanically deboned poultry meat | A two-day shelf life extension of meat inoculated with starter cultures | Raccach *et al.*, 1979 |
| *Microbacterium thermosphactum* (*B. thermosphacta*) or *Lb.* spp. (homofermentative) | Sliced luncheon meats, VP | Rapid spoilage and off-odours observed with *B. thermosphacta*; Delayed spoilage with lactobacilli, no off-odours for 21 days at $10^8$ CFU/g | Egan *et al.*, 1980 |
| *Lactobacillus* spp. (isolated from VP beef) | Beef steaks, VP | Inoculation of steaks with lactobacilli did not improve shelf life or sensory properties | Hanna *et al.*, 1980; Smith *et al.*, 1981 |
| *Lb. brevis, Lb. viridescens, Lb. plantarum*, or *Leuconostoc mesenteroides* | Irradiated bologna, VP, inoculated with *B. thermosphacta* | Inhibition of *B. thermosphacta* in the presence of *Lb. brevis* or *Lb. plantarum.* | Collins-Thompson and Lopez, 1982 |
| *Lb. sake, Lb. curvatus, Lb. divergens* (*C. divergens*), *Leuconostoc* spp., or *L. raffinolactis* | Beef, VP | Minimal effect of inoculated organisms on shelf life of beef stored at 2°C | Schillinger and Lücke, 1987b |
| *Lb. curvatus* LTH 1174 ($bac^+$) | Fermented sausage | Better flavour when sausage fermented with $bac^+$ culture. Dominant microbiota by the third fermentation day | Vogel *et al.*, 1993 |
| FloraCarn L-2 (*Lb. alimentarius* BJ-33) | Sausage, VP, or MAP bacon | Suppression of background microbiota | Andersen, 1995 |

Table 1.4 *(continued)*

| Competitor and/or bacteriocin used | Product | Summary | Reference |
|---|---|---|---|
| $Bac^+$ strains: *C. maltaromicus* LV17 or UAL26, *Lc. gelidum* 187-22, or *Lb. sake* Lb 706 at $10^2$ or $10^4$ CFU/$cm^2$ | Vacuum-packed and aerobically-stored high pH raw beef | Reliable growth and production of bacteriocin by *Lc. gelidum*; no sensory deterioration of the raw beef samples | Leisner *et al.*, 1995 |
| *Lb. alimentarius* or *Staphylococcus xylosus* commercial starter cultures | Sliced VP cooked ham | Shelf life extension of VP cooked ham stored at 4°C in the presence of *Lb. alimentarius* | Kotzekidou and Bloukas, 1996 |
| *Lc. gelidum* UAL187 ($bac^+$, wild type), UAL187-22 ($bac^+$, slow grower variant) or UAL187-13 ($bac^-$) | Vacuum-packed raw beef slices inoculated with spoilage organism, *Lb. sake* | Delayed spoilage of meat by sulfide-producing *Lb. sake* for up to 8 weeks at 2°C. Slow growing $bac^+$ variant or $bac^-$ variant did not delay spoilage | Leisner *et al.*, 1996 |
| *Lc. mesenteroides*, $nis^r$, *L. lactis* ($bac^+$) and its $bac^-$ variant | Model sauerkraut fermentation system | Successful use of a nisin-producing culture to control fermentation processes by *Lc. mesenteroides* | Harris *et al.*, 1992 |
| *Lb. plantarum* LCPO10 ($bac^+$) or 55-1 ($bac^-$ variant) | Spanish-style green olive fermentations | Bacteriocin production established LCPO10 strain as the dominant organism: useful as starter culture | Ruiz-Barba *et al.*, 1994 |
| Nisin or *L. lactis* ($bac^+$)/*L. lactis* ($bac^-$ variant) | Brined cabbage | Inhibition of background microbiota by nisin or by nisin-producer, allowing controlled cabbage fermentation by nisin-resistant *Lc. mesenteroides* | Breidt *et al.*, 1995 |

Table 1.4 *(continued)*

| Competitor and/or bacteriocin used | Product | Summary | Reference |
|---|---|---|---|
| Bac+ *Lb. casei* (2), *P. acidilactici*, or other *Pediococcus* spp. | Ready-to-use vegetables | Inoculated strains of *Lb. casei* reduced mesophilic population on vegetables, particularly coliforms and enterococci; acidity of salads increased slightly | Vescovo *et al.*, 1995 |
| Pediocin AcH, nisin, Nisaplin (nisin), MicroGard, or sodium lactate | Raw beef VP; inoculated with *Lc. mesenteroides* at $10^3$-$10^4$ CFU/g or with a naturally-occurring microbiota | Reduction in numbers of spoilage organisms on beef stored at 3°C for up to 8 weeks, with lactate being the most effective and MicroGard the least effective biopreservative. Immediate bactericidal effect of Nisaplin and nisin on *Lc. mesenteroides* | Rozbeh *et al.*, 1993 |
| Pediocin PO2 or nisin | Cooked sliced corned beef VP; inoculated with *Lb. curvatus* at $10^4$ CFU/cm$^2$ | Inhibition of *Lb. curvatus* in a model system, however, loss of pediocin activity and minimal nisin activity in cooked meat at 5 or 10°C | Coventry *et al.*, 1995 |
| Nisin Z, carnocin UI49, or bavaricin A | Brined shrimp | Shelf life extension by nisin and bavaricin A, but not as effective as a benzoate-sorbate solution | Einarsson and Lauzon, 1995 |
| Nisin (Ambicin), free or immobilized in a calcium alginate gel | Beef carcass tissues inoculated with *B. thermosphacta* | Reduced growth of spoilage organism, with the use of nisin. Inhibition was improved by the use of encapsulated nisin | Cutter and Siragusa, 1996a |

Table 1.4 *(continued)*

| Competitor and/or bacteriocin used | Product | Summary | Reference |
|---|---|---|---|
| Nisin (Ambicin) in spray water | UV-sterilized beef tissues inoculated with *B. thermosphacta* and VP | Inhibition of spoilage organism below detection limit: potential shelf life extension of fresh meat | Cutter and Siragusa, 1996b |
| Nisin | Precooked VP beef stored at 4 or 10°C for 21 or 70 days | Reduced spore outgrowth of *C. sporogenes* | Hague *et al.*, 1997 |

### 1.3.4 Biopreservation against pathogenic bacteria

The antagonistic activities of LAB and/or their bacteriocins against pathogenic organisms in foods are being investigated by several research groups. Bacteriocins of LAB are mainly active against gram-positive organisms and interest has focused mainly on two foodborne pathogens, *Li. monocytogenes* and *C. botulinum*.

Of the bacteriocins produced by LAB, nisin is the best characterized and has a long history of use in food. It is produced by *L. lactis*, and is a GRAS (generally recognized as safe) substance permitted in some countries for use in dairy products and some canned foods as an anti-clostridial agent (Delves-Broughton, 1990). With the exception of nisin, bacteriocins produced by different genera of lactic acid bacteria are still in the investigative stages and are not used as food additives in a pure form. In recent years, however, LAB fermentation products have been available on the market. Quest ALTA™ and PerLac™ (Quest International Canada Inc., PQ) are labeled as all-natural ingredients produced from cultured whey, dairy solids, or corn syrup solids. They are labeled as flavour enhancers and shelf life extenders in meat and poultry products. Although the information about these products is proprietary, it is believed that bacteriocin-producing LAB are used for the fermentation process. Jack *et al.* (1996) reported ALTA™ to contain pediocin, a byproduct of fermentation by *P. acidilactici.*

Use of LAB as protective cultures in RTE MAP chilled meats is not a novel idea. Riemann *et al.* (1972) recommended the addition of 1% glucose to semi-preserved meats, such as ham and bacon, as an added barrier to temperature abuse. If temperature increased, the indigenous flora would metabolize the glucose, lowering the product pH

below that which would support the growth of pathogens. Tanaka *et al.* (1980) refined this so-called "Wisconsin" process and demonstrated that addition of a fermentable carbohydrate source along with a lactic acid starter culture and 40 or 80 ppm sodium nitrite to bacon had better antibotulinal activity than 120 ppm nitrite without added sucrose or lactic acid bacteria (Tanaka *et al.* 1985b). Product pH dropped rapidly when the product was temperature abused. Sensory evaluation indicated that the Wisconsin process bacon was as acceptable as the control bacon, even after 6-8 weeks of refrigerated storage (Tanaka *et al.* 1985a). The FDA recommended that retail store production of VP refrigerated foods not be allowed unless antibotulinal barriers are present (Gombas, 1989). One of the barriers specified was the presence of high levels of non-pathogenic competing organisms (Gombas, 1989). To be effective, LAB should provide protection against pathogenicity under all conditions of temperature abuse, during all stages of product shelf life.

#### 1.3.4.1 *Carnobacterium piscicola*

Carnobacteria have not been studied as extensively as pediococci, *L. lactis* or lactobacilli, possibly because they are non-aciduric which may limit their functionality in fermented foods, and their bacteriocins do not have as broad a spectrum of activity as pediococci, nisin, or bacteriocins of lactobacilli. Buchanan and Klawitter (1992b) studied the effect of *C. piscicola* LK5, a raw ground beef isolate, on *Li. monocytogenes* in a variety of food products at 5 and 19°C. Inoculated foods included UHT milk, canned dog food (all-beef), raw ground beef, irradiation-sterilized raw ground beef, chicken roll, pasteurized

crab meat, canned creamed corn, and frankfurters. Growth inhibition was observed in several food systems, more effectively in products with minimal or no background microbiota and at the lower storage temperature. No growth of the pathogen was observed in non-sterile raw ground beef or irradiated raw ground beef kept at 5°C even in the absence of *C. piscicola*, while it readily proliferated in the cooked meat.

*C. piscicola* CP5 was isolated from Brie cheese and produces an antilisterial bacteriocin, carnocin CP5 (Mathieu *et al.*, 1993). Activity of the carnocin in crude or partially purified form, or of the producer organism against *Li. monocytogenes* was tested in skimmed milk at 4, 7, or 15°C. Addition of carnocin CP5 caused an initial decrease in numbers of *Li. monocytogenes*, followed by recovery at 7 or 15°C. Co-cultures of *C. piscicola* CP5 and *Li. monocytogenes* caused an initial reduction of the pathogen by 1 to 2-log units at 4 and 7°C, followed by growth inhibition for 21 days. No further reductions in numbers of *L. monocytogenes* were observed, although carnocin could be detected. At 15°C, *Li. monocytogenes* counts were reduced by over 6-log units after 21 days. Bactericidal and inhibitory effects were more extensive when tests were done in broth (Mathieu *et al.*, 1994).

Inhibitory effects of piscicolin 126, produced by a spoiled ham isolate, *C. piscicola* JG 126, were tested in a devilled ham paste food system. Growth of *Li. monocytogenes* ($10^3$ CFU/g inoculum) was inhibited for 14 days at 10°C. Nisaplin (nisin, Aplin and Barrett, UK) in the same system failed to inhibit *Li. monocytogenes*, while inhibition by ALTA™ 2341 was observed until day 7 (Jack *et al.*, 1996). Added to full cream milk, piscicolin 126 inhibited the growth of *Li. monocytogenes* at low inoculum levels ($10^2$ CFU/ml). At

higher inoculum levels ($10^4$ and $10^6$ CFU/ml), an initial drop in pathogen numbers was followed by regrowth within 24 h. In Camembert cheese with added piscicolin 126, counts of *Li. monocytogenes* were 3-4 log units lower than controls without piscicolin, however, initial inhibition was followed by regrowth, attributed to enzyme inactivation of the bacteriocin and emergence of a resistant *Li. monocytogenes* population (Wan *et al.*, 1997).

### 1.3.4.2 Lactobacilli

Successful inhibition of *Li. monocytogenes* by bacteriocin-producing *Lb. bavaricus* MN, a meat isolate, was observed in a model beef gravy system at 4 and 10°C (Winkowski and Montville, 1992). Inhibition was enhanced in the presence of a fermentable carbohydrate. When *L. lactis* 11454 ($Nisin^+$) or *P. pentosaceus* ($bac^+$) were co-inoculated with *Li. monocytogenes* in the gravy system, no inhibition was observed, although bacteriocins produced by these LAB were detected in the gravy. Winkowski *et al.* (1993) further tested the effects of $bac^+$ *Lb. bavaricus* MN ($10^3$ or $10^5$ CFU/g) on growth of *Li. monocytogenes* ($10^2$ CFU/g) in VP, minimally heat-treated beef systems (beef cubes, beef cubes with gravy, and beef cubes with gravy and added glucose). At 4°C, the pathogen was inhibited more effectively by the higher inoculum level of the antagonistic culture. Some inactivation of *Li. monocytogenes* was observed in the system with added glucose. Growth of the pathogen was not inhibited but delayed at 10°C, and more effectively with higher levels of *Lb. bavaricus* MN.

Schillinger and Lücke (1989a) screened several strains of LAB isolated from meat and meat products for antagonistic activity. They focused on *Lb. sake* Lb 706 which produces sakacin A, a bacteriocin with a narrow inhibitory spectrum, active against various LAB and *Li. monocytogenes*. The lag phase of *Li. monocytogenes* was extended to 6 days in pasteurized minced meat inoculated with *Lb. sake* Lb 706 ($bac^+$) compared with minced meat inoculated with a $bac^-$ variant of *Lb. sake* Lb 706 where no lag phase was observed (Schillinger *et al.*, 1991). The inhibition did not last beyond one week of storage at 8°C, suggesting inactivation of the bacteriocin. Exponential growth was delayed by 2 days in high pH (6.3) comminuted cured raw pork ('fresh mettwurst'), while the inactivation of the pathogen observed at normal pH (5.7) was more extensive in the presence of *Lb. sake* Lb 706, potentiating the hurdle effect. Loss of bacteriocin activity was observed after one week at 8°C in minced meat, possibly due to adherence to meat particles, enzyme activity, or uneven distribution in meat. Similar inactivation of pediocin was reported by Pucci *et al.* (1988) and of carnocin by Mathieu *et al.* (1993) in dairy products.

Comparable results were observed by Hugas *et al.* (1995) when using a $bac^+$ *Lb. sake* (CTC494) and a $bac^-$ *Lb. curvatus* (CTC371) during sausage fermentation. In a model sausage system, *Li. monocytogenes* was inactivated to a greater extent in the presence of the bacteriocin-producing strain. When tested as a starter culture during the manufacture of dry fermented sausages, *Lb. sake* CTC494 inhibited growth and achieved a greater reduction of viable cells of *L. innocua* compared to the $bac^-$ *Lb. curvatus*. Satisfactory results were obtained when sensory properties of the test sausages were investigated.

Addition of a bac$^+$ *Lb. plantarum* did not influence toxigenesis by *C. botulinum* in a model gravy system, compared to a bac$^-$ strain (Crandall and Montville, 1993). Both strains inhibited toxigenesis when a fermentable carbohydrate was added to gravy incubated at 15°C.

### 1.3.4.3 *Lactococcus lactis*

Nisin is a broad spectrum bacteriocin, active against several gram-positive bacteria and can prevent the cutgrowth of *C. botulinum* spores. The addition of nisin or its producer culture *L. lactis* have been investigated most often in cured VP meats or in dairy products (Tables 1.5 and 1.6). Due to its antibotulinal effectiveness, nisin was evaluated as an additive in cured and canned meats to replace or reduce use of nitrites (Rayman *et al.*, 1981, 1983; Taylor and Somers, 1985; Taylor *et al.*, 1985). Evidence for the efficacy of nisin in cured meat was not conclusive. More recently, addition of a nisin-producing *L. lactis* did not influence toxigenesis by *C. botulinum* in a model gravy system, compared to a bac$^-$ strain. Both strains inhibited toxigenesis only when a fermentable carbohydrate was added to gravy incubated at 15°C (Crandall and Montville, 1993).

Rogers and Montville (1994) have recently shown loss of nisin activity in high concentrations of proteins and phospholipids, and as storage temperature increases. Cutter and Siragusa (1994) indicated that nisin may be effective in spray washes as sanitizers for beef carcasses, while Mahadeo and Tatini (1994) reported that a decrease in numbers of *Li. monocytogenes* in the presence of nisin was more pronounced for cells suspended in scald water than cells attached to poultry skin. The preservative action of

nisin or bacteriocin-producing *L. lactis* against *Li. monocytogenes* has been tested in several dairy products (Tables 1.5 and 1.6).

Studies on the effect of nisin or *L. lactis* in RTE meat products has been minimal, possibly because of its reduced activity in protein foods. Addition of *L. lactis* 11454 to a gravy model system failed to inhibit growth of *Li. monocytogenes* (Winkowski and Montville, 1993). However, addition of nisin ($10^3$ and $10^4$ IU/ml) to cooked pork stored in air, 100% $CO_2$, or 80:20 $CO_2$:air, after the cooking step, caused a 2-log drop in numbers of *Li. monocytogenes*, originally inoculated at levels of $10^3$ CFU/g (Fang and Lin, 1994). The pathogen did not grow during 30 days of storage at 4°C, while numbers reached approximately $10^6$- $10^7$ CFU/g in nisin-free controls. In pork stored at 20°C for 10 days, similar inhibition was observed only in samples containing $10^4$ IU/ml of nisin. At the lower nisin concentration ($10^3$ IU/ml), *Li. monocytogenes* was growing actively by the second incubation day and reached levels 1.5-log cycles higher than nisin-free controls by day 10, in all storage environments. Residual nisin activity was minimal by the end of the storage period, and was lost more rapidly at 20°C. In raw beef meat, addition of nisin delayed growth of *Li. monocytogenes* by 2 weeks at 5°C (Chung *et al.*, 1989). Over 70% of measurable nisin activity was lost after 4 days at 5°C, while minimal activity was detected from meat stored at room temperature. Attempts to control growth of *Li. monocytogenes* on RTE cold-smoked salmon with nisin-producing *L. lactis* 11454 were not successful at 10°C (Wessels and Huss, 1996). The 'protective' culture was inhibited more extensively than the pathogen in this study. Numbers of *L. lactis* dropped from $10^6$ to $10^5$ CFU/g after 2 days at 10°C and did not grow appreciably by 22 days of storage. Inhibition of *L. lactis* may be due to the salt content of the product. Cooked

sliced meats (luncheon meat, ham, chicken breast) co-inoculated with a nisin-producing *L. lactis* and *Li. monocytogenes*, were VP or MAP (30:70 $CO_2$:$N_2$) and stored at 7°C (Beumer *et al.*, 1996). Minimal inhibition of the pathogen was observed in the presence of the nisin-producing culture.

The mesophilic nature of *L. lactis* may preclude its use in chilled meats. As well, nisin is most soluble and active at a lower pH. The pH of non-fermented meat products may not allow for optimal nisin activity. Use of this bacteriocin in dairy products or fermented meats with an acidic pH may be a more viable option. Another possibility might be the use of protein engineering to modify properties of nisin (Dodd *et al.*, 1992). Nisin Z is a naturally occurring variant of nisin, with one amino acid substitution (Mulders *et al.*, 1991), which seems to exhibit increased solubility at pH > 6.0 (De Vos *et al.*, 1993).

#### 1.3.4.4 Pediococci

Pediocins or bacteriocin-producing pediococci have been the most frequently tested systems for biological control in the past few years. Pediococci are used in fermented sausage manufacturing and although most studies have tested their effectiveness against *Li. monocytogenes* or *C. botulinum* in fermented meats, the inhibitory potential of pediococci or their bacteriocins has been investigated in several other food systems (Tables 1.5 and 1.6).

Use of pediocin in meat products to control the growth of *Li. monocytogenes* has been variably successful. Addition of pediocin AcH or *P. acidilactici* JBL1095 ($bac^+$) to wiener exudates held at 4 or at 25°C was effective in inhibiting growth of *Li.*

*monocytogenes* at 25°C (Yousef *et al.*, 1991). Pediocin AcH had an immediate listericidal effect at 4 and 25°C. Neither the pathogen nor the *Pediococcus* culture grew at 4°C. Numbers of *Li. monocytogenes* increased by 2 to 3-log units within the first 2 days in wiener exudates held at 25°C and co-inoculated with *P. acidilactici*. Thereafter, numbers dropped sharply, from $10^6$ CFU/ml to less than 10 CFU/ml. This delay likely reflects the time required for the competitor to grow and produce bacteriocin. No growth of either *Li. monocytogenes* or *P. acidilactici* (JBL1095, bac+ or LB42, bac-) was observed in co-inoculated or control samples of VP wieners at 4°C, and the pediococci did not produce either acid or bacteriocin at the lower storage temperature (Degnan *et al.*, 1992). In packages stored at 25°C, numbers of *Li. monocytogenes* decreased by 2.7-log units when co-inoculated with JBL1095 while the pediocin-negative strain had a listeriostatic effect (Degnan *et al.*, 1992).

Bacteriocin-producing strains of pediococci used in sausage fermentation trials achieved additional reductions in numbers of *Li. monocytogenes* compared to bacteriocin-negative isogenic variants (Foegeding *et al.*, 1992; Luchansky *et al.*, 1992; Baccus-Taylor *et al.*, 1993) or bacteriocin-negative strains (Berry *et al.*, 1990). Numbers of *Li. monocytogenes* did not decrease, however, growth of *Li. monocytogenes* was inhibited on vacuum-packed frankfurters co-inoculated with high levels ($10^7$ CFU/g) of *P. acidilactici* during storage at 4°C or 15°C (Berry *et al.*, 1990). Minimal or no differences were observed between the $bac^+$ culture and its $bac^-$ isogenic derivative.

Crude bacteriocin powder derived from *P. acidilactici* M inhibited growth of *Li. monocytogenes* for 16 days during kimchi (spiced cabbage) fermentation at 14°C (Choi and Beuchat, 1994).

Bacteriocin PA-1, in cell-free culture supernatants of *P. acidilactici*, exhibited an immediate bactericidal effect of up to 2-log units on *Li. monocytogenes* in fresh meats. The degree of inhibition was highly dependent on the bacteriocin concentration and *Listeria* inoculum levels. Storage at 5°C for 28 days did not inactivate the bacteriocin, although no further decreases in pathogen numbers were observed (Nielsen *et al.*, 1990). Skyttä *et al.* (1991) investigated the antibacterial activity of three bacteriocin-producing strains of *Pediococcus* spp. in minced meat inoculated with four organisms, *Y. enterocolitica*, *Li. monocytogenes*, *P. fragi*, and *P. fluorescens* at levels of $10^2$ CFU/g. Levels of pediococci ranged from $10^3$-$10^8$ CFU/g. Meat samples were stored at 15°C for 2 days, followed by storage at 6°C for 2 weeks. Inhibition of test organisms by the pediococci occurred at the higher inoculation levels. *P. damnosus* cell-free extracts had a significantly higher inhibitory potential against test organisms than viable *Pediococcus* cells. A possible explanation could be that the 2-day storage period at 15°C was too brief for pediococci to start bacteriocin production. Pediocin $PO_2$ reduced numbers of *Li. monocytogenes* by 0.6-log units after 48 hours of storage at 4°C (El-Khateib, 1993). Addition of pediocin to turkey slurries caused an initial 1.1-log units drop in viable counts and growth inhibition for 7 days at 4°C (Schlyter, 1993b). The pathogen grew actively by the second week of storage at 4°C. When held at 25°C, no inhibition was detected beyond the initial drop in numbers observed upon addition of the bacteriocin.

The effect of pediocin or pediococci has been investigated in dairy products with variable results (Pucci *et al.*, 1988; Motlagh *et al.*, 1992; Raccach and Geschell, 1993). Addition of pediocin PA-1 to cottage cheese, half-and-half cream, and cheese sauce could inhibit *Li. monocytogenes* (Pucci *et al.* 1988). However, resurgence of the pathogen was observed after 7 days of storage. Motlagh *et al.* (1992) reported an initial reduction in numbers of *Li. monocytogenes* in sterile ground beef, sausage mix, cottage cheese, ice cream, and reconstituted dry milk kept at 4 or 10°C. Their results suggest that pediocin AcH is bactericidal to *Listeria* spp., however, it will not control the growth of surviving *Listeria* cells by itself. Sensitivity to pediocin AcH was dependent on the strain of *Listeria*, initial pathogen level, and concentration of the bacteriocin.

ALTA™ 2341, a commercial shelf life extender produced by pediococcal fermentation of corn syrup solids had minimal effects on growth of *Li. monocytogenes* in turkey slurries stored at 25°C when added singly, however, it enhanced the inhibitory effects of sodium diacetate in combination treatments. Counts of *Li. monocytogenes* on blue crab meat, treated in a wash containing 20,000 AU/ml of ALTA 2341, decreased by 2 to 3-log units within 0.04 day at 4°C. Minimal recovery was observed after storage at 6°C for 6 days (Degnan *et al.*, 1994). Inhibitory effects of ALTA 2341 were minimal at 2,000 or 10,000 AU/ml.

Ability of a $bac^+$ *P. pentosaceus* strain or its $bac^-$ isogenic variant to inhibit *C. botulinum* ($10^2$, $10^4$, or $10^6$ CFU/ml) outgrowth and toxigenesis, was tested in a model gravy system incubated at 15, 25, or 35°C (Crandall and Montville, 1993). When 0.5% glucose was added to the media, no toxin was detected at all inoculum levels, regardless of the

pediococcal strain present. In the absence of glucose, the bacteriocin-producing culture inhibited toxigenesis at the lowest *C. botulinum* inoculum level ($10^2$ CFU/ml), however, the authors indicated that pH of the medium with the bac$^+$ strain was lower than pH of the gravy with the corresponding bac$^-$ strain. Inhibition cannot be conclusively attributed to bacteriocin production. These effects were observed at 15°C. Toxin was detected under all treatment conditions at 25 and 35°C. In a subsequent study, bac$^+$ *P. pentosaceus* did not inhibit *C. botulinum* toxigenesis in VP, sous-vide cooked beef with gravy stored at 4 and 10°C, even though the gravy contained glucose (Crandall *et al.*, 1994). The bac$^+$ and bac$^-$ strains of *P. pentosaceus* did not grow nor produce acid or bacteriocin in a similar sterile system, stored at 4 and 10°C (Crandall *et al.*, 1994). *P. acidilactici* was able to inhibit *C. botulinum* toxigenesis in temperature abused (15°C or higher) chicken salad formulated with dextrose (Hutton *et al.*, 1991). Inhibition was attributed to acidification (Wisconsin process). The pH of chicken salad stored at 10°C did not change after 14 days, indicating that *P. acidilactici* did not grow at that temperature in the salad, although it could grow weakly in MRS broth at 10°C (Hutton *et al.*, 1991).

### 1.3.4.5 Enterococcal bacteriocins or bac$^+$ enterococci

Recent reports (Parente and Hill, 1991; Ben Embarek *et al.*, 1994; Olasupo *et al.*, 1994; Torri Tarelli, 1994; Giraffa, 1995) of bacteriocin-producing enterococcal food isolates has prompted research into their inhibitory potential in food systems. To date, activity of enterococci or their bacteriocins in food systems has been restricted to milk. Parente and Hill (1992) observed reduced growth of *Li. monocytogenes* in enterocin-containing skim

milk held at 30°C. After 24 h incubation, numbers of *Li. monocytogenes* in test milk were approximately 1-log unit lower than the control. Inhibitory activity of enterocin 1146 was more effective in milk stored at 6°C. The potential of bac$^+$ *E. faecium* 7C5 to serve as a cheese starter adjunct was investigated by Giraffa *et al.* (1995). In milk co-inoculated with *L. innocua* and bac$^+$ *E. faecium* 7C5, numbers of *L. innocua* were reduced from $10^3$ CFU/ml to less than 10 CFU/ml within 25 h at 37°C. Potential uses of enterococcal bacteriocins in dairy products were reviewed by Giraffa (1995).

Sterilized blue crab, inoculated with *Li. monocytogenes*, was dipped in an enterocin 1083 wash and stored at 4°C for 6 days (Arihara *et al.*, 1993). Enterocin 1083 at the highest levels tested (10,000 or 20,000 AU/ml wash) caused an initial reduction of 2- to 3-log units in counts of *Li. monocytogenes* within an hour. Minimal recovery of the pathogen was observed after 6 days at 4°C (Degnan *et al.*, 1994).

Table 1.5 Use of bioprotective cultures to enhance safety of food products

| Competitor | Study summary | Reference |
|---|---|---|
| ***Listeria monocytogenes*** | | |
| *C. piscicola* LK5 ($bac^+$) | Co-inoculation of UHT milk, canned meat, raw ground beef and irradiated raw ground beef (sterile), chicken roll, pasteurized crabmeat, canned creamed corn, and frankfurters. Growth reduction in products where the background microbiota was suppressed. No activity of *C. piscicola* LK5 in non-sterile beef or in chicken roll. Inhibitory activity more effective at 5°C than at 19°C. | Buchanan and Klawitter, 1992a |
| *C. piscicola* CP5 ($bac^+$) at $10^7$ CFU/ml | Initial reduction in population of *Li. monocytogenes* in skimmed milk at 4, 7, or 15°C | Mathieu *et al.*, 1994 |
| *E. faecium* 7C5 ($bac^+$) | Complete inhibition of *L. innocua* in milk for soft cheese manufacture; observed when bacteriocin-producer and starter culture co-inoculated in milk; synergistic effect of pH decrease and bacteriocin production | Giraffa *et al.*, 1995 |
| *Lb. sake* Lb 706 at $10^6$ CFU/g, sakacin A-producer, or its $bac^-$ negative variant | Extension of lag phase by bacteriocin producer in sterile minced meat and in comminuted cured raw pork (pH 6.3). Growth inhibition in comminuted raw pork at pH 5.6-5.8. Loss of bacteriocin activity with time | Schillinger *et al.*, 1991 |
| *Lb. plantarum* MCS ($bac^+$), its $bac^-$ variant *Lb. plantarum* MCS1, or commercial starter cultures | No effect of LAB cultures on growth of pathogen in artificially-contaminated salami. Inhibition of *Li. monocytogenes* by $bac^+$ *Lb. plantarum* in naturally-contaminated salami | Campanini *et al.*, 1993 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| Bac$^+$ *L. lactis* 11454, *P. pentosaceus* 43200, or *Lb. bavaricus* MN at $10^5$ CFU/ml | Inhibitory activity by bac$^+$ *Lb. bavaricus* MN in a model meat gravy system at 4 and 10°C; enhanced by addition of glucose | Winkowski and Montville, 1992 |
| Bacteriocin-producing *Lb. bavaricus* MN at $10^3$ or $10^5$ CFU/g | Inhibition of pathogen in VP beef cubes, beef cubes in gravy, and beef cubes in gravy with 0.5% glucose. More effective at 4 than at 10°C, and at the higher inoculum levels of *Lb. bavaricus*. No loss of bacteriocin activity | Winkowski *et al.*, 1993 |
| FloraCarn L-2 (*Lb. alimentarius* BJ-33) at $10^7$ CFU/g | Reduced pathogen growth in VP sausages; Growth inhibition for 5 weeks, followed by lower counts in MAP bacon | Andersen, 1995 |
| *Lb. sake* CTC494 (bac$^+$) or *Lb. curvatus* CTC371 (bac$^-$) at $10^6$ CFU/g | Listericidal and listeriostatic effect of bac$^+$ *Lb. sake* in a model sausage systems and during dry fermented sausage manufacture | Hugas *et al.*, 1995 |
| *Lb. curvatus*, or nisin-producing *L. lactis* at 10-100 CFU/g | Minimal effect of the competitive microbiota on growth of *Li. monocytogenes* in vacuum or MAP sliced cooked roast beef at 7°C | Beumer *et al.*, 1996 |
| *Lb. casei*, *Lb. plantarum*, and *Pediococcus* spp. isolated from commercial salads, at $10^4$-$10^5$ CFU/g | Inhibition of pathogens in salads and salad juice; most effective by *Lb. casei* | Vescovo *et al.*, 1996 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| *Lb. casei* CRL 705 or lactocin 705 or *Lb. casei* CRL 686 (bac-) at $10^9$ CFU/ml | Inhibition of pathogen by addition of bacteriocin to sterile or non sterile meat slurries at 20°C. No inhibition observed with the producer strain | Vignolo *et al.*, 1996 |
| *L. lactis* ($bac^+$) produces nisin or its isogenic derivative ($bac^-$) | Initial decrease in numbers during the first two days of manufacture and ripening of brie cheese, followed by recovery, slower in the core (lower pH) than in the crust. More effective at lower pathogen inoculum levels | Maisnier-Patin *et al.*, 1992 |
| *L. lactis* ssp. *cremoris* or *L. lactis* ssp. *lactis* | Acid-induced inactivation of *Li. monocytogenes* and *S. aureus* enhanced by the presence of nisin. Delayed recovery of *C. sporogenes* in the presence of nisin | Zottola *et al.*, 1994 |
| *L. lactis* ATCC 11454, nisin-producer at $8x10^7$ CFU/g | Slight initial reduction of *Li. monocytogenes* population on slices of cold-smoked salmon at 10°C in the presence of *L. lactis*. Counts of *Li. monocytogenes* were lower than the control over a 3-week storage period. | Wessels and Huss, 1996 |
| *Leuconostoc* spp. (V6) or *Lb. plantarum* LKE5 at $10^6$ CFU/ml | Growth reduction or bactericidal effect observed in sterile shrimp extracts, dependent on inoculum level and competitive organism | Jeppesen and Huss, 1993 |
| *Pediococcus* starter cultures, bacteriocin producer JD1-23 and non-producer MP1-08 | 1-2 $\log_{10}$ reduction in numbers of *Li. monocytogenes* after 12 hours of sausage fermentation, observed with bacteriocin-producing starter culture | Berry *et al.*, 1990 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| Bacteriocin-producing *P. acidilactici* JD1-23 and its plasmid-cured derivative JD-M | Growth inhibition of *Li. monocytogenes* in processed frankfurters by the protective cultures at $10^7$ CFU/g but not at $10^3$-$10^4$ CFU/g. JD1-23 more effective | Berry *et al.*, 1991 |
| *P. acidilactici* H (JBL 1095) or pediocin AcH | Inhibition of the pathogen in wiener exudate at 4 and 25°C | Yousef *et al.*, 1991 |
| *P. acidilactici* JBL 1095 (produces pediocin) or *P. acidilactici* LB42 (nonproducer) | Listericidal effect by strain JBL 1095 and listeriostatic by strain LB42 in vacuum-packaged wieners at 25°C; No growth of pathogen or LAB at 4°C | Degnan *et al.*, 1992 |
| *P. acidilactici* ($bac^+$) or its isogenic ($bac^-$) derivative at $10^8$ CFU/g | Pediocin-producer enhanced inhibition of *Li. monocytogenes* during dry sausage manufacturing | Foegeding *et al.*, 1992 |
| *P. acidilactici* JBL1095 ($bac^+$) or JBL 1350 ($bac^-$) at $10^7$ CFU/g | Greater listericidal effect of $bac^+$ *P. acidilactici* during sausage manufacture. Pediocin activity recovered during storage for 60 days at 4°C | Luchansky *et al.*, 1992 |
| *P. acidilactici* commercial starter cultures, pediocin-producer and non-producer at $10^7$ CFU/g | Greater inhibition of the pathogen by the pediocin-producing culture | Baccus-Taylor *et al.*, 1993 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| *P. acidilactici* ($bac^+$ and $bac^-$ strains) and *P. pentosaceus* ($bac^+$ strains) | Reduction in pathogen numbers observed with all $bac^+$ strains of pediococci in sterile reconstituted milk stored at 32°C and at 4.5°C | Raccach and Geshell, 1993 |
| *E. faecalis*, *Lb. paracasei*, or *L. lactis strains*; all $bac^+$ | Inhibition observed in Camembert cheese when antagonistic culture is used as a starter culture in cheese manufacture, and in the presence of low pathogen numbers | Sulzer and Busse, 1991 |
| Background microbiota: reduced and not reduced by disinfection | Higher levels of *Li. monocytogenes* on fresh endive leaves with reduced background microbiota | Carlin *et al.*, 1996 |
| Pseudomonads, *Enterobacteriaceae*, or natural endive population | Slower growth of *Li. monocytogenes* in the presence of pseudomonads or *Enterobacteriaceae* on fresh endive leaves; Complete inhibition by natural population in sterile leaf exudates | Carlin *et al.*, 1996 |
| *Lb. sake* CTC494 ($bac^+$) or *Lb. curvatus* CTC371 ($bac^-$) at $10^6$ CFU/g | Listericidal and listeriostatic effect of $bac^+$ *Lb. sake* in a model sausage systems against *Li. monocytogenes* and during dry fermented sausage manufacture against *L. innocua* | Hugas *et al.*, 1995 |
| *Leuconostoc* spp. (V6) or *Lb. plantarum* LKE5 at $10^6$ CFU/ml | Growth reduction or bactericidal effect observed in sterile shrimp extracts, dependent on inoculum level and competitive organism | Jeppesen and Huss, 1993 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| ***Yersinia enterocolitica*** | | |
| *Lb. plantarum, P. pentosaceus,* or *P. acidilactici* | Reduced growth in the presence of lactic acid bacteria in a sausage-like product made with plain or cured meat; effect not due to pH | Raccach and Henningsen, 1984 |
| *Leuconostoc* spp. (V6) or *Lb. plantarum* LKE5 at $10^6$ CFU/ml | Growth reduction or bactericidal effect observed in sterile shrimp extracts, dependent on inoculum level and competitive organism | Jeppesen and Huss, 1993 |
| Normal background microbiota | Growth reduction in the presence of competitive microbiota at 4, 10, and 15°C in raw, minced ground beef | Kleinlein and Unterman, 1990 |
| Commercial starter cultures containing *Lb. pentosus, Lb. plantarum,* or *P. acidilactici* | Bactericidal effect in the presence of *Lb. pentosus* and *Lb. plantarum*; Effect likely due to decrease in pH of fermented sausages | Asplund *et al.*, 1993 |
| *Lb. casei, Lb. plantarum,* and *Pediococcus* spp. isolated from commercial salads, at $10^4$-$10^5$ CFU/g | *A. hydrophila, L. monocytogenes, S. typhimurium,* or S. aureus at $10^4$-$10^5$ CFU/g. Inhibition of pathogens in salads and salad juice; most effective by *Lb. casei* | Vescovo *et al.*, 1996 |
| ***C. botulinum*** | | |
| *P. acidilactici* | Prevention of toxigenesis in chicken salad in the presence of a LAB and dextrose. Inhibitory effect due to pH reduction | Hutton *et al.*, 1991 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| ***C. botulinum*** | | |
| *P. pentosaceus* ATCC 43200 (bac$^+$)or its isogenic derivative, 43NP1 (bac$^-$) at $10^4$ CFU/g | No inhibition of toxigenesis by *P. pentosaceus* in minimally heat-treated VP beef with gravy, stored at 4 or 10°C | Crandall *et al.*, 1994 |
| *Lb. plantarum* at $10^6$ CFU/g | Inhibition of toxigenesis by *Lb. plantarum* in VP bacon containing sucrose and stored at 27°C. Inhibition due acid production | Tanaka *et al.*, 1980 |
| *P. acidilactici* $PO_2$ at $10^7$ CFU/g | Inhibition of toxigenesis in the presence of sucrose, in VP bacon stored at 27°C | Tanaka *et al.*, 1985 |
| *L. lactis* 11454, *P. pentosaceus* ATCC 43200, *Lb. plantarum* BN at $10^4$ CFU/ml | Inhibition of toxigenesis in a model gravy system due to acid production in the presence of glucose. Effective at 15°C but not at 25 or 35°C; bacteriocin production had little or no inhibitory effect | Crandall and Montville, 1993 |
| Various | | |
| *L. lactis* ssp. *cremoris* or *L. lactis* ssp. *lactis* | *Li. monocytogenes*, or *S. aureus* in cheese<br>Acid-induced inactivation of *Li. monocytogenes* and *S. aureus* enhanced by the presence of nisin. | Zottola *et al.*, 1994 |
| *P. damnosus* or *P. pentosaceus* | *C. botulinum*, *Li. monocytogenes*, *S. infantis*, and *Y. enterocolitica*<br>Reduced growth of pathogens at 6 or 15°C. No differentiation between pH effect or proteinaceous agents | Mattila-Sandholm *et al.*, 1991 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| Various | | |
| Bac+ *P. pentosaceus* or *P. damnosus* at $10^3$-$10^8$ CFU/g or cell-free culture extracts | *Y. enterocolitica*, *Li. monocytogenes*, *P. fragi*, or *P. fluorescens*<br>Inhibition of pathogens and spoilage organisms in minced meat at the higher inoculum levels of pediococci only. Cell-free crude extracts of pediococci showed more inhibitory potential than live producer cells. | Skÿtta *et al.*, 1991 |
| Normal background microbiota, or lactobacilli | *S. aureus*, *B. cereus*, *S. typhimurium*, *S. enteritidis* or *Y. enterocolitica* on VP sausages.<br>Competitive microbiota affected growth of pathogens to different degrees; inhibitory activity more effective at lower temperatures | Nielsen and Zeuthen, 1985 |
| Normal background microbiota, or lactobacilli | *C. perfringens* in VP frankfurters.<br>Growth was negatively influence by the competitive organisms. | Nielsen and Zeuthen, 1985 |
| Competitive microbiota, mostly LAB: $10^3$ CFU/g on 'new' ham and $10^7$-$10^8$ CFU/g on 'old' ham | *B. cereus*, *C. perfringens*, *E. coli*, *S. typhimurium*, *S. aureus* on sliced ham, VP and held at 4°C for 24 h ('new') or 30°C ('old') prior to inoculation. Growth of all pathogens except *C. perfringens* on 'new' ham under severe temperature abuse (21 or 30°C); survival but no growth on 'old' ham. | Steele and Stiles, 1981 |
| Minced meat with a natural spoilage microbiota or inoculated with different spoilage organisms. | *S. aureus*, *B. cereus*, *Li. monocytogenes*, *Y. enterocolitica*, and *S. infantis* in naturally contaminated meat samples<br>Inhibition of pathogens by background microbiota, and by *Lactobacillus* and *Pseudomonas* spp. *P. damnosus* inhibited growth of salmonellae | Mattila-Sandholm and Skÿtta, 1991 |

Table 1.5 *(continued)*

| Competitor | Study summary | Reference |
|---|---|---|
| Various | | |
| Pseudomonads, lactobacilli, *P. damnosus*, *B. thermosphacta* | As above, however, in irradiated minced beef; Inhibition of pathogens by pseudomonads and lactobacilli; inhibition of salmonellae by *P. damnosus* | Mattila-Sandholm and Skÿtta, 1991 |

Table 1.6 Bacteriocin-mediated biopreservation of food products against foodborne pathogens

| Competitor and/or bacteriocin used | Results summary | Reference |
|---|---|---|
| ALTA 2341 and/or sodium diacetate | ALTA alone not effective; however, enhances inhibitory action of sodium diacetate on *Li. monocytogenes* in turkey slurries | Schlyter *et al.*, 1993a |
| ALTA 2341, enterocin 1083, MicroGard, PerLac 1911, or Nisin (also acetate, diacetate and TSP) | ALTA, enterocin, and Nisin decreased *Li. monocytogenes* numbers initially, in blue crab washed with LAB fermentation products and stored at 4°C. No effects thereafter | Degnan *et al.*, 1994 |
| Carnocin CP5 | Initial reduction in population of *Li. monocytogenes* in skimmed milk at 4, 7, or 15°C | Mathieu *et al.*, 1994 |
| Enterocin 1146 | Initial drop in numbers of *Li. monocytogenes*, followed by recovery, at levels slightly lower than controls in milk | Parente and Hill, 1992 |
| Bacteriocins of *Lb. sake* Lb 706 or *P. acidilactici* | Growth of *Li. monocytogenes* in kimchi inhibited by bacteriocin from *P. acidilactici* only. Initial drop in pathogen population observed | Choi and Beuchat, 1994 |
| *Lb.. casei* CRL 705 or lactocin 705 or L. casei CRL 686 (bac-) at $10^9$ CFU/ml | Inhibition of *Li. monocytogenes by* addition of bacteriocin to sterile or non sterile meat slurries at 20°C. No inhibition observed with the producer strain | Vignolo *et al.*, 1996 |
| Nisin (Nisaplin) | Bacteriocin did not prevent outgrowth of *C. botulinum* spores in cured pork slurry model system at pH 5.8 | Rayman *et al.*, 1983 |
| Nisin (Nisaplin) | Delayed *C. botulinum toxin* formation by 1-wk at 27°C in the presence of high levels of nisin (150 ppm), in bacon | Taylor and Somers, 1985 |

Table 1.6 *(continued)*

| Competitor and/or bacteriocin used | Results summary | Reference |
|---|---|---|
| Nisin | Shelf life extension and delayed *C. botulinum* toxin formation in chicken frankfurter emulsions; lower levels of nitrites required | Taylor *et al.*, 1985 |
| Nisin | *Li. monocytogenes* not recovered from nisin-containing cottage cheese, even in high inoculum numbers | Benkerroum and Sandine, 1988 |
| Nisin (Nisaplin) | Initial decrease in numbers of gram-positive bacteria (*Li. monocytogenes*, *S aureus*, *S. marcescens*, *S. typhimurium*, *P. aeruginosa*) attached to fresh beef at 5°C or room temperature, followed by recovery. Loss of nisin activity on meat, more pronounced at room temperature | Chung *et al.*, 1989 |
| Nisin | Inhibition of *Li. monocytogenes* more effective in skim milk. Presence of fat (in half-and-half) reduced activity of nisin | Jung *et al.*, 1992 |
| Nisin (commercial) or pediocin $PO_2$ (in sterilized culture supernatant fluid) | Immediate listericidal action upon exposure; no delayed inhibition. Nisin more effective than pediocin on beef muscle | El-Khateib *et al.*, 1993 |
| Nisin | Initial reduction of *Li. monocytogenes* numbers and inhibition of growth during storage at 4°C on cooked pork. Less effective at 20°C. Loss of nisin activity on pork with time | Fang and Lin, 1994 |
| Nisin (Nisaplin) | Listericidal effect upon application; nisin more effective against *Li. monocytogenes* cells in scald water than on turkey skin | Mahadeo and Tatini, 1994 |

Table 1.6 *(continued)*

| Competitor and/or bacteriocin used | Results summary | Reference |
|---|---|---|
| Heat-treated, bac$^+$ *L. lactis* culture suspensions | Initial decrease in numbers of *Li. monocytogenes* followed by recovery at 5°C in Mozzarella but not in skim milk. | Stecchini *et al.*, 1995 |
| Nisin (in spray water) | Initial reduction in *L. innocua* numbers on sterile beef carcasses, VP, followed by growth. Population numbers lower than untreated control | Cutter and Siragusa, 1996a |
| Nisin, Nisaplin | Decrease in numbers of nisin-resistant *Li. monocytogenes* more rapid in the presence of nisin at 20°C. No evidence of loss of activity in long-life cottage cheese | Ferreira and Lund, 1996 |
| Nisin (in combination with sorbate) | Initial decrease in numbers of *Li. monocytogenes* on 100% $CO_2$-packed beef. Minimal growth observed after 4 weeks at 4°C likely not due to preservatives since pathogen numbers were very close on treated and untreated beef. | Avery and Buncic, 1997 |
| Nisin (Nisaplin) | Immediate listericidal effect, followed by listeriostatic effect in ricotta-type cheese for 8 or more weeks at 6-8°C at pH 5.8 and with higher levels of nisin. Inhibition is reduced at pH 6.0 and lower nisin levels. Retention of nisin activity (10-32% loss) | Davies *et al.*, 1997 |
| Nisin (with and without other preservatives) | Synergistic effect of nisin and sodium benzoate at 4°C against *S. aureus* or *B. cereus* in sterile, VP vegetarian food. Loss of 65-75% of nisin activity after 28 days at 4 or 30°C | Fang and Chen, 1997 |
| Pediocin PA-1 | Initial decrease in *Li. monocytogenes* numbers at 4 and 30°C in cottage cheese, cream or cheese sauce, followed by recovery in non-acidic food systems | Pucci *et al.*, 1988 |

Table 1.6 *(continued)*

| Competitor and/or bacteriocin used | Results summary | Reference |
|---|---|---|
| Bacteriocin produced by *P. acidilactici* LACTACEL 110 | Inhibition of the *Li. monocytogenes* by the bacteriocin on fresh beef is dependent on initial pathogen inoculum and bacteriocin concentration. The bacteriocin was not inactivated by the meat at 5°C | Nielsen *et al.*, 1990 |
| *P. acidilactici* H (JBL 1095) or pediocin AcH | Inhibition of *Li. monocytogenes* in wiener exudate at 4 and 25°C | Yousef *et al.*, 1991 |
| Pediocin AcH | Initial reduction in population of *Li. monocytogenes* in ground beef or milk treated with pediocin AcH and stored at 4 or 10°C | Motlagh *et al.*, 1992 |
| Pediocin AcH, free with or without an emulsifier, or encapsulated | Initial reduction in the population of *Li. monocytogenes* in dairy and meat products, within the first 2 minutes. Loss of activity of free pediocin reduced by presence of an emulsifier and by encapsulation. | Degnan *et al.*, 1993 |
| Pediocin (alone or in combination with other antimicrobials) | Initial decrease of *Li. monocytogenes* in pasteurized turkey slurries, followed by recovery at 4 and at 25°C | Schlyter *et al.*, 1993b |
| Pediocin AcH bound to heat-killed *producer P. acidilactici* H | Inhibition of *Li. monocytogenes* in raw chicken meat at 5°C and slower growth in cooked meat at 8°C | Goff *et al.*, 1996 |
| Piscicolin 126, compared to ALTA 2341 or nisin (Nisaplin) | Inhibition of *Li. monocytogenes* in sterile devilled ham paste for up to 14 days at 10°C by piscicolin; nisin least effective for extended storage | Jack *et al.*, 1996 |

Table 1.6 *(continued)*

| Competitor and/or bacteriocin used | Results summary | Reference |
|---|---|---|
| Piscicolin 126, produced by *C. piscicola* JG126 | Inhibition of *Li. monocytogenes* by piscicolin at lowest inoculum level in milk. Initial decrease followed by recovery of pathogen at the two higher inoculum levels. Growth reduction in Camembert cheese made from the inoculated milk. Inactivation of bacteriocin in cheese over storage period | Wan *et al.*, 1997 |

### 1.3.4.6 Effect of a background microbiota in biopreservation

The effects of background microbiota on growth of pathogenic organisms in food have not been investigated extensively, due to the logistic problems involved in obtaining a sterile, natural food system, and a similar contaminated system without altering the nature of the food by steaming, autoclaving or other harsh treatment. Consequently, the inhibitory activity, or lack thereof, of a natural microbiota on pathogens can seldom be evaluated by itself.

Kleinlein and Unterman (1990) found that growth of *Y. enterocolitica* was delayed in raw beef with a high background microbiota ($10^5$ CFU/g) compared to beef with low counts ($10^2$ CFU/g). Presence of a high population on 'old' ham ($10^7$-$10^8$ CFU/g) inhibited growth of *B. cereus*, *C. perfringens*, *E. coli*, *S. typhimurium*, and *S. aureus* (Steele and Stiles, 1981). All pathogens grew on 'new' ($10^3$ CFU/g) ham at 21 or 30°C. No growth was observed at 4°C for 24 h. *B. cereus*. *S. aureus*, *Li. monocytogenes*, *Y. enterocolitica*, and *S. infantis* were inhibited at 6°C by the background microbiota naturally present in minced meat or by inoculated spoilage organisms (Mattila-Sandholm, 1991a). In another study, growth of *B. cereus*, *S. aureus*, *S. typhimurium*, *S. enteritidis*, or *Y. enterocolitica* was affected to different degrees by the presence of a normal background microbiota on VP sausages (Nielsen and Zeuthen, 1985). *B. cereus* and gram-negative pathogens were more severely affected by the background population. *C. perfringens* did not grow in VP franks with a background microbiota (Nielsen and Zeuthen, 1985). A reduced background microbiota on fresh endive leaves was associated with higher numbers of *Li. monocytogenes* (Carlin *et al.*, 1996).

### 1.3.5 Factors affecting activity of bacteriocin or bac$^+$ cultures

When reviewing the application of bacteriocin-producing LAB as natural preservatives in foods, several factors have to be taken into consideration. The previous sections give an overview of the applications of bacteriocins or producer cultures in different food matrices. Most review articles stress that protective cultures or bacteriocins may be most useful as an additional hurdle in minimally processed foods; however, very few studies have focused on the use of biopreservation of RTE, minimally preserved, MAP chilled foods. These include the work by Winkowski *et al.* (1993), Crandall *et al.* (1994), Fang and Lin (1994), and Beumer *et al.* (1996). Fermented or cured meat products have gathered more attention, although these have built-in hurdles (spices, curing mixture, fermentation step) that are absent in minimally preserved meat products.

A protective culture has to be selected carefully for use as a biopreservative. One of the most important factors should be its ability to grow at refrigerated temperatures. Originally, the Wisconsin process was based on the addition of a fermentable carbohydrate and a mesophilic starter culture to MAP chilled products. In the event of temperature abuse, the starter would grow and metabolize the carbohydrate, causing acidification of the product. With the emergence of pathogens able to grow at refrigeration temperatures, this concept needs to be reformulated. The need for a protective culture that grows at a faster rate than a pathogen during refrigerated storage would preclude use of *L. lactis* or pediococci in RTE chilled foods. Carnobacteria, lactobacilli, and leuconostocs may be a better option, although some leuconostocs produce extracellular polysaccharides in the presence of fermentable carbohydrates, while

some lactobacilli may produce hydrogen sulfide and cause greening in VP and $CO_2$-packed meats (Egan and Shay, 1982; Schillinger and Lücke, 1987b; Egan *et al.*, 1989; Borch and Agerhem, 1992; McMullen and Stiles, 1996).

When LAB are tested as protective cultures in food products, seldom is a sensory evaluation study carried out in parallel to evaluate the effect of the added culture on the sensory properties of the food.

Rapid inactivation of bacteriocins has often been reported upon addition to a food matrix, most often in meat products (Bell and De Lacy, 1986; Chung *et al.*, 1989; Schillinger and Lücke, 1991; Nielsen *et al.*, 1990; Jung *et al.*, 1992; Fang and Lin, 1994; Fang and Chen, 1997). Degnan *et al.* (1992), monitored the activity of pediocin AcH in meat slurries of beef tallow or beef muscle. Pediocin was added either in free form or liposome-encapsulated. Approximately 40-76% of pediocin activity was lost within 1.5 min of addition. More activity was recovered from foodborne components with the least amount of proteins and from heated slurries. When pediocin was encapsulated within liposomes, significantly greater recovery of pediocin from meat components was achieved. In a subsequent study, Degnan *et al.* (1993) evaluated the antilisterial activity of pediocin AcH in different food system slurries, either free in the presence of an emulsifier, or encapsulated within liposomes. Greater activity was recovered from dairy-based and from diluted slurries than meat-based slurries. Greater pediocin activity was recovered from slurries containing encapsulated pediocin AcH or an emulsifier. Cutter and Siragusa (1996b) compared the effect of free nisin and nisin immobilized in a calcium alginate gel on the reduction of *B. thermosphacta* on beef surfaces. Immobilized nisin

achieved greater reductions in numbers of *B. thermosphacta*, and activity was recovered for a longer period of time.

Other factors to be considered in the use of bacteriocins or producer cultures in foods are the emergence of resistant populations, uneven distribution in a solid meat system, insufficient quantity of bacteriocin being added or produced to achieve complete inhibition, and increased effectiveness of bacteriocins when low inoculum levels of the target organism are present. Effectively, in most systems where a bacteriocin or a producer culture is added, a sharp initial decrease in numbers of the target pathogens occurs, most often followed by recovery or by growth inhibition. When pathogen inoculum levels are high, regrowth of the pathogen often follows. If pathogen inoculum levels are low (less than $10^3$), growth is seldom observed before the end of the storage period.

Loss of bacteriocin activity could be caused by production of proteolytic enzymes in the system. Addition of the producer culture to a meat system may be the best option to get continuous production of bacteriocin, however, the onset of bacteriocin synthesis is critical. Some bacteriocins are produced early in the growth cycle, while others are produced during the stationary phase. Numbers of *Li. monocytogenes* increased by 2-log units in wiener exudates held at 25°C during the first two days of storage in the presence of a bacteriocin-producing *P. acidilactici* (Yousef *et al.*, 1991). Thereafter, numbers of the pathogen decreased dramatically. That 2-day period during which *Li. monocytogenes* was multiplying actively before *P. acidilactici* exerted an inhibitory influence, probably

reflects the time required for the producer strain to grow and produce sufficient amounts of bacteriocin.

Hanlin *et al.* (1993) suggested adding bacteriocins in combination may have a greater antibacterial effectiveness, particularly if the bacteriocins added have different mechanisms of actions. Emergence of resistant variants of the pathogens could be minimized considerably.

Previous work indicates that use of biopreservation to enhance safety of RTE MAP foods has good potential keeping in mind, however, that protective cultures or bacteriocins may be useful as an added barrier, and not as a complete preservation system. Extensive research is needed to identify the different organisms that may be appropriate in different food systems. The mechanism by which bacteriocins exert their effects on target organisms is still not fully understood. Clarifying the mechanism of action maybe the single most important step in improving the activity of bacteriocins. Molecular techniques and protein engineering, already initiated with nisin, probably spell the future in bacteriocin research, for the production of antimicrobials with increased effectiveness or lethality against pathogenic bacteria.

### 1.3.6 Other 'natural' preservation methods

#### 1.3.6.1 Lactates

Sodium lactate is the sodium salt of L(+) lactic acid which occurs naturally in living muscle tissues. Sodium lactate has a neutral pH, is a GRAS substance and is used in meat and poultry products as a humectant, flavour enhancer, and antimicrobial agent.

Lactates increase the cooking yield of meat products by improving the water-holding capacity. Sensory evaluation of cooked beef top rounds treated with sodium lactate indicated that test samples had a stronger beefy, meaty flavour than control samples (Papadopoulos *et al.*, 1991).

Inhibitory activity is attributed to a decrease in water activity, and to the action of the lactate ion. The antimicrobial properties of lactates were reviewed by Shelef (1994). Sodium lactate has been used to extend the shelf life of cooked (Duxbury, 1990) and fresh meat products (Lamkey *et al.*, 1991), and control the growth of various pathogens on cooked meat products (Maas *et al.*, 1989; Shelef and Yang, 1991; Stillmunkes *et al.*, 1993; Weaver and Shelef, 1993). Wederquist *et al.* (1994), found a 2% solution of lactate to delay growth of *Li. monocytogenes* on vacuum-packed refrigerated turkey bologna. Growth of the pathogen was suppressed for 28 days on vacuum-packed bologna-type sausages stored at 5°C (Qvist *et al.*, 1994). Rapid growth of the pathogen occurred in samples stored at 10°C. Minimal reductions in counts of *Li. monocytogenes* were achieved in blue crab meat washed with a 1M solution of sodium lactate (Degnan *et al.*, 1994), however, Na-lactate exhibited a synergistic effect when combined with sodium diacetate, in delaying the growth of *Li. monocytogenes* in turkey slurries (Schlyter *et al.*, 1993b). Addition of lactate to sous-vide chicken, beef or salmon delayed toxigenesis of proteolytic and non-proteolytic spores of *C. botulinum* (Meng and Genigeorgis, 1994).

Lactates are considered to be good antibacterial agents since their antimicrobial activity is not restricted to gram-positive pathogens, but affects gram-negative organisms as well

(Miller and Acuff, 1994; Shelef and Potluri, 1995). They appear to work best when combined with another hurdle system (Buncic *et al.*, 1995).

### 1.3.6.2 Hazard analysis critical control points (HACCP)

To improve the microbiological safety of RTE chilled food products, a HACCP approach is recommended through all stages of processing, production, storage, and distribution (Agriculture Canada, 1990b). A practical application of a HACCP system applied to a sous-vide processed meat and pasta product was given by Smith *et al.* (1990). The Meat and Poultry Working Group of the National Advisory Committee on the Microbiological Criteria for Foods defined nine general process types and categories for refrigerated prepared foods and recommended the use of HACCP for proper process control, proper labeling for refrigerated products, and the use of time/temperature indicators (TTI) wherever feasible (Adams, 1991).

### 1.3.6.3 Predictive microbiology

Challenge and accelerated storage tests have been traditionally used to assess safety of foods and of MAP products (Hotchkiss, 1988). These are the most common approaches to studying the effects of different variables on the behavior of spoilage and pathogenic organisms, through inoculation of microbiological media or a food matrix with a particular organism, and following survival over time under storage conditions thought to be representative of those encountered during distribution. The intrinsic and extrinsic variables governing growth are defined, and maximum and minimum limits permitting

growth are established. One or more of those parameters could be varied at one time. The data collected from such experiments allow the food microbiologist to make a certain statement regarding the safety and stability of a particular food product. Such studies are of limited value since any change in the formulation, packaging, storage, or use of the product would necessitate a new set of tests. They often do not take into consideration the interactive effects exerted by different intrinsic and extrinsic parameters on the behaviour of microorganism in foods. In recent years, research groups in the United States (at the USDA), the UK (various research institutes coordinated by the Ministry of Agriculture, Fisheries and Food), and Australia (University of Tasmania) have focused on the use of mathematical models to predict growth responses of microorganisms to one or more variables. Knowledge of how intrinsic and extrinsic factors, individually and in combination, influence the rate of growth of specific organisms, should allow food scientists to forecast the behaviour of the organism in a food matrix.

## 1.4 IDENTIFICATION OF LACTIC ACID BACTERIA

Due to their considerable importance in food fermentations and their antagonistic properties, the identification of LAB has elicited a lot of attention. The taxonomy of LAB associated with foods was reviewed by Stiles and Holzapfel (1997). Classification by traditional identification schemes based on morphological and physiological features is a tedious and time-consuming process. As well, morphologies of LAB may be misleading to the inexperienced microbiologist, with lactococci often appearing as elongated cells which might lead to incorrect classification as rods. In the past decade,

the use of molecular techniques in taxonomy has resulted in major changes in the classification schemes of LAB with several new species characterized, new genera created and several rapid methods of identification being proposed and investigated. Streptococci were originally split into two serological groups, N and D. Nucleic acid hybridization of 23S rRNA indicated that serological group N streptococci, the lactic streptococci, fell into one rRNA homology cluster while the serological group D streptococci were divided in two different rRNA homology clusters (presently, the enterococci and the streptococci) (Kilpper-Bälz *et al.*, 1982). The serological group N streptococci were soon transferred to a new genus, *Lactococcus* (Schleifer *et al.*, 1985), and *S. faecium* and *S. faecalis* (formerly group D streptococci) were transferred to the genus *Enterococcus* (Schleifer and Kilpper-Bälz, 1984). The basis for the classification of lactococci, enterococci and streptococci were later reviewed by Schleifer and Kilpper-Bälz (1987). Other changes took place in the genera *Lactobacillus*, and atypical strains *Lb. divergens* and *Lb. piscicola*, isolated from poultry, were classified into a new genus: *Carnobacterium* (Collins *et al.*, 1987).

Cellular fatty acids profiles have been, and are, a valuable taxonomic key in the classification of LAB (Decallone *et al.*, 1991) and were used by Dainty *et al.* (1984), Shaw and Harding (1989), and Dykes *et al.* (1995) to cluster strains of lactic acid bacteria isolated from vacuum-packaged meats. Recently, databases and libraries have been constructed since the development of the automated Microbial Identification System (MIS; Microbial ID Inc., Newark, Del.). This system has become a tool for automated identification of bacteria, however, it is only as good as its databases. The MIS system and automated identification systems based on carbohydrate fermentation patterns are

mainly restricted to species of bacteria of medical importance. This trend is changing as the importance of 'innocuous' bacteria is becoming more obvious due to their role in spoilage, fermentation and biological control in foods, and their use for biodegradation and environmental bioremediation for example.

Lactococci, particularly strains of *L. lactis*, are one of the best known groups of LAB due to their importance as starter cultures in fermentation and ripening of dairy products. Due to their extensive use in the dairy industry, reliable identification of this class of organisms is important. Pulsed-field gel electrophoresis was used to evaluate genome size and compare restriction patterns of *L. lactis*, *E. faecalis*, *S. salivarius*, and *S. sanguis* (Le Bourgeois *et al.*, 1989). Köhler *et al.* (1991) used rRNA gene restriction patterns and chemiluminescent labeling and detection methods to differentiate strains and subspecies of *L. lactis*. Chromosome mapping of LAB was recently reviewed by Le Bourgeois *et al.* (1993). Closely-related strains of *L. lactis* had almost identical genomic fingerprints (Cancilla *et al.*, 1992).

Use of the polymerase chain reaction (PCR) and 16S or 23S rRNA-targeted oligonucleotide probes may be the most popular and reliable method for the identification of food-related LAB. Two oligonucleotide probes, one genus-specific for lactococci, and another subspecies-specific for *L. lactis* ssp. *cremoris* were designed and used in hybridization experiments by Salama *et al.* (1991). Klijn *et al.* (1991; 1995) used PCR to amplify variable regions of the 16S rRNA of lactococci and leuconostocs, followed by hybridization of labeled species-specific probes to the PCR products. Betzl *et al.* (1990), in a more direct approach, used 23S rRNA probes for identification of *L. lactis* strains

and several *Enterococcus* species in dot-blot and colony hybridization experiments. *L. lactis*, enterococci, and streptococci were identified by whole cell colony hybridization using labeled rRNA-targeted probes and epifluorescent microscopy (Beimfohr *et al.*, 1993). This last technique required pretreatment of gram-positive cells to achieve optimal probe permeability.

A similar approach to Klijn *et al.* (1991) was followed for the identification of *Carnobacterium* spp. (Brooks *et al*, 1992) and *B. thermosphacta* (Grant *et al.*, 1993). Nissen *et al.* (1994) used 16S rRNA genus-specific probes for carnobacteria and leuconostocs. Numerical analysis of total soluble cell protein patterns was more reliable than conventional techniques for the differentiation of *C. piscicola* and *C. divergens* (Dicks *et al.*, 1995).

Ribotyping, randomly amplified polymorphic DNA and pulsed-field gel electrophoresis patterns were used for the identification of *Lb. sake* strains associated with the spoilage of VP cooked meat products (Björkroth and Korkeala, 1996a; 1996b; 1996c). In a subsequent study, rRNA gene restriction patterns were used as a tool to evaluate LAB contamination patterns of a VP, cooked sliced meat product during all production in a meat processing plant, from raw mass to the slicing-packaging step (Björkroth and Korkeala, 1997). Ehrmann *et al.* (1994) used a reverse dot blot hybridization assay for the direct identification of LAB in fermented foods. Oligonucleotide probes for 16 different species of lactobacilli, one *L. lactis* and one *S. thermophilus* probes were used as membrane-bound capture probes.

Ahrné *et al.* (1989) studied the plasmid profiles of lactobacilli isolated from meat products, while Hertel *et al.* (1993) identified lactobacilli occurring in fermented milk products using oligonucleotide probes and electrophoretic protein profiles. Nissen and Dainty (1995) found rRNA probe-based identification of *Lb. sake* and *Lb. curvatus* to be more reliable than conventional methods. The identification of lactobacilli and bifidobacteria by nucleic acids-based techniques was recently reviewed by Charteris *et al,* (1997). Tilsala-Timisjärvi and Alatossava (1997) reported the identification of several *Lactobacillus* spp. and *S. thermophilus* using PCR primers targeted to the 16S-23S rRNA intergenic sequence.

Although different molecular-based techniques have been developed for the identification of LAB from foods, they have not displaced conventional methods used in most food laboratories. These rapid techniques require an investment in terms of equipment and material, and the maintenance of oligonucleotide libraries. Additionally, methods developed in one laboratory, may have to be 'adapted' slightly to give satisfactory results in another laboratory. Of the techniques developed, PCR and oligonucleotide probing seem to be the most rapid and effective for identification purposes, and they will gain more importance as researchers are constantly on the lookout for protective cultures, phage-resistant strains for fermentation purposes, and probiotic organisms.

# CHAPTER 2 SHELF LIFE AND MICROBIAL ECOLOGY OF PRECOOKED POULTRY STORED UNDER MODIFIED ATMOSPHERE AT 3.5 AND 10°C

## 2.1 INTRODUCTION

The shelf life of ready-to-eat (RTE), refrigerated foods has been considerably extended in recent years by the use of modified atmosphere packaging (MAP) technologies. Originally used for the refrigerated transport of bulk raw beef and pork, the advances in packaging technologies and impermeable films have extended the application of MAP to consumer size packages. The technology relies on the exclusion of oxygen from the package and the presence of elevated concentrations of carbon dioxide. Growth of psychrotrophic aerobic bacteria, such as *Pseudomonas* spp., responsible for slime and offensive odour production, is inhibited. The specific inhibitory action of $CO_2$ is not known but appears to be bacteriostatic, increasing both lag phase and generation time of microorganisms (Daniels *et al.*, 1985). The anaerobic atmosphere in MAP favours the establishment of a gram-positive, facultative anaerobic population dominated by lactic acid bacteria.

Although an extensive literature exists on the shelf life extension, spoilage patterns and spoilage microbiota of MAP raw meat and processed, cured meat products (Church, 1993; Hood and Mead., 1993; Borch *et al.*, 1996), little published information is available about cooked, uncured RTE MAP meat products with minimal amounts of preservatives, and their spoilage and safety are not well known. The few studies available indicate that shelf life varies with the type of food and the type of packaging

atmosphere. Processed meat or roast beef sandwiches were acceptable for up to 35 days of storage at 4°C (McMullen and Stiles, 1989). Shelf life of the sandwiches was considerably shortened by the presence of oxygen in the atmosphere. The lactic acid bacteria developing in the different MAP sandwiches produced off-flavours generally after achieving a maximum population of approximately $10^8$ CFU/g (McMullen and Stiles, 1989).

Microbial counts were lowest on cooked beef roasts packaged under 100% $CO_2$, followed by roasts stored under 15:30:55 $CO_2$:$O_2$:$N_2$, and highest on VP samples after 21 days at 4°C, however, VP beef received higher sensory ratings and had lower moisture losses at day 14 and day 21 (McDaniel *et al.*, 1984). Subjective observations of precooked beef slices packaged under atmospheres containing varying levels of $CO_2$, $O_2$, and $N_2$ or VP, indicated that off-odours and colour deterioration occurred earlier in gas-packed samples (Carr and Marchello, 1986; 1987). Hintlian and Hotchkiss (1987b) found that cooked roast beef with gravy, packaged under 75:15:10 $CO_2$:$N_2$:$O_2$ and stored at 4.4°C, had a microbiological shelf life of 42 days (no aerobes or anaerobes recovered) while sensory evaluation showed deterioration, possibly due to warmed-over flavour, within one week of storage. Penney *et al.* (1993) compared the effects of VP and $CO_2$-saturated packaging on the shelf life of sliced roast beef stored at -1.5, 3, or 10°C. Carbon dioxide-packed samples had a shelf life of 10 weeks and 16 weeks at 3 and -1.5°C, respectively, while VP samples were rejected after 3 weeks at 3°C and 8 weeks at -1.5°C. At an abusive temperature (10°C), VP samples were rejected after 3 days and $CO_2$-packed samples after 4 days (Penney *et al.*, 1993).

Little information is available on the effects of MAP on RTE, minimally preserved poultry products. Chilled storage (0 and 4°C) of chicken à la king, VP or MAP under 70:30 $CO_2$:$N_2$, extended the lag phase of the naturally occurring microbiota by 10 to 15 days as compared to cling-wrapped samples (Young *et al.*, 1987). In fried chicken drumsticks, under similar storage conditions, the lag phase of naturally occurring microbiota was extended by 5 to 15 days in cling-wrapped and VP samples as compared to the MAP samples (Young *et al.*, 1987). The authors concluded that inhibition of microbial growth at chilled storage temperatures was dependent on the initial microbiota. *Pseudomonas* spp. and lactobacilli were dominant on cling-wrapped and MAP samples, respectively. The dominant microbiota of VP samples could not be determined. Quantitative descriptive analyses profiles of VP, MAP, and cling-wrapped fried chicken drumsticks and chicken à la king indicated that vacuum and MA packaging treatments are not significantly different (Young *et al.*, 1989). Sensory shelf life of vacuum and MAP samples was improved over cling-wrapped samples, however, MAP had deleterious effects on some components of chicken à la king, a complex dish with several ingredients.

Characterization of the major spoilage organisms is important for predicting the quality and safety of the final product. The purpose of this study was to determine the shelf life and identify the dominant microbiota of minimally preserved, oven-roasted MAP poultry, stored at 3.5 and 10°C ± 0.5°C.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Product preparation

Fresh chicken legs were prepared at a commercial poultry processing plant located in southwestern Ontario. Raw samples were injected with a commercial brine (sodium tripolyphosphate; sodium chloride, flavours) to approximately 30 to 35% of the original weight, steam-cooked to an internal temperature of 82°C as measured with a digital thermometer (PDT300, UEI, Beaverton, OR) inserted at the thigh joint. The oven time was approximately 26 to 27 min. Samples were cooled to an internal temperature of 12°C in a blast freezer (approximate freezer time of 22-23 min). Individual pieces were aseptically packaged in barrier foam trays with a gas impermeable lining and top film (W. R. Grace & Co., Cryovac Division, Mississauga, ON). The internal film liner of the tray had a moisture vapor transmission rate (MVTR) of 13.85 $gm/m^2$ in 24 h at 37.8°C and 100% relative humidity (RH) and an $O_2$ transmission rate (OTR) of 5.5 $cc/m^2$ in 24 h at 22.8°C and 0% RH. The top film had a MVTR of 7.69 $gm/m^2$ in 24 h at 37.8°C and 100% RH, and an OTR of 20 $cc/m^2$ in 24 h at 22.8°C and 0% RH. The top film and the film lining the interior of the tray were laminates of linear low density polyethylene (LLDPE) and ethylene vinyl alcohol (EVOH) polymers. Packages were evacuated and back flushed with a food grade mixture of 40:60 $CO_2$:$N_2$ (Liquid Carbonic Inc., Mississauga, ON) using a Multivac packaging machine (Multivac Space, Sepp Hagenmüller KG, Wolfertschwenden, Germany). MAP samples were stored at 3.5 or 10°C ± 0.5°C for 7 weeks or 5 weeks, respectively.

### 2.2.3 Sample analysis

At weekly intervals, duplicate samples were tested for different microbial populations (Table 2.1), pH, and $CO_2$ and $O_2$ concentrations in each package. A sample (11 g) was aseptically cut from the joint area with a sterile scalpel (Fisher Scientific, Pittsburgh, PA) and homogenized in 99 ml of sterile 0.1% peptone water for 2 min using a Stomacher Homogenizer (Colworth Stomacher 400, Seward Medical, London, UK). Serial dilutions were prepared in sterile 0.1% peptone water. Spore populations were determined by heating 20 ml of the initial dilution for 10 min at 80°C, followed by pour plating in tryptic soy agar and incubation at 37°C, either aerobically or anaerobically.

The pH of the chicken slurry was determined using a Fisher Acumet 915 Meter (Fisher Scientific). Prior to microbiological analysis, gas samples were withdrawn from each package through sampling patches (Fisher Scientific) using a gas-tight syringe, and gas composition ($O_2$ and $CO_2$) was determined using a gas chromatograph equipped with a thermal conductivity detector (GOW-MAC 69-150, Gow Mac Instruments Co., Bridgewater, NJ) and an electronic integrator (Hewlett Packard, 3077A). Oxygen and $CO_2$ were separated with stainless steel columns packed with Molecular Sieve 5A (0.92 m, 60/80, GOW-MAC) and Chromosorb 102 (1.83 m, 80/100, GOW-MAC), respectively. The flow rate of the carrier gas (He) was 50 and 30 ml $min^{-1}$ for $O_2$ and $CO_2$, respectively Isothermal separation was done at 50°C with an injection port temperature of 80°C. The detector current was set at 150 mA.

Unless otherwise specified, all media were supplied by Difco (Difco Laboratories Inc., Detroit, MI).

At weekly intervals, untrained panelists (employees at the production plant) evaluated the overall acceptability of the stored MAP product which was held at 3.5°C. MAP cooked poultry samples were heated in a microwave oven prior to serving. Panelists were asked to evaluate the acceptability of poultry samples based on odour and flavour.

Table 2.1 Media and incubation conditions used in testing for different microbial populations in MAP precooked poultry

| Population | Media | Incubation time & temperature |
|---|---|---|
| Total aerobic counts | Standard plate count agar (SPCA) | 22°C, 48 h |
| Total anaerobic counts | SPCA[a] | 22°C, 48 h |
| Lactic acid bacteria | Acidified MRS agar[a,b] | 30°C, 48 h |
| Psychrotrophs | SPCA | 3.5°C, 10 days |
| Pseudomonads | CFC agar[c] | 22°C, 24 h |
| Coliforms | Violet red bile agar | 37°C, 24 h |
| Aerobic spores | Tryptic soy agar | 37°C, 48 h |
| Anaerobic spores | Tryptic soy agar[a] | 37°C, 48 h |
| Yeasts and molds | Chloramphenicol agar[d] | 22°C, 5 days |

[a]Plates were incubated in anaerobic jars (Gas Pack Jars, BBL Microbiology Systems, Cockeysville, MD)
[b]MRS agar (Difco) acidified with 85% lactic acid (Fisher Scientific) to pH 5.6 (McMullen and Stiles, 1989)
[c]Cephaloridine Fusidin Cetrimide agar (CFC agar; Mead and Adams, 1977)
[d]SPC agar with 100 μg/ml chloramphenicol (Sigma)

### 2.2.4 Statistical Analysis

The microbiological data obtained from three replicates conducted on three separate occasions were analyzed by ANOVA using the General Linear Model procedure (SAS Institute Inc., Cary, NC).

## 2.3 RESULTS

### 2.3.1 Storage at 3.5 ± 0.5°C

All microbial populations were <100 CFU/g for the first 2 weeks at 3.5°C, with the exception of replicate three, where low counts were detected following one week of storage (Table 2.2). Counts started to increase after the second week to reach maximal levels of $10^8$ CFU/g by the seventh week of storage. Populations of aerobes, anaerobes, psychrotrophs, and lactics were similar over the duration of the study (Table 2.2), suggesting that they all may be the same population growing under four different conditions. Counts from duplicate packages were highly variable, ranging from <1000 CFU/g to $>10^5$ CFU/g between duplicate packages on a sampling day (Table 2.2). Yeast and moulds counts and spore counts were <100 CFU/g for the duration of the study. Pseudomonads and coliform counts varied markedly between duplicate samples for each trial and between trials (Table 2.3). No off-odours were detected upon opening the packages prior to microbiological analyses for the duration of the study.

Panelists reported that poultry meat samples had an acceptable appearance, odour, and flavour during the storage period; however, most samples did not taste as 'fresh' after 21

days of storage. Samples were deemed acceptable by panelists, even following achievement of maximal population numbers ($10^8$ CFU/g).

Table 2.2 Counts of aerobes, anaerobes, psychrotrophs, and lactic acid bacteria ($log_{10}$ CFU/g) on duplicate packages of precooked MAP poultry, stored at 3.5°C, from week 0 to week 7, for three separate trials[ab]

| | Aerobic counts | | | Anaerobic counts | | | Psychrotroph counts | | | MRS counts | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 |
| Week 0 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| Week 1 | < 2.00 | < 2.00 | 2.60 | < 2.00 | < 2.00 | 2.70 | < 2.00 | < 2.00 | 2.60 | < 2.00 | < 2.00 | 2.65 |
| | < 2.00 | < 2.00 | 2.60 | < 2.00 | < 2.00 | 2.48 | < 2.00 | < 2.00 | 2.50 | < 2.00 | < 2.00 | 2.60 |
| Week 2 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| | 3.67 | < 2.00 | < 2.00 | 3.64 | < 2.00 | < 2.00 | 3.28 | < 2.00 | < 2.00 | 3.65 | < 2.00 | < 2.00 |
| Week 3 | < 2.00 | > 5.75 | < 2.00 | < 2.00 | > 5.75 | < 2.00 | < 2.00 | > 5.75 | < 2.00 | < 2.00 | > 5.75 | < 2.00 |
| | > 5.75 | > 5.75 | 5.63 | > 5.75 | > 5.75 | 5.95 | > 5.70 | > 5.75 | 5.47 | < 2.00 | > 5.75 | 5.65 |
| Week 4 | > 5.75 | 4.45 | < 3.00 | 4.97 | 4.33 | < 3.00 | NA | 4.45 | < 3.00 | < 2.00 | 4.18 | < 3.00 |
| | > 5.75 | 6.90 | 5.68 | > 5.75 | 6.89 | 5.66 | NA | 6.93 | 5.72 | > 5.75 | 6.71 | 5.26 |
| Week 5 | 3.00 | 7.91 | 7.16 | 3.00 | 7.86 | 7.12 | < 3.00 | 7.86 | NA | < 3.00 | 7.82 | 7.15 |
| | 7.54 | 7.54 | 6.00 | 7.53 | 7.60 | 5.97 | 7.57 | 7.57 | NA | 6.77 | 7.41 | 5.98 |
| Week 6 | 7.16 | 6.98 | 7.61 | 7.25 | 6.96 | 7.64 | 7.17 | 7.02 | 7.63 | 7.11 | 6.96 | 7.57 |
| | 7.72 | 7.84 | 6.00 | 7.80 | 7.80 | 6.02 | 7.78 | 7.74 | 6.04 | 7.61 | 7.73 | 5.93 |
| Week 7 | 8.13 | 8.29 | 8.20 | 8.00 | 8.32 | 8.27 | 8.75 | 8.24 | 8.27 | 7.83 | 8.15 | 8.12 |
| | 8.57 | < 6.00 | 7.89 | 8.59 | < 6.00 | 7.93 | 7.97 | < 6.00 | 7.94 | 7.39 | < 6.00 | 7.90 |

[a] < or >, estimated counts (Swanson *et al.*, 1992)

[b] NA, data not available

Table 2.3 Populations of pseudomonads and coliforms ($log_{10}$ CFU/g) on duplicate packages of precooked MAP poultry, stored at 3.5°C from day 21 to day 49, for three separate trials

| | Pseudomonads | | | Coliforms | | |
|---|---|---|---|---|---|---|
| Time | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 |
| Week 3 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| | < 2.00 | 3.70 | < 2.00 | < 2.00 | 3.70 | < 2.00 |
| Week 4 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| | 4.50 | 5.80 | < 2.00 | 4.60 | 5.80 | < 2.00 |
| Week 5 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| | 4.50 | < 2.00 | < 2.00 | 4.60 | < 2.00 | < 2.00 |
| Week 6 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| | < 2.00 | 5.20 | 5.50 | 6.80 | 5.60 | 6.40 |
| Week 7 | < 2.00 | < 2.00 | 5.60 | < 2.00 | < 2.00 | 6.50 |
| | < 2.00 | > 5.00 | 3.20 | 6.70 | > 4.00 | 4.70 |

### 2.3.2 Storage at 10 ± 0.5°C

Figure 2.1 shows the mean results obtained for the various populations enumerated on the MAP poultry stored for up to 35 days at 10°C. Sporeformers, yeasts and molds were not recovered (< 100 CFU/g) from the samples. Aerobes, anaerobes, lactics, and psychrotrophs were similar in their growth pattern, increasing to levels of $10^3$-$10^4$ CFU/g within the first week of storage and achieving levels of $10^7$-$10^8$ CFU/g after 4 weeks at 10°C. The growth of coliforms was slightly slower, and the population was 0.5 to 2-log units lower than total counts of aerobes. Pseudomonads were inhibited in the first week of storage, picking up afterwards to reach levels of $10^7$ CFU/g by the end of the storage period. Upon opening packages for microbiological analysis, strong off-odours were within the fourth week of storage.

There were no significant differences ($p > 0.05$) between replicate trials for populations of aerobes, anaerobes, and psychrotrophs at each storage temperature. Growth curves of the microbiota were significantly different at 3.5 and 10°C ($p < 0.01$).

### 2.3.3 pH and $CO_2$ concentrations

The pH of the meat was 6.5 at the beginning of the study and did not change significantly over the storage period for samples stored at either temperature.

Initial $CO_2$ concentration was measured 6 to 8 hours following production and packaging. An initial decrease from 36% to 30-32% after the first week was observed at 3.5°C (Fig. 2.2). Thereafter, carbon dioxide levels remained relatively stable ranging from 26 to 30%

in packages stored at 3.5°C and from 32 to 34% in packages kept at 10°C (Fig. 2.2). Oxygen was seldom detected in the packages and never exceeded 1% of the atmosphere.

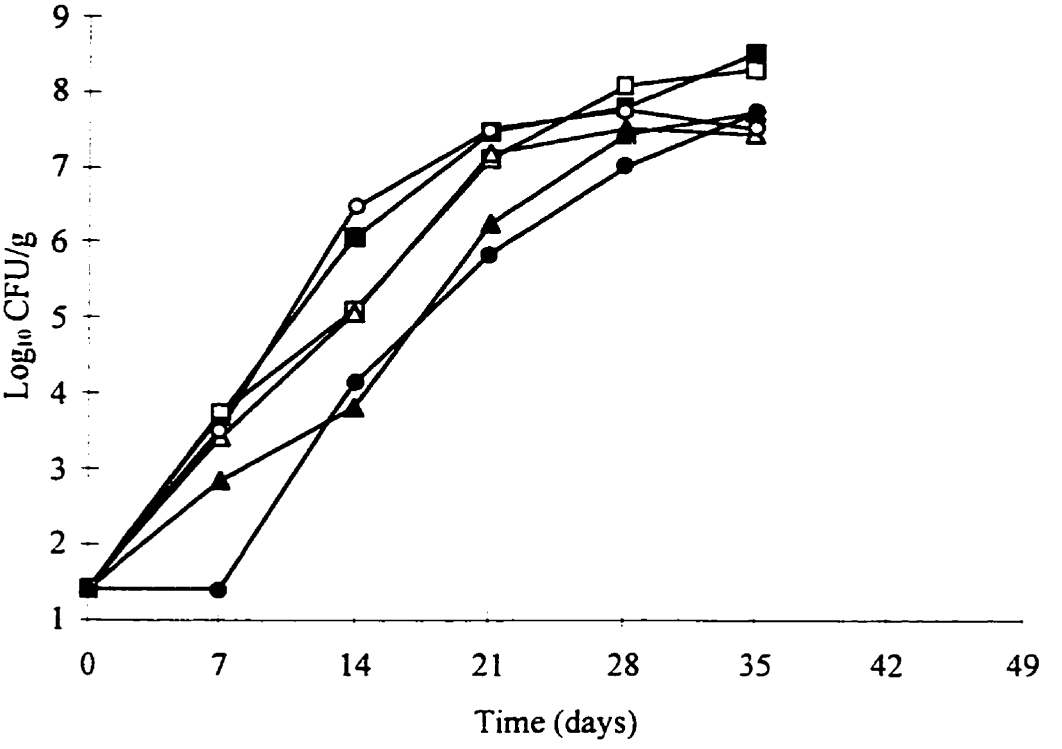



Figure 2.1 Growth of different populations on cooked refrigerated MAP poultry stored at 10°C; total aerobes (■), total anaerobes (□), psychrotrophs (ↄ), lactics (Δ), coliforms (▲), pseudomonads (●)

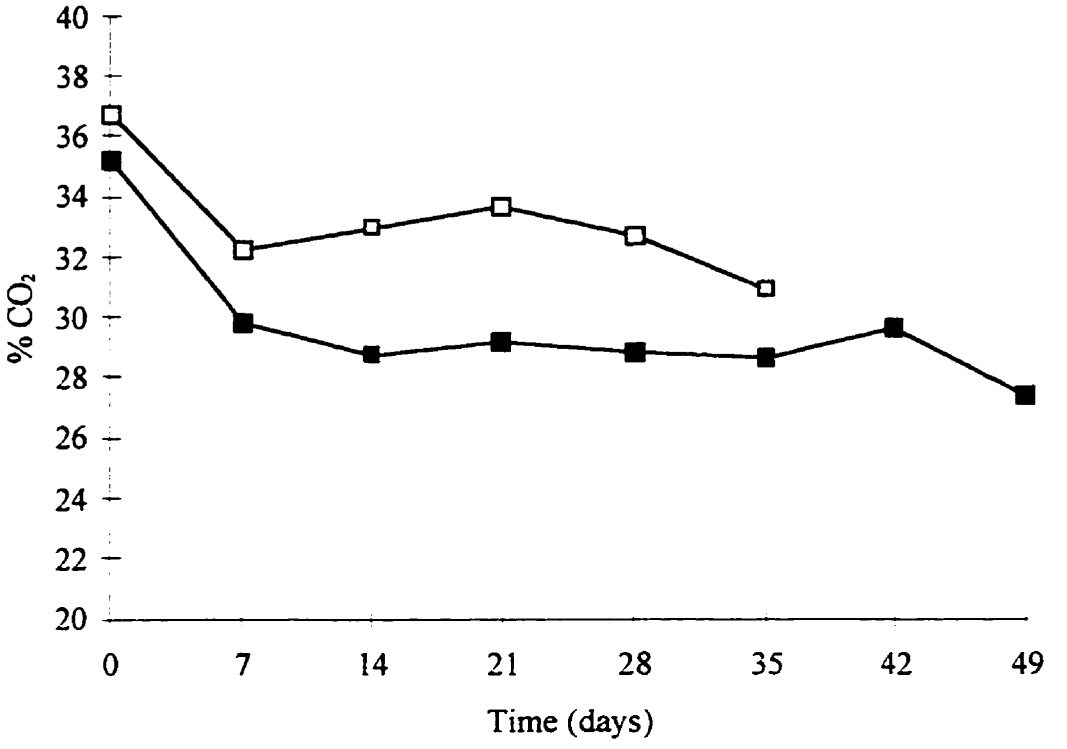



Figure 2.2 Average concentration of $CO_2$ in packages of cooked modified atmosphere packaged poultry stored at 3.5°C (■) and at 10°C (□)

## 2.4 DISCUSSION

Counts for aerobes, anaerobes, presumptive lactic acid bacteria, and psychrotrophs were similar for the duration of the study in samples stored at 3.5°C suggesting that they were the same population enumerated under four different plate storage conditions. The absence of spoilage indicators (appearance and odour) after 7 weeks of storage and the achievement of maximal population levels is consistent with findings by McMullen and Stiles (1989) who reported that off-flavours were not detected in some MAP sandwiches until well after maximal lactic acid bacteria populations were reached. Egan *et al.* (1980) compared the effect of *B. thermosphacta* (previously *M. thermosphactum*), heterofermentative, and homofermentative lactobacilli on the spoilage of VP luncheon meats. In the presence of *M. thermosphactum*, spoilage was detected by a trained sensory panel at about the time counts reached $10^8$ CFU/g. Meat inoculated with homofermentative or heterofermentative LAB was acceptable for up to 21 days and up to 11 days, respectively, after LAB populations reached $10^8$ CFU/g. When slices of raw beef were inoculated with a homofermentative *Lactobacillus* or a heterofermentative *Leuconostoc* sp. and modified-atmosphere packaged, flavour scores of heat-treated beef inoculated with the heterofermentative species decreased before achieving maximal bacterial load (Borch and Agerhem, 1992). In the present study, no off-odours were detected by the experimenter and by other laboratory members when left-over packages of poultry stored for 3 months at 3.5°C were opened.

Off-aromas could be detected upon opening packages stored at 10°C after 3 to 4 weeks. The modified atmosphere did not inhibit the growth of the pseudomonads and coliform

population as in the product kept at 3.5°C. These organisms may be responsible for the spoilage of the product stored at 10°C as their increase in numbers from $10^6$ CFU/g to $10^7$ CFU/g coincides with detection of spoilage aromas. The efficiency of carbon dioxide in inhibiting the gram-negative spoilage flora decreased as the storage temperature increased, possibly due to decreased solubility of the gas in the meat tissue (Baker *et al.*, 1986; Daniels *et al.*, 1985). A slight inhibition of *Pseudomonas* spp. could be observed at the beginning of the study (Fig. 2.1) but did not extend beyond the first week of storage.

The carbon dioxide composition of the atmospheres inside the packages stored at 3.5 or at 10°C showed an initial drop in the first week of storage which could be attributed to an initial absorption of the gas by the meat. This drop was followed by a stabilization of the $CO_2$ concentration over the rest of the test period. The concentration of $CO_2$ in packages stored at 10°C was slightly more elevated than in packages stored at 3.5°C for the duration of the study, suggesting that the solubility of the gas decreased at the higher storage temperature. McMullen and Stiles (1989) attributed the initial decrease in $CO_2$ concentrations in MAP sandwiches kept at 4°C to absorption of the gas by the product. Thereafter, $CO_2$ concentrations increased in packages of roast beef, ham, or bologna sandwiches, but decreased in MAP hamburgers. The increase may have been due to the presence of a heterofermentative lactic acid microbiota in the first three products, while a different population of lactic acid bacteria was possibly predominant on the hamburgers.

This work has shown that precooked RTE MAP poultry has an extended shelf life at refrigeration temperatures. Abusive storage temperature however, may severely alter the

quality of the product. As expected, the microbiota present on chilled poultry samples consisted mainly of presumptive lactic acid bacteria which are the dominant organisms on MAP chilled meat products, whether raw, cured, or cooked. No off-flavours were detected on samples stored at 3.5°C and the poultry product was deemed acceptable, even after achievement of maximal counts ($10^8$ CFU/g).

No changes in pH were observed after 7 weeks of storage which may be due to the absence of a readily fermentable carbohydrate source, or the presence of non-aciduric organisms.

The presumptive lactic acid bacteria enumerated on the chilled MAP cooked poultry stored at 3.5°C would be expected to belong to the genera *Lactobacillus, Carnobacterium* and *Leuconostoc*, which are most often isolated from MAP meat products (Borch *et al.*, 1996). To determine whether isolates from cooked meat products fall within this group the identification of the microbiota that predominated on the MAP chilled poultry samples will be addressed in the next chapter.

What immediately elicits concern is the safety of these uncured, cooked meat products that rely on refrigeration, a fragile barrier, and on the change in package atmosphere for preservation. The extended shelf life may allow psychrotrophic pathogens such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, and *Clostridium botulinum* to grow and produce toxin, particularly if the aerobic spoilage flora is inhibited by carbon dioxide, thus providing no warning to the consumer about a potential hazard associated with consumption of the product. MAP products have been shown capable of supporting the growth of pathogenic organisms (Glass and Doyle, 1989; Hudson and

Mott, 1993a; 1993b; Hudson *et al.*, 1994) and it is imperative to determine the safety of minimally preserved RTE chilled products, and to investigate different options to reduce the associated hazards.

# CHAPTER 3 IDENTIFICATION OF *CARNOBACTERIUM, LACTOCOCCUS,* AND *ENTEROCOCCUS* SPP. ISOLATED FROM COOKED MODIFIED ATMOSPHERE PACKAGED POULTRY MEAT

## 3.1 INTRODUCTION

It is well established that lactic acid bacteria (LAB) are the predominant spoilage population that develops on modified atmosphere packed (MAP) and vacuum-packed meats, whether raw (Hitchener *et al.*, 1982; Shaw and Harding, 1984; Grant and Patterson, 1991b), fermented or cooked (Borch and Molin, 1988; McMullen and Stiles, 1989; von Holy *et al.*, 1992). *Carnobacterium* spp., *Lactobacillus* spp., *Pediococcus* spp., and *Leuconostoc* spp. are the main genera associated with spoilage of these products. *Brochothrix thermosphacta* is also frequently found on vacuum-packed meats. Several identification methods and keys have been proposed for different LAB (Schillinger and Lücke, 1987a; Döring *et al.*, 1988; Montel *et al.*, 1991), however, atypical strains are often encountered and many of these have been reclassified in new genera such as *Carnobacterium* spp. (Collins *et al.*, 1987) and *Weissella* spp. (Collins *et al.*, 1993), or described as new species such as *Leuconostoc gelidum* and *Leuconostoc carnosum* (Shaw and Harding, 1989). Recently, strains of *Lactococcus lactis*, an organism traditionally associated with dairy and vegetable products, were isolated from fermented sausages (Rodriguez *et al.*, 1995), raw pork (Garver and Muriana, 1993) and vacuum-packed seafood (Mauguin and Novel, 1994). Another atypical *Lactococcus* strain isolated from salmonid fish was classified as *L. piscium* sp. nov. (Williams *et al.*, 1990). The purpose of this study was to identify and characterize strains of LAB, mainly

*Lactococcus* and *Carnobacterium* spp., isolated from cooked, ready-to eat MAP poultry stored at 3.5°C. These LAB did not spoil the MAP poultry meat even at levels of $10^8$ CFU/g, and they may have potential applications as biopreservation agents.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Source of organisms

Dominant organisms were isolated from cooked, MAP, refrigerated poultry stored at 3.5°C (Chapter 2). Colonies were selected based on appearance from standard plate count agar (SPCA, Difco) plates of the highest enumerated dilution of aerobes, anaerobes, and psychrotrophs. Preliminary analysis (morphology, Gram-staining, catalase and oxidase tests) suggested that a similar population was present on the SPCA plates from different incubation conditions. Thus, characterization was continued on aerobic population isolates only from trials 1 and 2. All isolates (aerobes, anaerobes, and psychrotrophs) were characterized from trial 3. A total of 206 colonies was selected, checked for purity, and differentiated by observation of cellular morphology using phase-contrast microscopy, Gram-staining, catalase, and oxidase reactions. Colonies were kept at -80°C in tryptic soy broth with 20 % glycerol (w/v) for long term storage. A total of 197 gram-positive, catalase and oxidase-negative isolates was further identified. The other nine isolates were gram-negative, catalase-positive, oxidase-negative rods and were identified using the Vitek Jr. (Vitek Systems, bioMérieux Vitek, Inc., Hazelwood, MO) according to the manufacturer's instructions.

Microscopic examination of gram-positive strains showed 36.5% of the isolates (72 strains) were short to medium rods, occurring either as single cells or in pairs. The rest of the isolates (125 strains) were single, pairs, or chains of coccobacilli. Based on these results, isolates were further differentiated using various phenotypic and chemotaxonomic tests.

### 3.2.2 Cultures and cultivation

The reference strains used are listed in Table 3.1. Gram-positive isolates were initially cultivated in Lactobacilli MRS broth (Difco). Rod-shaped isolates did not grow optimally in that medium and when preliminary tests indicated that the isolates might be *Carnobacterium* spp., MRS broth (De Man *et al.*, 1960) with acetate omitted (modified MRS, MMRS) was used, as suggested by Hammes *et al.*, (1991). The same medium was used to culture reference strains of carnobacteria. The remainder of the poultry isolates and the reference strains were maintained in MRS broth (Difco) at 28°C, and *Listeria monocytogenes* in TSA broth at 30°C. It was later observed that all gram-positive isolates grew well in M17 medium (Difco) supplemented with 0.5% glucose (M17G) and in APT medium (Difco).

Table 3.1 References strains used in characterization tests

| Species | Reference number | Source and comments |
|---|---|---|
| *Carnobacterium divergens* | LV13[a] | UAL9[b]; bac$^{+c}$ |
| *C. divergens* | ATCC[d] 35677 | Type strain |
| *C. divergens* | UAL 278[be] | |
| *C. gallinarum* | ATCC 49517 | Type strain |
| *C. piscicola* | LV17[a] | UAL8[b]; bac$^{+}$ |
| *C. piscicola* | ATCC 35586 | Type strain |
| *C. piscicola* | ATCC 43224 | |
| *C. piscicola* | UAL 26[be] | |
| *C. mobile* | ATCC 49516 | Type strain |
| *Enterococcus faecalis* | ATCC 19433 | Type strain |
| *E. faecalis* | ATCC 33186 | |
| *E. faecium* | ATCC 19434 | Type strain |
| *E. durans* | ATCC 19432 | |
| *Lactococcus lactis* ssp. *lactis* | ATCC 11454 | |
| *L. lactis* ssp. *lactis* | ATCC 7962 | |
| *L. lactis* ssp. *lactis* | NCK 401[f] | Bac$^{+}$ (nisin) |
| *L. lactis* ssp. *cremoris* | ATCC 14365 | |
| *L. raffinolactis* | NCFB 617[g] | Type strain |
| *L. piscium* | NCFB 2778 | Type strain |
| *L. garvieae* | NCFB 2155 | Type strain |
| *L. plantarum* | NCFB 1869 | Type strain |
| *Leuconostoc mesenteroides* | Y105[bh] | Bac$^{+}$ (mesentericin Y105) |
| *Pediococcus acidilactici* | PAC 1.0[bi] | Bac$^{+}$ (pediocin PA1) |
| *Listeria monocytogenes* | LI 512[j] | Serotype (ST) 1/2b - Meat isolate |
| *Li. monocytogenes* | LI 527 | Scott A - ST 4b - Patient/Outbreak |
| *Li. monocytogenes* | LI 521 | Murray B - ST 4b - Meat isolate |
| *Li. monocytogenes* | LI 500 | ATCC 15313, ST 1/2b, rabbit |

Table 3.1 (*continued*)

| Species | Reference number | Source and comments |
|---|---|---|
| *Li. monocytogenes* | LI 503 | ATCC 19113, ST 3a, human isolate |
| *Li. monocytogenes* | LI 514 | ST 1/2b, meat isolate |
| *Li. monocytogenes* | LI 531 | ST 4b; Switzerland/outbreak |
| *Li. monocytogenes* | LI 533 | ST 4b; LCDC; Coleslaw/outbreak |
| *Li. monocytogenes* | LI 540 | ST 1/2b; salami |
| *Li. monocytogenes* | LI 550 | ST 4b; Health & Welfare Canada; Food |
| *Li. monocytogenes* | LI 564 | ST 4b; cheese/outbreak |
| *Li. monocytogenes* | LI 576 | ST 1/2b; meat isolate |
| *Li. welshimerii* | ATCC 35897 | Type strain |
| *Li. innocua* | ATCC 33091 | |
| *Li. grayi* | ATCC 19120 | Type strain |
| *Li. murrayi* | ATCC 25401 | Type strain |
| *Li. ivanovnii* | ATCC 19119 | Type strain |

[a]Strains originally from Dr. B. G. Shaw, Institute of Food Research, Bristol, UK; obtained from Dr. M. E. Stiles, University of Alberta (Edmonton, AB)
[b]UAL, University of Alberta; Lactic Acid Bacteria Collection
[c]$Bac^+$, bacteriocin-producer
[d]ATCC, American Type Culture Collection (Rockville, MD)
[e]Strains were obtained from Dr. L. M. McMullen, University of Alberta
[f]Harris *et al.*, 1992; North Carolina State University (Raleigh, NC)
[g]NCFB, National Collection of Food Bacteria, Agricultural and Food Research Council, Institute of Food Research (Reading, UK)
[h]Héchard *et al.*, 1992; strain was obtained from Dr. M. E. Stiles, University of Alberta
[i]Gonzalez and Kunka, 1987; strain was obtained from Dr. M. E. Stiles, University of Alberta
[j]*Listeria* spp. were obtained from Dr. A. Lammerding (Health of Animals Laboratory, Health Canada, Guelph, ON)

### 3.2.3 Physiological and biochemical tests

All broth tests were inoculated with 0.05% of a bacterial culture grown in broth at 28°C for 18 to 24 h. Chemicals used for biochemical tests were purchased from Sigma (Sigma Chemical Co., St. Louis, MO), and growth media from Difco (Difco Laboratories, Detroit, MI) unless otherwise specified. Broth cultures and inoculated agar plates were incubated aerobically unless otherwise specified.

Anaerobic growth was tested by streaking overnight cultures onto MRS or MMRS agar plates for coccoid and rod-shaped isolates, respectively. Plates were incubated anaerobically (Gas Pack Jars, BBL Microbiology Systems, Cockeysville, MD) at 28°C for 3 days.

The presence of *meso*-diaminopimelic acid (DAP) in cell walls was determined using thin-layer chromatography on cellulose plates (Fisher Scientific) as described by Kandler and Weiss (1986).

Oxidation/Fermentation (O/F) of glucose was observed in Hugh-Leifson media (Difco) containing 1% glucose. Duplicate tubes were stab-inoculated with each strain and one of the tubes was overlaid with 2 ml of sterile mineral oil. Tubes were incubated at 28°C and examined periodically for growth and glucose fermentation patterns for up to 5 days.

Motility was determined by examination of growth pattern along the stab line of the Hugh-Leifson O/F media. Non-motile organisms grew along the stab line while motile strains diffused through the agar, away from the line of inoculation.

Acid tolerance was tested in MMRS broth adjusted to pH 5.0 and 5.5 with 1N HCl, and to pH 9.0 and 9.5 with 10N and 1N NaOH following autoclaving. Cultures were incubated at 28°C for up to 7 days.

Production of dextran from sucrose was assessed on MMRS agar with 10% sucrose replacing glucose. Plates were incubated at 28°C for 3 days. Growth on acetate agar (Rogosa SL media, Difco) was followed for a maximum of 7 days.

Growth in the presence of 3% or 6.5% NaCl was observed in MMRS broth held at 28°C for 7 days. Growth at 3.5 and 40°C was followed for 15 and 7 days, respectively, in MMRS broth. Isolates that grew at 40°C were tested for growth at 45 and 50°C.

Production of $CO_2$ from glucose was determined in basal MMRS broth (with acetate, citrate, and beef extract omitted) with inverted Durham tubes, incubated for up to 7 days at 28°C.

Hydrolysis of arginine (L-arginine) was determined by the method of Niven *et al.* (1942).

Differentiation of enterococci from *L. garvieae* was determined by inoculation in KF broth containing 0.04% sodium azide and 0.01% 2,3,5-triphenyl tetrazolium chloride (TTC) (Devriese *et al.*, 1991).

Growth and acidification of litmus milk was followed for 7 days at 28°C.

Carbohydrate fermentation patterns were determined by a miniaturized method. Overnight cultures at 28°C (APT broth) were pelleted and washed once in sterile 0.85% saline. The pellet was resuspended in the basal media to a point 4 of the McFarland scale. The basal medium consisted of double-strength MRS with meat extract, glucose,

and acetate omitted and with 0.34 g/l bromocresol purple as an indicator. A 100 µl volume of filter-sterilized solutions (4% w/v stock) of different sugars was dispensed in sterile microtiter plates (Fisher Scientific). After addition of an equal volume of basal media, the final concentration of each carbohydrate was 2%. The carbohydrates tested were N-acetyl glucosamine, adonitol, esculin, D-amygdalin, L(+)-arabinose, D(+)-arabitol, L(-)-arabitol, arbutin, D(+)-cellobiose, dulcitol, D-fructose, D(+)-fucose, D(+)-galactose, D-gluconic acid lactone, D-glucose, glycerol, inulin, myo-inositol, α-lactose, maltose, D-mannitol, D(+)-mannose, D(+)-melezitose, D(+)-melibiose, methyl α-D-glucopyranoside, methyl β-D-xylopyranoside, D(+)-raffinose, L-rhamnose, D(-)-ribose, salicin, D-sorbitol, L(-)-sorbose, sucrose, D(+)-trehalose, xylitol, D(+)-xylose, and L(-)-xylose. Following inoculation, individual wells were immediately overlaid with 75 µl of sterile mineral oil to create an anaerobic environment and prevent evaporation during incubation. Plates were kept at 28°C for 5 days and checked daily for growth and colour changes. A total of 12 strains was further retested for carbohydrate fermentation patterns using the API 50 CHL system (bioMérieux).

The lactic acid isomer formed and end-point pH were measured in cultures incubated for 3 days in basal MRS broth at 28°C. The enantiomers of lactic acid were determined enzymatically in cell-free culture supernatant fluids with a D-, L-lactic acid assay kit (Boehringer Mannheim GmbH, Mannheim, Germany). The test was adapted for use in microtiter plates by reducing all reagent and sample volumes by 10-fold. The end-point pH of the supernatant was measured with a Fisher Acumet 915 Meter (Fisher Scientific).

Inhibition by bacteriocin-producing lactic acid bacteria was tested by a deferred antagonism method. Strains of bacteriocin-producing lactic acid bacteria, *C. divergens* LV13, *C. piscicola* LV17, *Lc. mesenteroides* Y105, *P. acidilactici* PAC 1.0, and *L. lactis* ssp. *lactis* (Table 3.1) were inoculated onto APT agar using a Cathra™ Systems Replicator (AutoMed, Arden Hills, MN). Plates were incubated at 28°C for 48 h, until growth was visible on the agar. Soft APT agar (4 ml, 0.75% agar) tubes were seeded with a 1% inoculum (APT broth, 18 to 24 h, 28°C) of the isolates. The seeded agar was rapidly poured over the test organisms. After 48 to 72 h at 28°C, the agar overlay was examined for zones of inhibition surrounding the buttons of growth of the test strains.

All poultry isolates were screened for production of antibacterial substances by the deferred antagonism method (Harris *et al.*, 1989). Isolates were screened against *Carnobacterium* and *Enterococcus* reference strains, several strains of *L. monocytogenes*, and other *Listeria* spp. and against 10 representative strains among the isolates. To determine the nature of the inhibitory substance produced, isolates that inhibited any of the indicator cultures were grown for 24 hours in APT broth, centrifuged for 5 min at 6000 X g and the pellet discarded. Following pH measurement, the supernatant was filter-sterilized through 0.2 µm surfactant-free cellulose acetate, low-protein binding Nalgene filters (Nalgene catalogue number 190-2520, Fisher Scientific). The supernatant was divided into 1-ml aliquots and either left untreated (control) or treated with pronase (0.5 mg/ml; Sigma), catalase (100 units/ml; Sigma), or heated at 64°C for 1 h. Samples treated with pronase or catalase were incubated at 37°C for 30 min. The spot-on-lawn assay was used to determine presence or absence of inhibitory activity (Hastings and Stiles, 1991). Following treatment, 10 µl of the supernates were spotted onto APT agar

plates overlaid with soft APT agar (0.75%) inoculated with 1% of overnight cultures of either *C. divergens* LV13 or *C. piscicola* LV17. To ensure that no protein-binding occurred during filter-sterilization, a portion of the supernatant was treated with chloroform to inactivate bacterial cells as described by Hastings and Stiles (1991) and used in a spot-on-lawn assay as described above.

### 3.2.4 Scanning electron microscopy (SEM) of lactococci

The procedure used for the preparation of bacterial cells was developed by Ms. A. K. Smith (Department of Food Science, University of Guelph; Smith, 1995). Cell suspensions were prepared in Sorensen's Phosphate Buffer (SPB, 0.07 M, pH 6.8), from 18-24 h growth on MMRS agar plates, and filtered through 0.20 µm polycarbonate membrane filters (Poretics Corp., Livermore, CA). Filters were fixed in 2% glutaraldehyde (in phosphate buffered saline) overnight. Filters were rinsed in SPB and post-fixed in 1% osmium tetroxide for 1 h. Samples were rinsed in SPB, dehydrated through an ethanol concentration gradient (30 to 100%), and critical point dried. Filters were adhered to aluminum pins with sticky tabs, sputter-coated with 20 nm of gold/palladium in a Hummer VII Sputter Coater (Anatech Corp., Alexandria, VA), and scanned in a Hitachi S-570 scanning electron microscope.

### 3.2.5 Numerical analysis

Results of morphological and biochemical tests were recorded as binary characters, '1' for positive and '0' for negative. Identical characters for all isolates were omitted from the

analysis. The CLUSTER procedure of SAS was used for the numerical analysis. The distance matrix between pairs of isolates was calculated using Jaccard's index, $S_j=a/(a+b+c)$, where ***a*** represents the number of positive matches, while ***b*** and ***c*** represent the number of non-matching characters between pairs of isolates. Isolates were clustered using the average linkage method (unweighted pair group method with arithmetic average [UPGMA]) where a taxonomic unit joins a cluster at the average similarity between that unit and all other members of the cluster.

### 3.2.6 Whole-cell fatty acid analysis

Selected poultry isolates and reference strains were analyzed using the Microbial Identification System (MIS; Microbial ID Inc., Newark, DE) according to the manufacturer's instructions (MIDI). Organisms were grown on Yeast Glucose Phosphate agar (Garvie, 1978) for 24 h at 28°C, and 45 mg of cells (wet weight) were harvested from the third quadrant of growth. Samples were saponified, methylated, extracted, and washed. The extracted fatty acids were separated on a Hewlett-Packard 5890 series II gas liquid chromatography column equipped with a flame-ionization detector. Signals from the detector were processed and the data analyzed by the MIS library software. Quantitative multivariate analyses of cellular fatty acid profiles generated similarity indices based on Euclidean distances between pairs of isolates and/or reference strains. The unweighted pair-group method with arithmetic average (UPGMA) was used to compute the distance matrix and construct a similarity dendrogram.

## 3.3 RESULTS

Gram-positive isolates (197) were subdivided to the genus level according to the scheme shown in Figure 3.1. Organisms were identified to the species level according to differential properties listed in Bergey's Manual of Determinative Bacteriology (1994b; 1994c). Table 3.2 lists the source of isolates. Differentiating phenotypic properties of gram-positive organisms are summarized in Table 3.3. Fermentation patterns that were positive or negative for all isolates are not included. Glucose, N-acetyl glucosamine, cellobiose, fructose, maltose, mannose, and salicin fermentations were positive for all. (D)-arabitol, (L)-arabitol, adonitol, fucose, xylopyranoside, xylitol, (L)-xylose, sorbose, and dulcitol fermentations were negative.

### 3.3.1 Identification of gram-negative isolates

Of the nine organisms isolated from MAP cooked poultry, eight were identified as *Serratia liquefaciens*, and one as *Pantoea agglomerans* (synonym for *Enterobacter agglomerans*; Bergey's Manual of Determinative Bacteriology, 1994a). These isolates were not associated with a specific week/trial combination.

### 3.3.2 Characterization of gram-positive rods

Isolates were slender rods, occurring singly, in pairs or short chains. Pairs of cells often formed a broad-angle V-shape. All isolates were facultatively anaerobic, had *meso*-DAP in the cell wall and produced only the L(+)-lactic acid enantiomer. They were

differentiated from lactobacilli by growth at pH 9.5 in MMRS broth and their inability to grow on acetate agar. No growth occurred at pH 5.0 in MMRS broth, but organisms grew well in 3% NaCl and more weakly in 6.5% NaCl. Growth in MMRS broth was observed at 3.5°C within 7 days, while no growth occurred at 40°C within 1 week. Strains were classified as *C. divergens* (61 isolates) or *C. piscicola* (11 isolates) based on carbohydrate utilization patterns, most importantly mannitol fermentation. Gas production was observed in *C. divergens* strains, however, this property was variable.

*C. divergens* isolates were dominant in trials one and two but absent from trial three. *C. piscicola* isolates were detected in trial three only.

*C. divergens* and *C. piscicola* isolates were inhibited by bacteriocin-producing reference strains of *P. acidilactici* PAC 1.0 (pediocin PA 1.0), *Lc. mesenteroides* Y105 (mesentericin Y105), *C. divergens* LV13, and by *L. lactis* ATCC 11454 (nisin).

### 3.3.3 Characterization of gram-positive cocci

A few of the isolates were either small sphaerical cocci occurring singly or in pairs, while the majority were ovoid, occurring singly, in pairs or short to long chains. Morphology was not easy to determine by phase-contrast microscopy, and it was necessary to resort to scanning electron microscopy to accurately determine morphology (Figures 3.2 and 3.3). Elongated cells were more common than sphaerical cells. Organisms were facultative anaerobes, did not have *meso*-DAP in the cell wall and produced only L(+)-lactic acid. No gas production was observed. Based on these characteristics, they were classified as either *Enterococcus* spp. or *Lactococcus* spp. The majority of isolates, 118 strains out of

125, did not grow at 40°C and were included in the *Lactococcus* genera. All but one of these presumptive lactococci grew at 3.5°C in broth within 10 days. The single isolate was classified as *L. lactis*, while the remaining 117 isolates were identified as *L. raffinolactis*, based on carbohydrate fermentation patterns. Although their growth characteristics were similar to those of *L. piscium*, they were differentiated from this organism by their inability to ferment gluconate. Presumptive *L. raffinolactis* strains did not grow at pH 5.0, in 6.5% NaCl, or on acetate agar. Growth was observed in 3% NaCl and at pH 9.0. Not all *L. raffinolactis* strains grew at pH 9.5. Isolates varied in their ability to ferment xylose, lactose, arabinose, amygdalin, melezitose, mannitol, and inulin. Day 7 readings of fermentation patterns were used for the cluster analysis, since reactions were delayed for some isolates.

The remaining seven isolates (out of 125) grew well at 40°C but not at 3.5°C. Out of these seven isolates, three grew at 45°C but not at 50°C. Based on carbohydrate fermentation patterns and ability to grow in the presence of sodium azide and TTC, those three isolates were identified as *Enterococcus faecalis*, while the remaining four were identified as *Lactococcus garvieae*.

*L. raffinolactis* isolates were collected from all three trials, however, they were dominant in the third trial.

All presumptive *L. raffinolactis* isolates were strongly inhibited by bacteriocin-producing *L. lactis* ATCC 11454 but weakly by *P. acidilactici* PAC1.0 and *Lc. mesenteroides* Y105. Bacteriocin-producing carnobacteria reference strains did not inhibit these isolates.

Nisin-producing *L. lactis* was inhibitory to *L. garvieae* poultry isolates, as were bacteriocin-producing *P. acidilactici* PAC 1.0 and *Lc. mesenteroides* Y105. Enterococci isolates were inhibited by *Lc. mesenteroides* Y105 and by *P. acidilactici* PAC 1.0. Bacteriocin-producing carnobacteria LV13 and LV17 did not inhibit either *L. garvieae* or *Enterococcus* poultry isolates.

### 3.3.4 Clustering of poultry isolates

The dendrogram obtained from fatty acid profiles of representative isolates is shown in Figure 3.4. Two separate clusters are observed, one grouping carnobacteria and the other grouping lactococci and enterococci. Presumptive *L. raffinolactis* isolates and the *L. raffinolactis* type strain formed a cluster distinct from other lactococci and from enterococci. Presumptive *L. lactis*, *L. garvieae*, and *E. faecalis* isolates were more closely related to each other than to *L. raffinolactis* isolates.

Clustering based on morphological and biochemical results indicated that poultry isolates fell into three major clusters (Figure 3.5). One cluster included all *L. raffinolactis* isolates and the reference strain of *L. raffinolactis* and *L. piscium*, the second cluster grouped *Carnobacterium* spp., and the third cluster grouped *L. lactis*, *L. garvieae*, and *Enterococcus* spp. Reference strains were clustered with poultry isolates of the same genus and species.

Table 3.2 Source of gram-positive organisms isolated during three trials from cooked, modified atmosphere packaged chilled poultry at 3.5°C

| Sampling periods for each trial | Number of selected colonies | Presumptive lactic acid bacteria isolates | | | | | |
|---|---|---|---|---|---|---|---|
| | | *C. divergens* | *C. piscicola* | *L. raffinolactis* | *L. garvieae* | *L. lactis* | *Enterococcus* |
| Trial 1 | | | | | | | |
| wk 4 | 8 | 4 | | 4 | | | |
| wk 5 | 4 | 4 | | | | | |
| wk 6 | 7 | 7 | | | | | |
| wk 7 | 11 | 5 | | 6 | | | |
| Trial 2 | | | | | | | |
| wk 4 | 16 | 10 | | 6 | | | |
| wk 5 | 12 | 9 | | 3 | | | |
| wk 6 | 16 | 16 | | | | | |
| wk 7 | 11 | 6 | | 5 | | | |
| Trial 3 | | | | | | | |
| wk 1 | 5 | | | 1 | 2 | | 2 |
| wk 2 | 14 | | | 11 | 2 | | 1 |
| wk 3 | 17 | | | 17 | | | |
| wk 4 | 17 | | 8 | 9 | | | |
| wk 5 | 9 | | | 9 | | | |
| wk 6 | 30 | | | 29 | | 1 | |
| wk 7 | 20 | | 3 | 17 | | | |

Table 3.3 Phenotypic characteristics of lactic acid bacteria isolated from cooked, modified atmosphere packaged poultry meat (number of isolates in each group is shown in parentheses)

| | Meat isolates (197) | | | | | |
|---|---|---|---|---|---|---|
| | *L. raffinolactis* (117) | *L. garvieae* (4) | *L. lactis* (1) | *C. divergens* (61) | *C. piscicola* (11) | *E. faecalis* (3) |
| Morphology | **Cocci/ovoid** | **Cocci/ovoid** | **Cocci/ovoid** | **Rods** | **Rods** | **Cocci** |
| Lactic acid isomer | **L-(+)** | **L-(+)** | **L-(+)** | **L-(+)** | **L-(+)** | **L-(+)** |
| *meso*-DAP | - | - | - | + | + | - |
| Motility | - | - | - | - | - | - |
| Gas from glucose | - | - | - | V[a] | - | - |
| Growth | | | | | | |
| at 3.5°C | + | - | - | + | + | - |
| at 40°C | - | + | - | - | - | + |
| at 45°C | - | - | - | - | - | + |
| at 50°C | - | - | - | - | - | - |
| in 3% NaCl | + | + | + | + | + | + |
| in 6.5% NaCl | - | + | - | +[wb] | +[w] | + |
| at pH 5.0 | - | +[w] | + | - | - | +[w] |
| at pH 5.5 | + | + | + | + | + | + |
| at pH 9.5 | V | + | + | + | + | + |

Table 3.3 (*continued*)

| | Meat isolates | | | | | |
|---|---|---|---|---|---|---|
| | *L. raffinolactis* (117)[a] | *L. garvieae* (4) | *L. lactis* (1) | *C. divergens* (61) | *C. piscicola* (11) | *E. faecalis* (3) |
| CHO utilization | | | | | | |
| Amygdalin | V | + | - | + | + | + |
| L-Arabinose | V | - | - | - | - | - |
| Arbutin | V | + | - | + | + | + |
| Galactose | + | + | + | + | V | + |
| α-$CH_3$-D-glucoside | V[c] | V[d] | - | - | +[w] | - |
| Glycerol | (-)[e] | - | - | + | + | + |
| Inositol | - | - | - | - | - | + |
| Inulin | V[c] | - | - | - | + | - |
| Lactose | V | - | + | V[d] | V | + |
| Mannitol | V | + | + | - | + | + |
| Melezitose | V | - | - | - | + | + |
| Melibiose | + | - | - | - | - | - |
| Raffinose | + | - | - | - | - | - |
| Rhamnose | - | - | - | - | - | + |
| Ribose | V[d] | + | + | + | + | + |
| Sorbitol | - | - | - | - | V | + |

Table 3.3 (*continued*)

| | Meat isolates | | | | | |
|---|---|---|---|---|---|---|
| | *L. raffinolactis* (117)[a] | *L. garvieae* (4) | *L. lactis* (1) | *C. divergens* (61) | *C. piscicola* (11) | *E. faecalis* (3) |
| Sucrose | + | + | - | + | + | + |
| Trehalose | V | + | + | + | + | + |
| D-xylose | V | - | - | - | - | - |
| Ammonia from arginine | - | + | + | + | + | + |
| Aesculin hydrolysis | + | + | + | + | + | + |
| Dextran from sucrose | - | - | - | - | - | - |
| Growth on acetate agar | - | - | - | - | - | - |
| in acetate broth | - | +[w] | +[w] | - | - | +[w] |
| End-point pH | 3.8-4.0 | 3.9-4.0 | 3.7 | 4.1-4.3 | 4.1-4.3 | 3.8 |
| Curd formation in Litmus milk | V | - | + | - | - | + |

[a]V, variable property in this group, positive or negative
[b]+[w] weakly positive
[c]positive,weakly positive, delayed positive, or negative
[d]Negative or delayed positive
[e]Negative, or delayed weakly positive

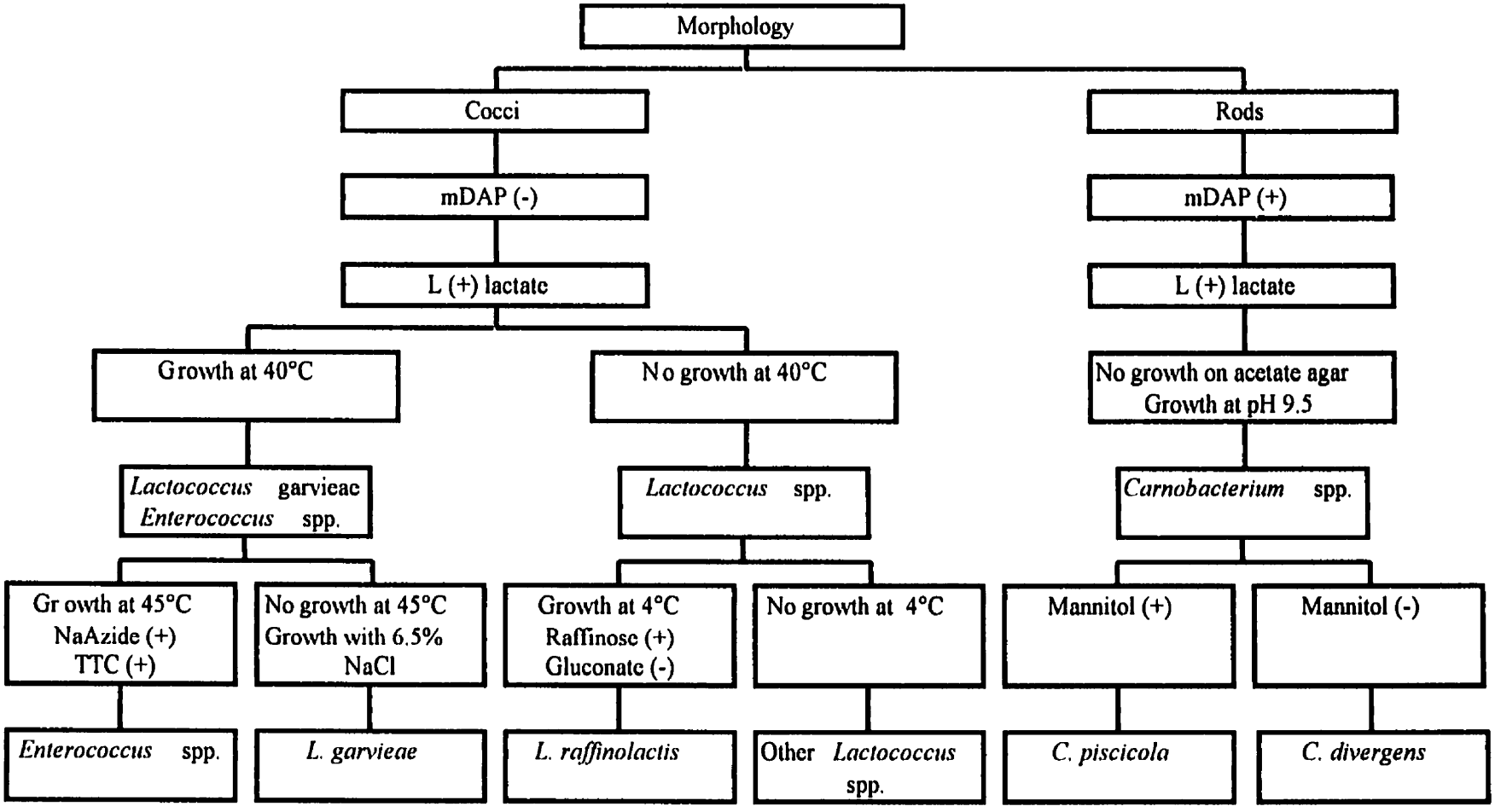



Figure 3.1 Scheme used for the preliminary identification of lactic acid bacteria isolated from cooked, modified atmosphere packaged, refrigerated poultry at 3.5°C.

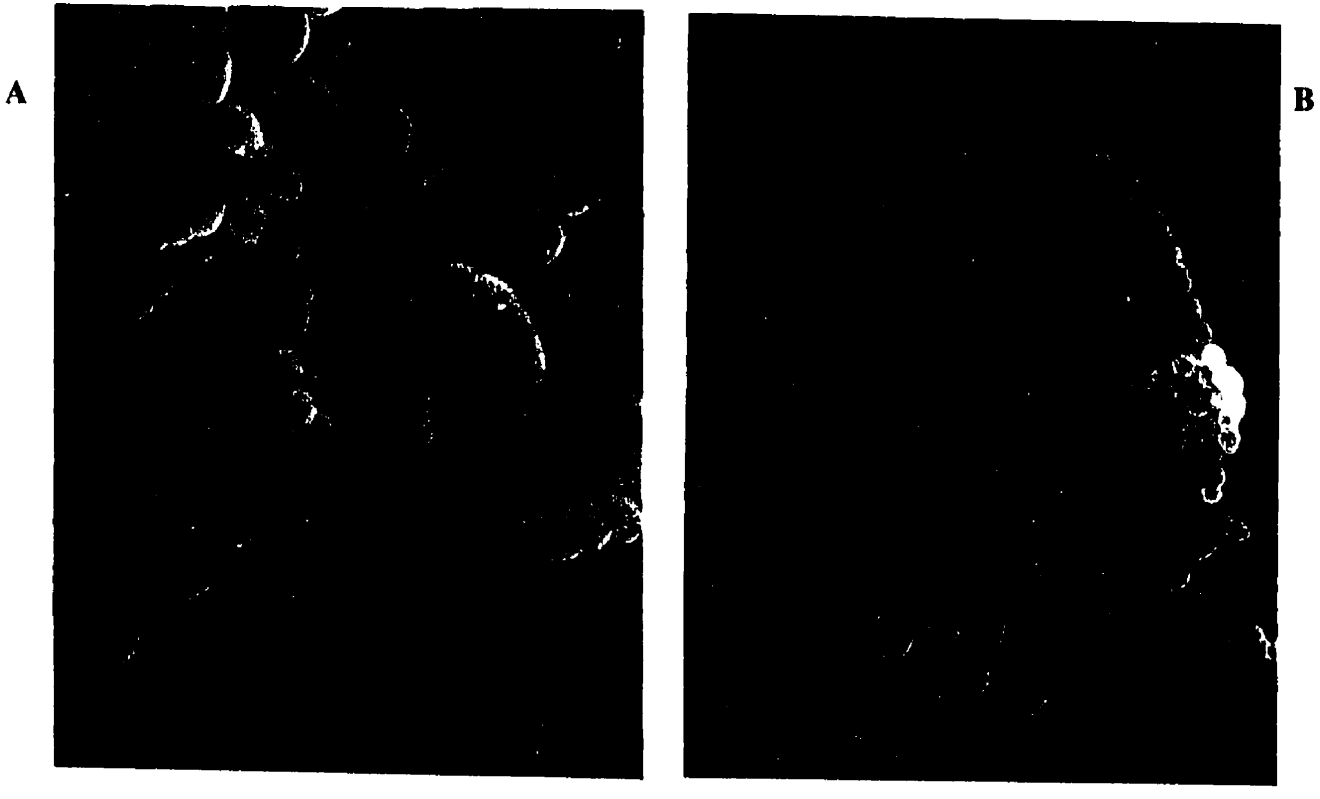



Figure 3.2 Scanning electron micrographs of *L. raffinolactis* poultry isolate 2113; (A) ovoid, rod-like morphological appearance (magnified 9000 X; bar = 3.0 μm); (B) formation of long chains (magnified 4500 X; bar = 6.0 μm).


Figure 3.3 Scanning electron micrographs of *L. raffinolactis* (magnified 9000 X; bar = 3.0 μm); (A) poultry isolate 1701; (B) *L. raffinolactis* type strain NCFB 617.

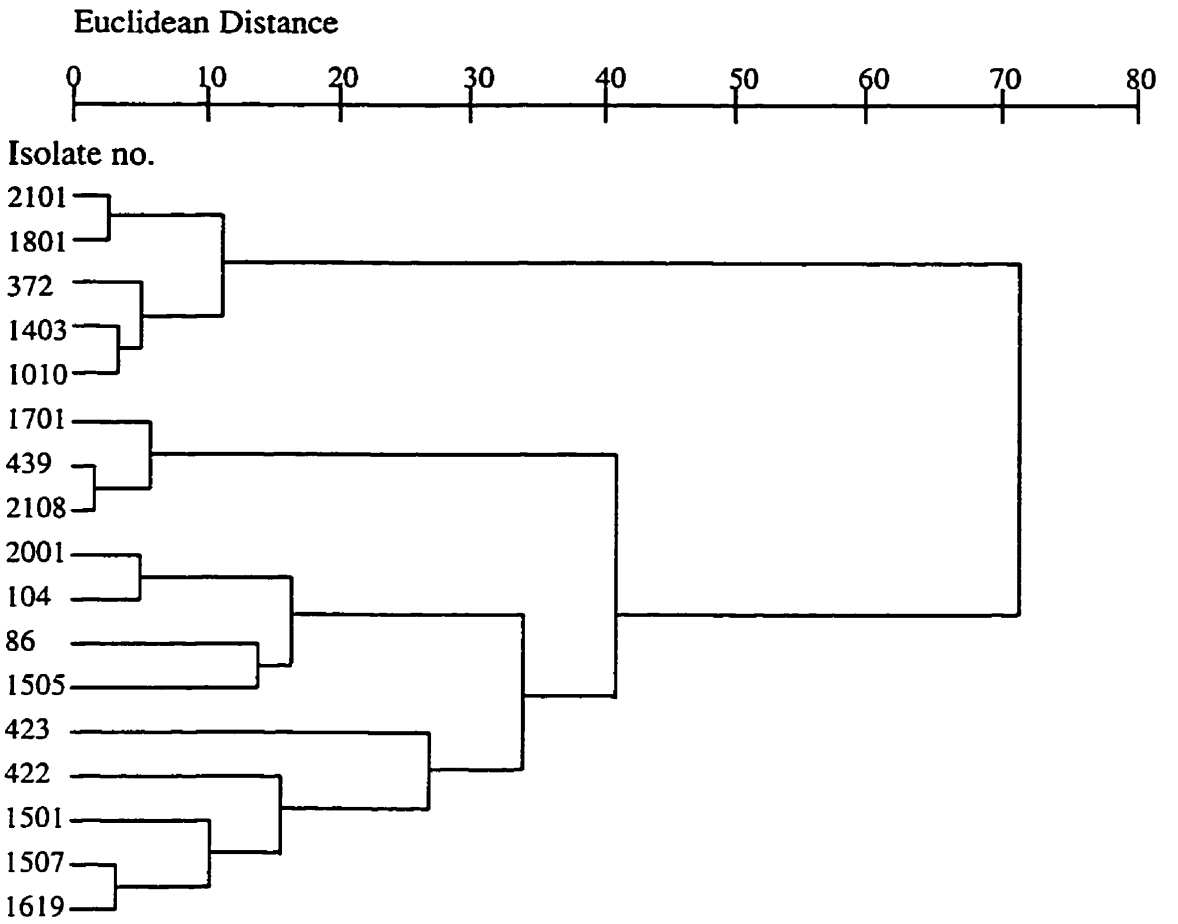



Figure 3.4 Dendrogram derived from the fatty acid profiles of selected poultry isolates and reference cultures, showing the relatedness of the different strains/species/genera

| Poultry isolates | | Reference strains | |
|---|---|---|---|
| *C. piscicola* | 1801, 2101 | *C. divergens* LV13 | 372 |
| *C. divergens* | 1403, 1010 | *L. raffinolactis* NCFB 617 | 439 |
| *L. raffinolactis* | 1701, 2108 | *L. lactis* NCK 401 | 104 |
| *L. lactis* | 2001 | *L. lactis* ATCC 7962 | 86 |
| *L. garvieae* | 1505 | *E. faecalis* ATCC 33186 | 423 |
| *E. faecalis* | 1501, 1507, 1619 | *E. faecium* ATCC 19434 | 422 |

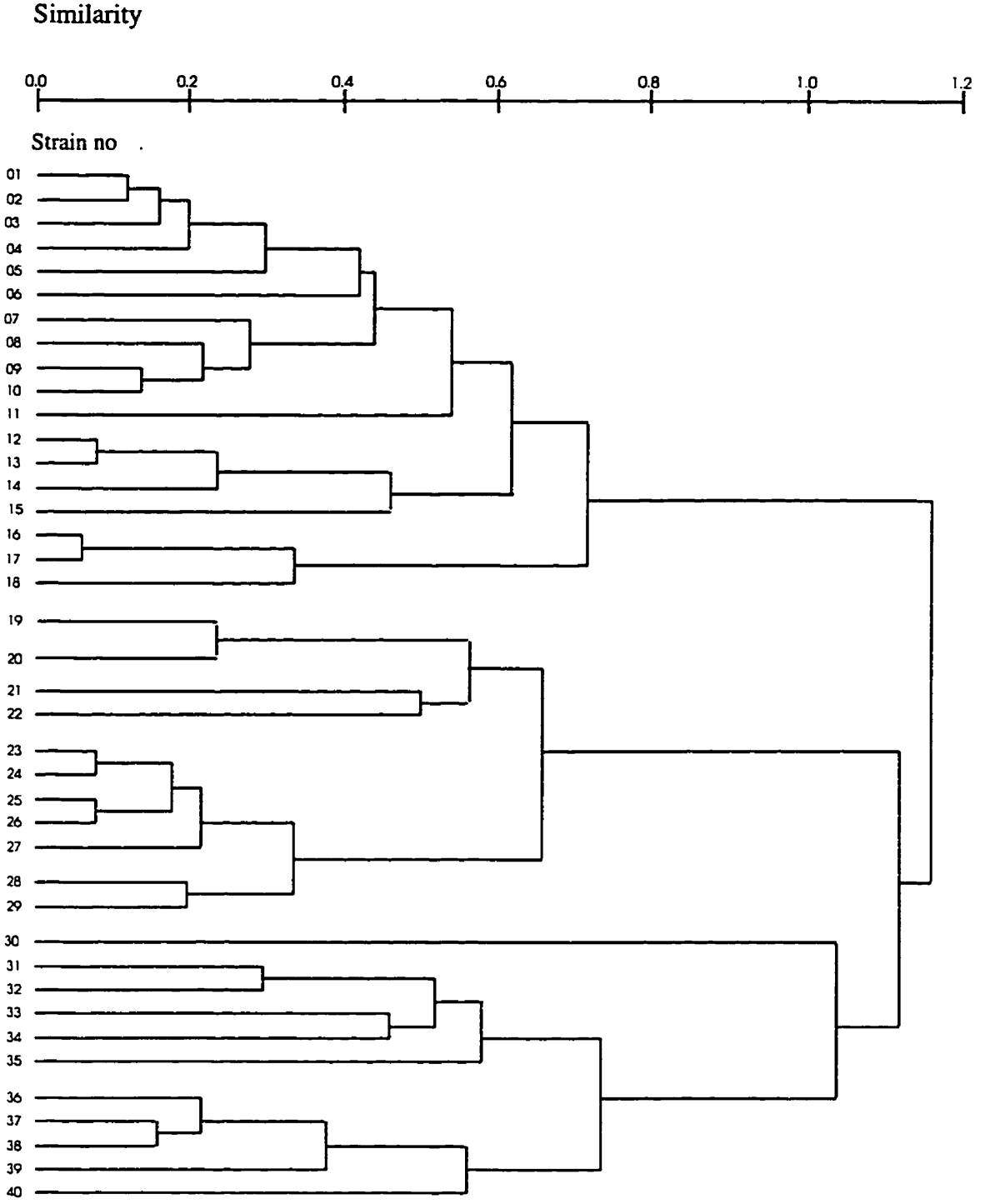



Figure 3.5 Dendrogram depicting the phenotypic relatedness among poultry isolates of the genera *Carnobacterium*, *Enterococcus*, and *Lactococcus* based on numerical analysis of biochemical and morphological data. Organisms were clustered using Jaccard's index and the UPGMA method (*Refer to table on following page for organism identification*)

Table 3.4 Identification table for organisms shown in Fig. 3.4

| Dendrogram number | Identity |
|---|---|
| 01 to 10 | *L. raffinolactis* poultry isolates |
| 11 | *L. raffinolactis* type strain |
| 12 to 14 | *L. raffinolactis* poultry isolates |
| 15 | *L. piscium* type strain |
| 16 to 18 | *L. raffinolactis* poultry isolates |
| 19 | *C. divergens* poultry isolate |
| 20 | *C. divergens* LV13, reference strain |
| 21 | *C. gallinarum* type strain |
| 22 | *C. mobile* type strain |
| 23 to 25 | *C. piscicola* isolates |
| 26 | *C. piscicola* type strain |
| 27, 28 | *C. piscicola* poultry isolates |
| 29 | *C. piscicola* reference strains |
| 30 | *L. plantarum* type strain |
| 31 | *L. lactis* ssp. *lactis* 14365 |
| 32 | *L. lactis* ssp. *lactis* 11454 |
| 33 | *L. lactis* poultry isolate |
| 34 | *L. lactis* ssp. *cremoris* 7962 |
| 35 | *L. garvieae* poultry isolate |
| | *L. garvieae* type strain (identical profiles) |
| 36 | *E. faecalis* 33186 |
| 37 (1619, 1501) | *E. faecalis* poultry isolates |
| 38 | *E. faecalis* type strain |
| 39 | *E. faecium* type strain |
| 40 | *E. durans* reference strain |

### 3.3.5 Production of inhibitory substances

Antibacterial substances were produced by all *C. piscicola* strains, active against reference carnobacteria *C. divergens* LV13 and *C. piscicola* LV17, *C. divergens* and *Enterococcus* spp. poultry isolates, *E. faecalis* ATCC 33186, *Li. grayi* ATCC 19120, *Li. ivanovnii* ATCC 19119, *Li. murrayi* ATCC 25401, *Li. innocua* ATCC 33091, and *Li. monocytogenes* reference strains LI 500, LI 503, LI 512, LI 514, LI 531, LI 533, LI 540, LI 550, LI 564, and LI 576. The antibacterial agent did not inhibit *Li. welshimerii* ATCC 35897, *Li. monocytogenes* LI527 (Scott A) or *Li. monocytogenes* LI521 (Murray B), *L. raffinolactis* or *L. garvieae* poultry isolates.

The activity of the antibacterial substances was not affected by treatment with catalase or by heating at 64°C. The activity of the inhibitory agents was lost when the supernatant was treated with pronase, indicating that inhibition was due to a proteinaceous agent.

## 3.4 DISCUSSION

Strains of *L. raffinolactis*, an organism seldom isolated or encountered in the literature, were isolated from the cooked modified atmosphere packaged poultry in all trials. Spontaneously soured milk isolates of *L. raffinolactis* were originally named and characterized by Orla-Jensen and Hansen (1932). Garvie (1979) proposed raw milk isolates (Garvie, 1953) as the neotype strain of *L. raffinolactis*. Strains of *L. raffinolactis* available in bacterial culture collections were originally isolated from raw milk, garden carrots and termite gut. Schillinger and Lücke (1987b) documented the isolation of *L. raffinolactis* from raw, vacuum-packed beef and pork meat and noted the highly

competitive behaviour of that species when inoculated on vacuum-packed raw meat with other LAB. It was the authors' belief that the microbiota of the cutting room influenced the development of the microbiota on the meat. *L. raffinolactis* were dominant in VP raw beef taken from one cutting room, but were seldom isolated from meat samples obtained from three other cutting rooms. More recently, Klijn *et al.* (1995) reported the isolation of *Lactococcus* species from dairy and non-dairy environments. Strains of *L. raffinolactis* were isolated from cheese plant samples (grass, wastewater tank, and wastewater disposal site soil), and from farm samples of soil and silage. Pot *et al.* (1996) isolated *Lactococcus* species from domestic animal species, including ruminants, cats, and dogs, and from milk samples taken from dairy cows. *L. raffinolactis* strains were isolated from goose intestine and bovine tonsils.

The cooking step in the preparation of the poultry samples was severe enough to result in a commercially sterile product. In a preliminary study, samples of cooked poultry were aseptically collected off-the-cooker or off-the-freezer belt in the production plant, MAP in 20:80 $CO_2$:$N_2$ and stored at 3.5°C for 8 weeks. Microbiological counts were below the detection limit (<100 CFU/g) for the duration of the study for all populations enumerated in both off-the-cooker and off-the-freezer samples. Meat samples for this study were collected off the packaging belt in the production room on three successive occasions within a period of 2 months (Chapter 2). Table 3.2 indicates that *L. raffinolactis* strains were present in poultry samples from all three trials. They were predominant in samples from trial three by the third week of storage.

The presence and dominance of *L. raffinolactis* spp. does not appear to be a chance occurrence. The scarcity of reports about the presence of *Lactococcus* spp. in meat products could be due to a failure to look for them and a belief that the organisms are associated with dairy and vegetable products. Isolates may be classified as 'atypical' strains or remain unidentified. The morphology of lactococci may be a factor that hinders their proper identification. Phase-contrast microscopic examination of *L. raffinolactis* isolates indicated that ovoid cells may be mistaken for rods. Morphology of selected strains of presumptive *L. raffinolactis* had to be confirmed by electron microscopy (Figures 3.2 and 3.3). Mauguin and Novel (1994) reported similar problems when identifying *Lactococcus* spp. from seafood.

Colonies selected from plates of aerobes, anaerobes and psychrotrophs in the third trial were the same population. Isolates of *L. raffinolactis* were clustered based on morphological and biochemical data. Isolates with identical profiles did not necessarily correspond to sampling weeks or to trials.

A review of the taxonomy and identification of LAB indicates that reports of 'atypical' organisms are not uncommon. Until recently, *Carnobacterium* spp, previously classified as 'atypical lactobacilli' (Shaw and Harding, 1985), were believed to be associated with MAP meat products and fish (Hiu *et al.*, 1984; Baya *et al.*, 1991). Millière *et al.* (1994) reported their isolation from the surface of mould-ripened soft-cheeses. In this study, *C. divergens* strains (61) were isolated from poultry samples in the first and second trial, while *C. piscicola* (11) isolates were present in samples from week five and week seven in the third trial. Presence of a specific *Carnobacterium* spp. does not appear to be as

consistent as presence of *L. raffinolactis* organisms. Isolates of *C. divergens* from trials one and two had identical carbohydrate fermentation patterns which may imply that the same strain was present on the different samples of poultry. The eight *C. piscicola* isolates from week four samples (trial three) had close carbohydrate fermentation patterns, and were clearly different from the week seven isolates. Week four isolates were able to ferment lactose and galactose while week seven isolates could ferment sorbitol.

*L. garvieae* and *E. faecalis* strains were isolated from poultry samples at the end of the first week of storage in trial three. No organisms were isolated from freshly packaged and one week-old samples in the first two trials. The absence of these organisms from subsequent packages in trial three could be explained by their mesophilic nature and, so, they were unable to grow at the storage temperature of the poultry products. Whether enterococci and species of lactococci other than *L. raffinolactis* are more common on meat products cannot be inferred from the results of this study.

The results of the cluster analysis based on carbohydrate fermentation patterns or on fatty acid methyl esters are quite different. While carnobacteria were clearly separated from cocci-shaped organisms in the composition of their cell wall fatty acids, the numerical analysis showed them to be more similar to enterococci, *L. garvieae,* and *L. lactis* in their biochemical properties. With both methods, *L. garvieae*, *L. lactis* and enterococci were more closely related to each other than to *L. raffinolactis*. Phylogenetic analyses of lactic acid bacteria indicate that carnobacteria and enterococci are more closely related to each other than to lactococci (Stiles and Holzapfel, 1997). Alignments of 16S rRNA

sequences of carnobacteria, enterococci, and lactococci using the PC/GENE® (IntelliGenetics, Mountain View, Inc., CA) program, result in higher similarity scores for carnobacteria and enterococci.

Bacteriocin-producing carnobacteria have been extensively studied (Ahn and Stiles, 1990; Schillinger and Holzapfel, 1990; Buchanan and Klawitter, 1992a) and are often isolated from meat products. All *C. piscicola* isolated from the cooked MAP poultry meat produced an inhibitory substance, active against enterococci, other carnobacteria, and several strains of *Li. monocytogenes*. Much interest has been directed towards the use of bacteriocin-producing carnobacteria as 'protective' cultures in food products. Although carnobacteria grew faster than *L. raffinolactis* isolates at refrigeration temperature (4°C) in a liquid medium and *C. piscicola* isolates produce inhibitory substances, these properties did not give them a competitive advantage over *L. raffinolactis* strains in the third trial. *L. raffinolactis* isolates were the most competitive of the species when *L. raffinolactis*, *Lb. sake* and *C. divergens* were inoculated onto raw meat which was subsequently vacuum-packaged and stored at 2°C for 30 days (Schillinger and Lücke, 1987b).

The method used in this study to select 'representative' colonies from total aerobic plate counts, based on colour and colony appearance was very subjective and depended solely on the judgment of the investigator. Different species of lactic acid bacteria often look similar on a general-purpose media such as plate count agar. Anyone familiar with the intensive labour required in traditional identification methods would understand the necessity for more rapid and more reliable identification methods such as nucleic-acid

based techniques. In the past years, several species of lactic acid bacteria have been reported to occur in various 'unexpected' food matrices and other habitats, and display uncharted growth patterns leading to 'atypical' strains reported in almost every taxonomy paper. The use of DNA-based techniques to identify bacterial organisms is becoming increasingly popular and LAB classification will be more easily achieved. For example, the use of 16S rRNA-targeted oligonucleotide probes in hybridization experiments would allow screening of whole plates within a fraction of the time required for traditional identification. Unfortunately, these methods are still restricted to large, well-equipped laboratories with a comprehensive database of oligonucleotide probes for the identification of lactic acid bacteria.

# CHAPTER 4 IDENTIFICATION OF CARNOBACTERIA AND LACTOCOCCI USING SPECIES-SPECIFIC 16S RRNA PCR PRIMERS

## 4.1 INTRODUCTION

Lactic acid bacteria (LAB) are widely distributed in the environment. They play a major role in the manufacture of fermented foods and are the dominant microbiota in modified atmosphere packaged meats (Shaw and Harding, 1984). Traditional classification methods based on morphology, physiology, and biochemical tests are time consuming and may be misleading. In recent years, several changes have occurred in the classification of LAB, and phylogenetic approaches to identification have become a major part of taxonomic studies (Stiles and Holzapfel, 1997). Automated sequencing methods and the availability of computational tools and publicly accessible genomic databases have accelerated the development of genetic-based methods for the identification of microorganisms. Several reports (Klijn *et al.*, 1991; Brooks *et al.*, 1992; Betzl *et al.*, 1990; Ehrmann *et al.*, 1994; Hertel *et al.*, 1991, 1993; Nissen *et al.*, 1994; Tilsala-Timisjärvi and Alatossava, 1997) have been published on the identification of different species of LAB using the polymerase chain reaction (PCR) to amplify ribosomal RNA sequences and specific DNA probes targeted to the 16S, 23S, or the 16S to 23S rRNA internal transcribed spacer regions.

The biochemical characterization of *Carnobacterium* spp. and *Lactococcus* spp., isolated from cooked, modified atmosphere packaged, refrigerated poultry products, was reported in Chapter 3. *Carnobacterium* species are generally associated with meat products (Shaw

and Harding, 1984, 1985); however, Millière *et al.* (1994) reported their isolation from mould-ripened cheese. They are mainly characterized by their inability to grow on acetate agar, low acid tolerance, and growth at pH 9.5. *Lactococcus* spp. are most often isolated from dairy and vegetable products. Recent reports have shown these organisms to be associated with meat products as well (Garver and Muriana, 1993; Mauguin and Novel, 1994; Rodriguez *et al.*, 1995). The morphology of lactococci may hinder correct identification. Cells are often ovoid and may be misclassified as rod-shaped lactobacilli or carnobacteria. While preliminary phenotypic characterization steps are still necessary, genetic-based techniques are invaluable to reduce the time required for correct identification to the species level.

This chapter reports the identification of *Carnobacterium* and *Lactococcus* spp. by PCR using 16S rDNA-targeted species-specific primers.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Sequence analysis

16S rRNA sequences were retrieved from the sequence databases at the National Center for Biotechnology Information (NCBI) using the RETRIEVE E-mail server (Bethesda, MD). Primer similarity searches were performed using the NCBI BLAST E-mail server (Altschul *et al.* 1990). The PC/GENE® (IntelliGenetics, Mountain View, Inc., CA) program was used for sequence alignments to determine variable regions of the 16S rRNA and to design primers.

### 4.2.2 Bacterial strains

Unless otherwise specified, all growth media were from Difco.

The reference strains used in this study are listed in Table 4.1. Representative strains of LAB isolated from cooked poultry meat packaged in 40:60 $CO_2$:$N_2$ (Chapter 2) and reference strains were maintained on APT agar (Difco) plates at 4°C and transferred monthly. For DNA extraction, bacteria were cultured overnight in APT broth or agar at 28°C for carnobacteria and strains of *L. raffinolactis*, 22°C for *Brochothrix thermosphacta*, and 30°C for all other strains.

Table 4.1 Reference strains used to test the specificity of the PCR primers

| Strain | Reference |
|---|---|
| *Bacillus cereus* | ATCC 14579 |
| *Brochothrix thermosphacta* | ATCC 11509; Type strain |
| *Carnobacterium divergens* | ATCC 35677[a]; Type strain |
| *C. divergens* | UAL 278[b] |
| *C. divergens* | LV13[bc] |
| *C. gallinarum* | ATCC 49517; Type strain |
| *C. mobile* | ATCC 49516; Type strain |
| *C. piscicola* | UAL 26[b] |
| *C. piscicola* | UAL 43225[b] |
| *C. piscicola* | UAL 43224[b] |
| *C. piscicola* | ATCC 35586; Type strain |
| *C. piscicola* | LV17[bc] |
| *C. piscicola* | UAL8[b] |
| *Enterococcus faecium* | ATCC 19434; Type strain |
| *E. faecalis* | ATCC 19433; Type strain |
| *E. durans* | ATCC 19432; Type strain |
| *Lactobacillus buchneri* | ATCC 9460; Type strain |
| *Lb. casei* | ATCC 11578 |
| *Lb. sake* | DSM 20017[d] |
| *Lb. acidophilus* | ATCC 4356; Type strain |
| *Lb. delbruckii* | ATCC 9649 |
| *Lb. brevis* | ATCC 3648 |
| *Lactococcus garvieae* | NCFB 2155[e]; Type strain |
| *L. piscium* | NCFB 2778; Type strain |
| *L. plantarum* | NCFB 1869; Type strain |
| *L. raffinolactis* | NCFB 617; Type strain |
| *L. lactis* ssp. *lactis* | NCK 401[f] |
| *L. lactis* ssp. *lactis* | ATCC 7962 |
| *L. lactis* ssp. *lactis* | ATCC 11454 |
| *L. lactis* ssp. *cremoris* | ATCC 14365 |
| *Leuconostoc mesenteroides* | Y105[bg] |
| *Lc. gelidum* | UAL-187[bh] |
| *Listeria monocytogenes* | LI 527; Scott A[i] |
| *Micrococcus luteus* | ATCC 7468 |
| *Pediococcus acidilactici* | PAC 1.0[bj] |

Table 4.1 *(continued)*

| Strain | Reference |
|---|---|
| *Streptococcus mitis* | NCFB 2495; Type strain |
| *Weissella viridescens* (previously *Lb. viridescens*) | ATCC 12706 |

[a]ATCC, American Type Culture Collection (Rockville, MD)
[b]UAL, University of Alberta Lactic Acid Bacteria Collection, University of Alberta, (Edmonton, AB)
[c]Strains originally from Dr. B. G. Shaw, Institute of Food Research, Bristol, UK; obtained from Dr. M. E. Stiles, University of Alberta
[d]DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany
[e]NCFB, National Collection of Food Bacteria (Agricultural and Food Research Council, Institute of Food Research, Reading, UK)
[f]North Carolina State University, Raleigh, NC; Harris *et al.*, 1992
[g]Héchard *et al.*, 1992; obtained from Dr. M. E. Stiles, University of Alberta
[h]Hastings and Stiles, 1991; obtained from Dr. M. E. Stiles, University of Alberta
[i]*Listeria* spp. were obtained from Dr. A. Lammerding (Health of Animals Laboratory, Health Canada, Guelph, ON)
[j]Gonzalez and Kunka, 1987; obtained from Dr. M. E. Stiles, University of Alberta

### 4.2.3 Crude DNA extraction

For DNA amplification, cells were either harvested from broth cultures or from agar plates. Cells from 50 µl overnight cultures (in APT or M17G broth) were pelleted, washed once with an equal volume of sterile ultrapure water (up-water), pelleted again and resuspended in 50 µl of sterile up-water, or colonies from agar plates were resuspended in 50 µl of sterile up-water. Suspensions prepared from agar or broth were placed in boiling water for 10 min and pelleted. The supernatant (0.5 µl) was used for PCR amplification reactions. Alternatively, 0.5 µl of the suspension was transferred to PCR amplification tubes (Gordon Technologies, Mississauga, ON) and heated to 99°C for 5 min in the thermal cycler prior to the addition of the PCR reaction mix.

### 4.2.4 PCR primers

Primer sequences, orientations and annealing positions are summarized in Table 4.2. For *Carnobacterium* species, a universal forward primer 27f and three specific reverse primers Cdi, Cmo, and Cpg were used to amplify target regions (198 to 199-bp) of the 16S rDNA of *C. divergens*, *C. mobile*, and *C. piscicola/C. gallinarum*, respectively. The reverse primers were designed from the domains of least homology among species of carnobacteria. Primers Cdi and Cmo were previously used by Brooks *et al.* (1992) for the identification of *Carnobacterium* spp. by DNA hybridization. *C. piscicola* and *C. gallinarum* share more than 96% homology in the 16S rRNA and the Cpg primer will amplify both *C. piscicola* and *C. gallinarum*. To differentiate between the two species, a

forward primer, Cga, was designed for *C. gallinarum*. Cga and Cpg were used to amplify a 128-bp region of the 16S rRNA of *C. gallinarum*.

For the identification of *Lactococcus* spp., forward primer 27f and three species-specific reverse primers were used to amplify target sequences of *L. lactis*, *L. garvieae*, and *L. raffinolactis*. Primer pairs 27f-Lla (for *L. lactis*), 27f-Lga (for *L. garvieae*) and 27f-Lra (for *L. raffinolactis*) should give products of 87-bp, 90-bp, and 203-bp respectively. Primers Lla and Lga were previously used by Klijn *et al.* (1991).

To ascertain the presence of genetic material in the crude DNA extracts, PCR reactions were set up using 16S rDNA-targeted universal primers 27-forward (27f) and 100-reverse (100r). Similar amplification conditions were used.

Table 4.2 Sequences and positions of the universal and specific primers used for PCR

| PCR primer | Primer sequence (5' to 3') | Position[a] | Orientation |
|---|---|---|---|
| 27f | AGAGTTTGATCMTGGCTCAG[b] | 8-27 | forward |
| 100r | ACTCACCCGTTCGCYRCTC[b] | 100-118 | reverse |
| Cdi[c] | GCGACCATGCGGTCACTTGAA | 185-206 | reverse |
| Cga | GGAAAGCTTNCTTTCTAACC[b] | 77-97 | forward |
| Cmo | TCCACCAGGAGGTGGTTGGAGT | 184-206 | reverse |
| Cpg | GAATCATGCGATTCCTGAAAC | 184-205 | reverse |
| Lla[d] | CAGTCGGTACAAGTACCAAC | 72-91 | reverse |
| Lga[d] | CATAAAAATAGCAAGCTATC | 75-94 | reverse |
| Lra | TGTCGAATATGCATCCAAC | 189-207 | reverse |

[a]*E. coli* numbering system
[b]Mixed mers symbols M=(A,C); Y=(C,T); R=(A,G); N=(A,C,G,T)
[c]Primer sequence similar to that of Brooks *et al.* (1992)
[d]Primer sequences similar or identical to those of Klijn *et al.* (1991)

### 4.2.5 Amplification of target DNA

*Taq* DNA polymerase, amplification buffers, and deoxynucleoside triphosphates were purchased from Boehringer Mannheim (Boehringer Mannheim GmbH, Mannheim, Germany).

PCR amplifications were carried out in 50 µl volumes, with the following reagents: 1 x Taq buffer, 1 µM of each dNTP mix, 25 ng of each primer, 0.5 µl of crude DNA extract or bacterial suspension, and 0.5 U of *Taq* DNA polymerase. Following an initial denaturation step of the template DNA at 94°C for 10 min, the remaining reaction components were added and the mixtures were subjected to 35 successive cycles of denaturation (94°C, 30 s), annealing (55°C, 45 s), and extension (72°C, 1 min) in an automated DNA thermal cycler (GeneAmp® PCR system 2400; Perkin-Elmer Cetus, Norwalk, CT). The amplification reactions were terminated by an elongation step of 3 min at 72°C. The whole reaction mixture was visualized on a 3% agarose gel in Tris-acetate buffer containing ethidium bromide (Sigma), and photographed on a UV transilluminator (2011 Macrovue Transilluminator, LKB, Bromma, Sweden).

## 4.3 RESULTS

The results for PCR amplification reactions using universal primers and species-specific primers are summarized in Tables 4.3 and 4.4. Amplification products using universal primers were obtained with all reference strains tested (Table 4.1). The PCR products expected are 199-bp in length for *C. divergens*, 198-bp for *C. mobile* and *C. piscicola*, and 128-bp for *C. gallinarum* (with the Cga-Cpg primer pair). Expected products sizes

are 87-bp in for *L. lactis* ssp. *lactis*, 90-bp for *L. garvieae*, and 203-bp for *L. raffinolactis*. No false positives were observed with any of the other bacterial species tested. Fig. 4.1 and 4.2 illustrate the results obtained with the *Carnobacterium* and *Lactococcus* species-specific primers, respectively.

Results of the PCR-amplification reactions from presumptive *Carnobacterium* poultry meat isolates were consistent with the biochemical test results. With mannitol-negative strains, identified as *C. divergens*, a PCR product was observed only with the 27-Cdi primer pair, while for mannitol-positive isolates identified as *C. piscicola*, a PCR product was obtained exclusively with the 27-Cpg primer pair.

Representative poultry isolates identified as *L. raffinolactis* gave PCR products of identical size to the product obtained with the type strain of *L. raffinolactis*. Similarly, the identity of suspect *L. garvieae* was confirmed using primer pair 27f-Lga. No product was observed with the poultry isolate identified as *L. lactis* using primer pair 27f-Lla. When control strains of *L. lactis* ssp. *lactis* and one strain of *L. lactis* ssp. *cremoris* were tested with this primer pair, only *L. lactis* ssp. *lactis* strains were positive. *L. lactis* ssp. *hordniae* was not tested, however, the work of Klijn *et al.* (1991) indicates that primer Lla is specific to *L. lactis* ssp. *lactis*.

Table 4.3 Differentiation of *Carnobacterium* species using 16S rDNA-targeted PCR primers

| Species | No. tested | Primer pairs | | | | |
|---|---|---|---|---|---|---|
| | | 27-Cdi | Cga-Cpg | 27-Cmo | 27-Cpg | 27-100 |
| *Carnobacterium divergens*[a] | 3 | + | - | - | - | + |
| *C. gallinarum*[a] | 1 | - | + | - | + | + |
| *C. mobile*[a] | 1 | - | - | + | - | + |
| *C. piscicola*[a] | 6 | - | - | - | + | + |
| Other species tested[b] | 26 | - | - | - | - | + |
| Poultry isolates | | | | | | |
| *C. divergens* | 20 | + | - | - | - | + |
| *C. piscicola* | 11 | - | - | - | + | + |

[a]Reference strains; Table 4.1
[b]Table 4.1

Table 4.4 Differentiation of *Lactococcus* species using 16S rDNA-targeted PCR primers

| Species | No. tested | Primer pairs | | | |
|---|---|---|---|---|---|
| | | 27-Lla | 27-Lga | 27-Lra | 27-100 |
| *Lactococcus lactis* ssp. *cremoris*[a] | 1 | - | - | - | + |
| *Lactococcus lactis* ssp. *lactis*[a] | 3 | + | - | - | + |
| *L. garvieae*[a] | 1 | - | + | - | + |
| *L. raffinolactis*[a] | 1 | - | - | + | + |
| Other species tested[b] | | - | - | - | + |
| Poultry isolates | | | | | |
| *L. lactis* | 1 | - | - | - | + |
| *L. garvieae* | 3 | - | + | - | + |
| *L. raffinolactis* | 30 | - | - | + | + |

[a]Reference strains; Table 4.1
[b]Table 4.1

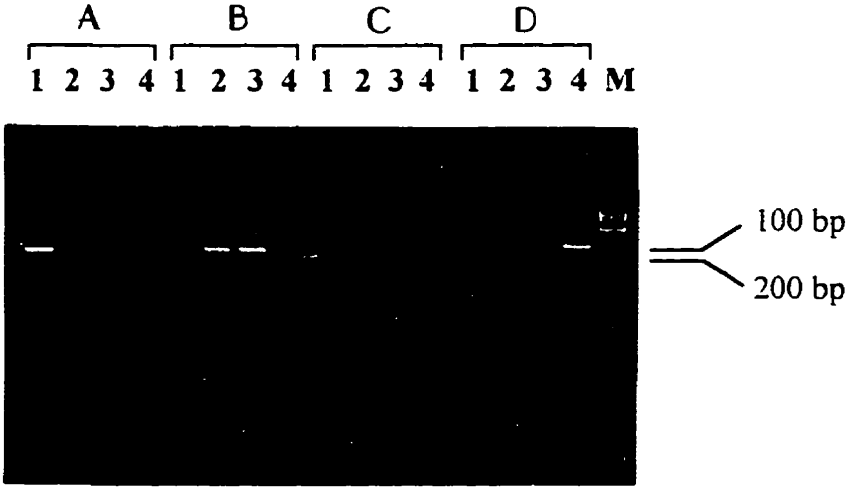



Figure 4.1 PCR products from *Carnobacterium* type strains, obtained using 16S rRNA-targeted, species-specific primers

| | | | |
|---|---|---|---|
| Series A | 27f-Cdi primer pair | Lane 1 | *C. divergens* ATCC 35677 |
| Series B | 27f-Cpg primer pair | Lane 2 | *C. piscicola* ATCC 35586 |
| Series C | Cga-Cpg primer pair | Lane 3 | *C. gallinarum* ATCC 49517 |
| Series D | 27f-Cmo primer pair | Lane 4 | *C. mobile* ATCC 49516 |
| | | Lane M | 100 bp DNA ladder |

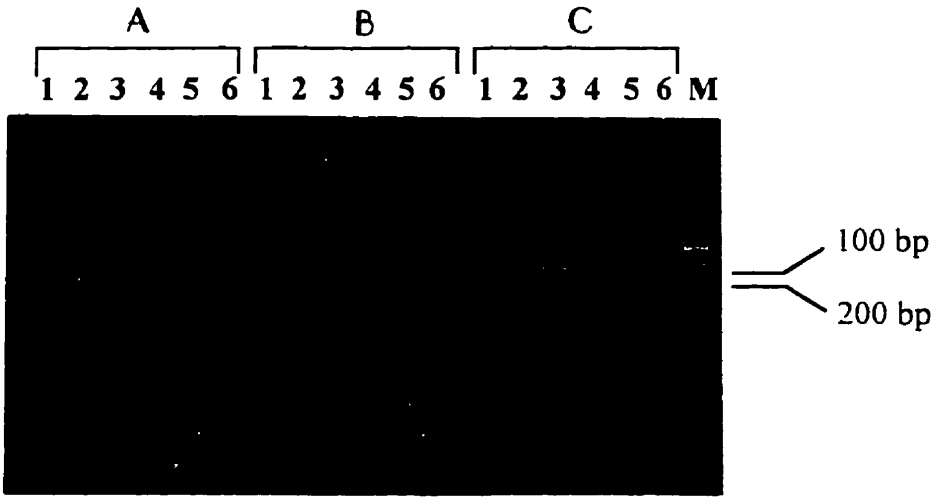



Figure 4.2 PCR products from *Lactococcus* species, obtained using 16S rRNA-targeted, species-specific primers

| | | | |
|---|---|---|---|
| Series A | 27f-Lga primer pair | Lane 1 | *L. lactis* ssp. *cremoris* ATCC 14365 |
| Series B | 27f-Lla primer pair | Lane 2 | *L. lactis* ssp. *lactis* ATCC 7962 |
| Series C | 27f-Lra primer pair | Lane 3 | *L. raffinolactis* NCFB 617 |
| | | Lane 4 | *L. piscium* NCFB 2778 |
| | | Lane 5 | *L. garvieae* NCFB 2155 |
| | | Lane 6 | *L. plantarum* NCFB 1869 |
| | | Lane M | 100 bp DNA ladder |

## 4.4 DISCUSSION

Similarity searches of the species-specific primers used for identification of *Carnobacterium* spp. showed that only the target organisms had a 100% identity with the specific primer used, with the exception of the Cpg primer. *C. gallinarum* and *C. piscicola* share 96.11% homology in their 16S rRNA sequences and the reverse Cpg primer anneals to both species. The Cga primer allowed the differentiation of *C. gallinarum* from *C. piscicola.* This method was very successful in differentiating between species of carnobacteria within three hours and was based on a modification of the procedure described by Brooks *et al.* (1992). Those investigators used a semi-universal primer and a *Carnobacterium* genus-specific primer to amplify a 259-bp region of the 16S rDNA, followed by hybridization with species-specific probes to the PCR products. However, when aligned with sequences from other species using the BLAST E-mail server, the genus-specific primer used by Brooks *et al.* (1992) showed 100% match with several bacterial genera including *Vagococcus* spp., *Enterococcus* spp., and *Listeria* spp. The PCR primers used were not specific enough to differentiate *Carnobacterium* spp. from other bacterial strains, however, hybridization of species-specific oligonucleotide probes to the PCR products allowed differentiation between *C. divergens*, *C. mobile*, and *C. piscicola/C. gallinarum*. The latter two species could not be differentiated by this technique. Nissen *et al.* (1994) were able to identify carnobacteria at the genus level by nucleic acid hybridization using 16S rRNA-targeted genus-specific oligonucleotide probes. The probes showed 100% identity only with *Carnobacterium* spp.

Several genetic-based techniques have been proposed for the identification of *Lactococcus* spp. by different groups over the past decade, although the interest has focused mainly on the identification of *L. lactis* species due to their importance in food processing applications. Betzl *et al.* (1990) used 23S rRNA-targeted oligonucleotide probes for the identification of different subspecies of *L. lactis* by colony hybridization. Later, Beimfohr *et al.* (1993) used 23S rRNA-targeted probes labeled with a fluorescent compound to identify single whole cells of *L. lactis* by epifluorescence microscopy. Klijn *et al.* (1991) used a PCR amplification step of the V1 region of lactococci, followed by hybridization of species-specific 16S rRNA-targeted oligonucleotide probes against the PCR products. This approach specifically differentiated between species of lactococci but again, requires a PCR amplification of the target region followed by dot-blot hybridization procedures. To confirm the identity of the lactococci isolated from poultry, we used one universal forward primer, and three species-specific primers. Poultry isolates that were classified as *L. lactis*, *L. garvieae*, or *L. raffinolactis* did not have biochemical profiles identical to the respective type strains and were classified based on closest match profile. Such classification methods are very time consuming and may be misleading. The method presented in this chapter is very specific and results can be obtained within 3 to 4 hours. Although *L. piscium* and *L. plantarum* were not tested, an analysis of the 16S rRNA sequences of these two species shows that species-specific primers can be easily designed.

# CHAPTER 5 GROWTH OF *LISTERIA MONOCYTOGENES* AND *YERSINIA ENTEROCOLITICA* ON COOKED POULTRY PACKAGED UNDER MODIFIED ATMOSPHERE IN THE PRESENCE AND ABSENCE OF NATURALLY OCCURRING LACTIC ACID BACTERIA DURING STORAGE AT 3.5, 6.5, AND 10°C

## 5.1 INTRODUCTION

Ready-to-eat (RTE), modified atmosphere packaged (MAP) foods are increasingly common on the refrigerated shelves of supermarkets and convenience stores in North America. Use of oxygen-free elevated carbon dioxide atmospheres considerably extends shelf life by inhibiting the gram-negative aerobic spoilage bacteria of cooked meat products (McDaniel *et al.*, 1984; McMullen and Stiles, 1989; Young *et al.* 1987, 1989).

The extended shelf life of MAP RTE refrigerated foods increases concern for the growth of facultative anaerobic psychrotrophic pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica*. In cooked foods, vegetative cells should be significantly reduced. However, the presence of *Li. monocytogenes* in cooked RTE foods is well documented (Gilbert *et al.*, 1989; Farber *et al.*, 1990; Kerr *et al.*, 1990; Green, 1990; Wang and Muriana, 1994) suggesting that either high initial populations or post-process contamination commonly occurs. *Y. enterocolitica* is less commonly isolated from RTE foods (Hudson *et al.*, 1992; Toora *et al.*, 1994). Both *Li. monocytogenes* and *Y. enterocolitica* grow in RTE meat products under refrigerated conditions often with no apparent signs of spoilage (Glass and Doyle, 1989; Manu-Tawiah *et al.*, 1993; Hudson

and Mott, 1993a, 1993b; Hudson *et al.*, 1994). The lack of obvious spoilage at high pathogen levels suggests that potentially harmful foods could be consumed.

RTE MAP foods often rely on proper refrigeration (≤4°C) to achieve adequate shelf life and as a deterrent to the growth of pathogens. Currently, this barrier is difficult to maintain after the food leaves the processing plant. Temperature abuse is prevalent in retail chill cabinets (Davidson, 1987; Hutton *et al.*, 1991) and in household refrigerators (Van Garde and Woodburn, 1987). Ideally, additional barriers or hurdles should be present in the food to inhibit or prevent the growth of pathogens. Acidification, reduction of water activity, addition of preservatives, and presence of a competitive microbiota have all been suggested as appropriate hurdles for RTE MAP foods (Gombas, 1989).

Sodium lactate is used in meat products to extend the shelf life of fresh and cured meats. It has little influence on pH, enhances meat flavour, and increases the water-holding capacity, thus increasing cooking yields. Sodium lactate can inhibit the growth of *Li. monocytogenes* in cured and uncured meats (Shelef and Yang, 1991; Qvist *et al.*, 1994, Wederquist *et al.*, 1994). The commercial shelf life extender, ALTA™ 2341 (Anon., 1997) was shown to have similar anti-listerial activity to pediocin (Schlyter *et al.*, 1993a). It was capable of reducing the growth of *Li. monocytogenes* in turkey slurries when combined with sodium diacetate (Schlyter *et al.*, 1993a). When blue crab meat was inoculated with *Li. monocytogenes* and subsequently washed with a solution of ALTA 2341, the population decreased by 1.2 to 2 $\log_{10}$ CFU/g when compared to the control (Degnan *et al.*, 1994).

The presence of a reliable competitive microbiota (particularly LAB) is believed to provide an effective barrier to the growth of pathogens in foods. In a previous study (Chapter 2) we demonstrated that a fully cooked, modified atmosphere packaged, refrigerated poultry product with a shelf life of greater than 6 weeks could be produced in a processing plant environment. Preliminary studies indicated that packaging hot poultry samples, directly out of the oven, resulted in a product with no background microbiota for over a 2-month period. If poultry samples were packaged, following cooling, in the processing plant, the background microbiota that developed consisted primarily of LAB. However, even when levels of the background microbiota reached $10^{10}$ CFU/piece (approximately $10^{8}$ CFU/g), there were no perceptible sensory changes to the product stored at 3.5°C (Chapter 2).

Hot packaging reduced the risk of post-process contamination but also eliminated a potential hurdle to pathogen growth. Cold-packaged samples developed a background microbiota but exposure of the product allowed for potential post-process contamination with other bacteria. In the present study, the fate of psychrotrophic pathogens, *Li. monocytogenes* and *Y. enterocolitica*, was determined in a cooked, MAP poultry product stored at refrigeration temperature (3.5°C) and under temperature abuse conditions (6.5 and 10°C). The effectiveness of two hurdles in the product was investigated, a combination of two antimicrobial agents, sodium lactate and ALTA 2341, along with the presence and growth of naturally occurring microbiota.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Sample collection

Poultry samples were prepared at a commercial poultry processing plant located in southwestern Ontario. A schematic of the procedure is shown in Fig. 5.1. Raw chicken legs (with skin) were injected, 32 to 35% of their initial weight, with either a control commercial brine formulation (spices, sodium chloride, sodium triphosphate) or a test brine with added sodium lactate (60%, pH 7.0, Wilke International, Inc., Olathe, KS) and a commercial shelf life extender ALTA™ 2341 (Quest International, Sarasota, FL) at levels of 3% w/w and 0.5% w/w, respectively, of the final cooked product. Leg quarters were oven-roasted (oven times of 26 to 27 min) to a minimal internal temperature of 82°C as measured at the thigh joint with a digital probe thermometer (PDT300, UEI, Beaverton, OR). Cooked legs were aseptically collected directly out of the oven (hot packed) or after passing through a cooling tunnel (cold packed). Hot-packed samples were placed, five at a time, in sterile stomacher bags (Seward Medical, London, UK) that were closed and sent through the cooling tunnel. Cold-packed samples, collected after cooling (cooling time approximately 22 min; internal temperature 12 to 16°C), were bulk packed (40 at a time) in sterile autoclave bags (Fisher Scientific). Samples were immediately placed on ice following cooling and transported to the University of Guelph for inoculation, MA-packaging, storage, and analyses.

### 5.2.2 Inoculum preparation

Unless otherwise specified, all media was supplied by Difco (Difco Laboratories, Detroit, MI).

Composite five-strain mixtures of *Li. monocytogenes* and *Y. enterocolitica* were used (Table 5.1). Bacteria were individually propagated in Brain Heart Infusion Broth (BHIB) incubated at 30°C. Overnight cultures (12 to 18 h) were pelleted and washed twice in 0.1% peptone. Cultures were diluted in 0.1% peptone to the same optical density at 600 nm and equal volumes of the five strains were mixed to prepare the inoculum. These five-strain composites were further diluted to approximately 1000 CFU/100 µl. The initial inoculum level was determined by plating the composite mixtures onto Plate Count Agar (PCA) followed by incubation for 24 h at 30°C. The inoculum (100 µl) was applied dropwise onto the surface of each cooked chicken leg and spread over most of the top area using a flame-sterilized glass rod. The surface covered by the inoculum consisted mainly of skin and meat to a lesser degree.

Table 5.1 Strains of *Li. monocytogenes* and *Y. enterocolitica* used in inoculum preparation

| Organism | Strain number | Serotype | Source |
|---|---|---|---|
| *Listeria monocytogenes*[a] | LI512 | 1/2b | Meat isolate |
| | LI514 | 1/2b | Meat isolate |
| | LI521 | 4b (Murray B) | Patient/Outbreak |
| | LI527 | 4b (Scott A) | Patient/Outbreak |
| | LI549 | 4b | Foodborne illness case/Cheese |
| *Yersinia enterocolitica*[b] | R-69 | O:8 | Human |
| | R-72 | O:3 | Pork |
| | RF18 | O:9 | Human |
| | ER-1261 | O:3 | Unknown |
| | PAA | O:8 | Human |

[a]Strains of *Li. monocytogenes* were obtained from Dr. A. Lammerding (Health of Animals Laboratory, Health Canada, Guelph, ON)

[b]Strains of *Y. enterocolitica* were obtained from Dr. M. W. Griffiths (University of Guelph, Department of Food Science, Guelph, ON)

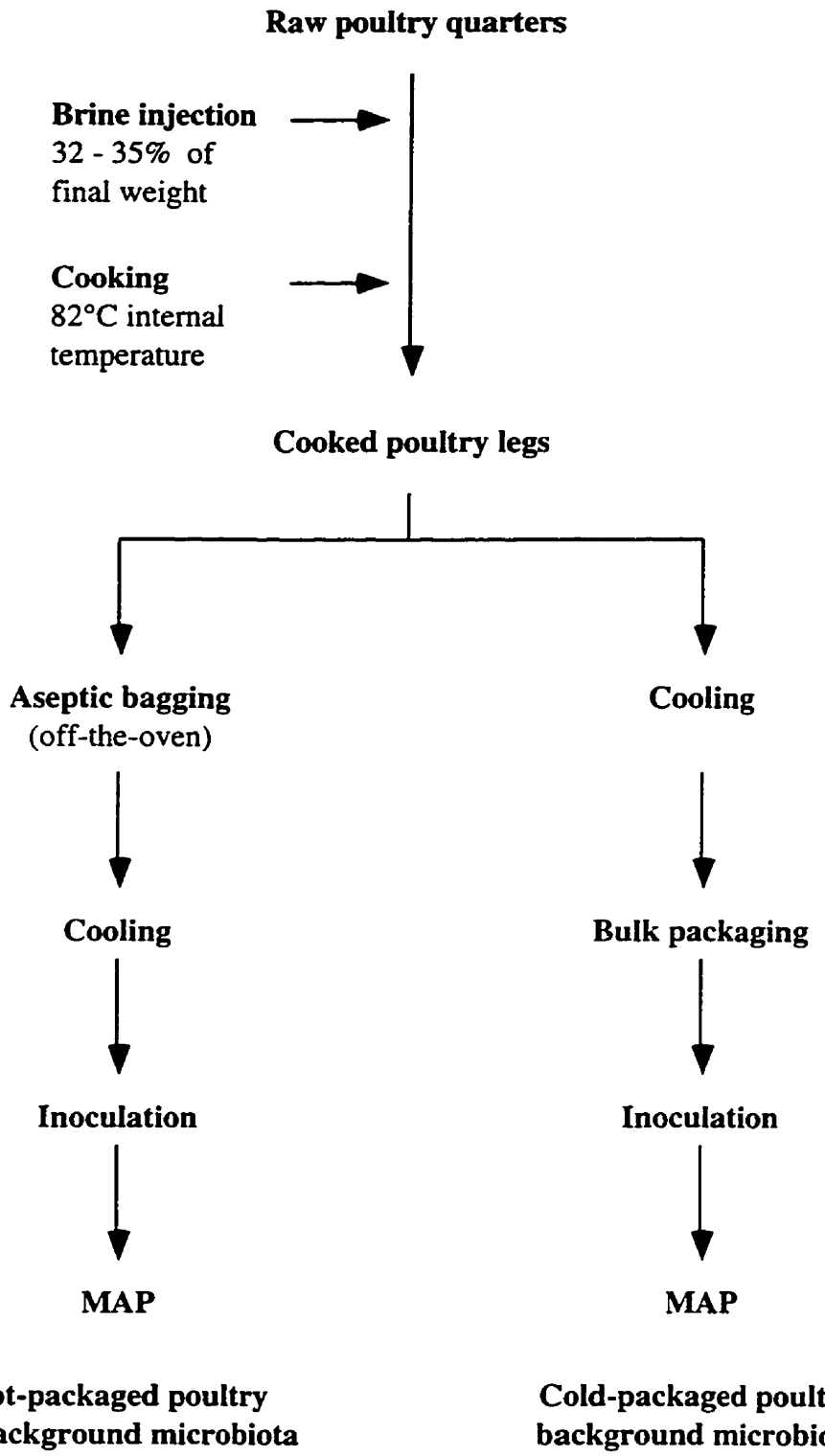



Figure 5.1 Flow diagram for production of cooked modified atmosphere packaged poultry

### 5.2.3 Sample packaging and storage

Following inoculation, chicken legs were individually packaged in Nylon/Ethylene vinyl acetate bags (OTR: 40 cc/$m^2$ in 24 h at 23°C and 0% RH; MVTR: 4.80 g/$m^2$ in 24 h at 37.8°C and 90% RH, Winpak Technologies, Toronto, ON) under an atmosphere of 44:56 $CO_2$:$N_2$ (Canox, Guelph, ON) using a tabletop Multivac packaging machine (Multivac A300, Sepp Hagenmüller KG, Wolfertschwenden, Germany). Packages were stored at one of three temperatures, 3.5, 6.5, and 10°C ± 0.5°C. Uninoculated controls were similarly packaged and stored. Duplicate samples were analyzed following inoculation and packaging and twice a week for a period of 2 to 5 weeks depending on the storage temperature. Each treatment was performed twice on two separate occasions with the exception of the *Y. enterocolitica* test at 10°C which was done once. Control samples (uninoculated) were packaged within 12 to 24 h of production, and test samples were inoculated and packaged within 15 to 24 h of production in the first trial and within 28 to 40 h in the second trial.

### 5.2.4 Microbiological analysis

Duplicate packages of each treatment were analyzed at every sampling time. The top of each package was swabbed with alcohol, opened with flame-sterilized scissors, and 100 ml of sterile 0.1% peptone diluent was added to each bag. Chicken legs were hand massaged for 2 min, followed by a vigorous shaking up-and-down 20 times. Serial dilutions were prepared and 100 µl was surface plated onto PCA and Oxford agar containing the Oxford selective supplement when samples were inoculated with *Li.*

*monocytogenes* or onto PCA and Cefsulodin-Irgasan-Novobiocin agar (CIN) when samples had been inoculated with *Y. enterocolitica*. PCA plates were incubated at 22°C for 48 h, and Oxford and CIN plates were incubated at 30°C for 48 h and 18 h, respectively. Uninoculated control samples were plated onto PCA, Oxford and CIN agar. Dark colonies on Oxford agar were counted as *Li. monocytogenes*, and small colonies on CIN agar with a dark red center surrounded by a transparent border were enumerated as *Y. enterocolitica*.

Immediately after inoculation and packaging, numbers of *Li. monocytogenes* and *Y. enterocolitica* were assessed by a 3-tube Most Probable Number (MPN) procedure (Peeler *et al.*, 1992; Donnelly *et al.*, 1992; Schiemann *et al.*, 1992). *Li. monocytogenes* MPN was determined by transferring 1-ml volumes of the wash and appropriate dilutions to 9 ml Listeria Enrichment Broth (LEB) followed by incubation at 30°C for 48 h. Samples (100 µl) from tubes showing growth were transferred to Fraser broth for 48 h at 30°C. Positive tubes of Fraser broth were streaked onto Oxford agar (30°C, 48 h) for confirmation. A similar procedure was used to determine initial levels of *Y. enterocolitica*. After 5 days at 22°C, positive tubes of Irgasan-Ticarcillin-Cefsulodin Broth (ITCB) were streaked onto CIN agar. Counts for all populations measured were reported as CFU/chicken leg.

The identification of selected colonies enumerated as either *Y. enterocolitica* or as *Li. monocytogenes* was confirmed using the Vitek Jr. (Vitek Systems, bioMérieux Vitek, Inc., Hazelwood, MO).

### 5.2.5 Identification of background microbiota

In some instances colonies were selected from the highest dilution of PCA plates from uninoculated samples and subcultured on tryptic soy agar. Colonies were purified and isolates were identified to the genus level based on Gram reaction, cell morphology, catalase, oxidase, motility, and for gram-positive isolates, growth on selective agar. Selected gram-negative organisms were identified further using the Vitek Jr. (Vitek Systems).

### 5.2.6 Headspace and pH

Gas samples were withdrawn from uninoculated packages through sampling patches (Fisher Scientific) using a gas-tight syringe. Gas composition ($O_2$ and $CO_2$) was determined by gas chromatography as previously described (Chapter 2) at the start of each trial, 24 h following packaging. At weekly intervals, uninoculated 11-g samples of chicken were homogenized for 2 min in 99 ml of 0.1% peptone using a Stomacher Lab Blender 400 (Seward Medical). The pH of the slurry was measured using a Fisher Acumet 915 Meter (Fisher Scientific).

### 5.2.7 Determination of antibacterial activity and heat resistance of ALTA 2341

ALTA 2341 is produced by culturing yeast extract, corn syrup, and vegetable protein using a food-grade fermentation process, and is marketed as a shelf life extender (Anon. 1997). It has been reported to contain pediocin (Jack *et al.*, 1996).

### 5.2.7.1 Preparation of ALTA 2341 solutions

A 3% solution of ALTA 2341 was aseptically prepared in sterile water. Aliquots (250 µl) were dispensed in sterile microfuge tubes, and placed in a water/polyethylene glycol bath at 85°C. Temperature was monitored using an Omega OM-160 Portable Intelligent Data Logger (Omega Engineering, Inc., Laval, PQ) equipped with a temperature sensor placed in a tube containing 250 µl of 3% ALTA 2341. Timing was started when the internal temperature of the control tube reached 84-85°C. Microfuge tubes were removed after 30, 60, 90, and 120 min at 85°C and cooled on ice.

### 5.2.7.2 Spot-on-lawn assay

Cultures of *Li. monocytogenes* and *Y. enterocolitica* (Table 5.1) were propagated in BHIB incubated at 30°C. Soft BHI agar (6 ml, 0.75% agar) tubes were seeded with a 1% inoculum (BHIB, 18 to 24 h, 30°C) of the pathogens and poured over plates of BHI agar. Bacterial lawns were also prepared from reference cultures of carnobacteria and lactococci (Table 5.2) and from representative strains of carnobacteria and lactococci isolated from cooked MAP poultry (Chapter 3) propagated in APT broth at 28°C and seeded in soft APT agar (6 ml, 0.75% agar).

Dilutions (2%, 1%, and 0.5%) were prepared from heat-treated and non heat-treated 3% solutions of ALTA 2341. Five-µl volumes were applied on lawn sections. Plates were incubated at 30°C at 24 h. The assay was repeated twice.

Table 5.2 Reference strains used in the activity assay for ALTA 2341[a]

| Strain | Reference |
|---|---|
| *Carnobacterium divergens* | ATCC 35677; Type strain |
| *C. divergens* | LV13 |
| *C. gallinarum* | ATCC 49517; Type strain |
| *C. mobile* | ATCC 49516; Type strain |
| *C. piscicola* | ATCC 35586; Type strain |
| *C. piscicola* | LV17 |
| *Enterococcus faecium* | ATCC 19434; Type strain |
| *E. faecalis* | ATCC 19433; Type strain |
| *E. durans* | ATCC 19432; Type strain |
| *Lactococcus garvieae* | NCFB 2155; Type strain |
| *L. piscium* | NCFB 2778; Type strain |
| *L. plantarum* | NCFB 1869; Type strain |
| *L. raffinolactis* | NCFB 617; Type strain |
| *L. lactis* ssp. *lactis* | ATCC 11454 |
| *L. lactis* ssp. *cremoris* | ATCC 14365 |

[a]Information pertaining to the source of organisms is available Table 4.1

### 5.2.8 Statistical analysis

Values from the replicate trials were used for the statistical analyses. Data were analyzed by ANOVA using the General Linear Model procedure of the SAS statistical package (SAS Institute Inc., Cary, NC). Replications and brine, packaging, and storage treatments were compared on the basis of $\log_{10}$ CFU/piece counts.

## 5.3 RESULTS

Data reported are averages of duplicate samples and replicate trials. There were no significant differences between replicate trials for growth of the background microbiota or *Li. monocytogenes*; however, there was significant differences ($p < 0.01$) in replicate

trials of *Y.* enterocolitica. Marked differences were observed between replicate treatments of the test brine. Inhibitory effects of the test brine on *Y. enterocolitica* were more significant in replicate two than in replicate one. Growth curves of *Y. enterocolitica* in replicate trials are presented separately in the appendix.

### 5.3.1 Growth of background microbiota

Counts of the background microbiota were similar at 6.5 and 10°C ($p > 0.05$), however, counts at both storage temperatures were significantly different ($p < 0.01$) from counts at 3.5°C. The lag phase increased and the growth rate decreased at 3.5°C (Fig. 5.2). The addition of sodium lactate and ALTA 2341 significantly affected counts of the background microbiota ($p < 0.01$). The effect was more pronounced at 3.5°C than at 6.5 or 10°C (Fig. 5.2). In test brine-injected samples, aerobic plate counts were 0.5- to 1-log cycles lower than controls at 6.5 and 10°C and 1 to 2 $\log_{10}$ cycles lower at 3.5°C.

### 5.3.2 Identification of background microbiota

With the exception of two packages that had approximately $10^5$ CFU/piece ($10^3$/g), hot-packed samples (approximately 200 packages) did not develop a background microbiota within the storage period. Total aerobic plate counts determined on PCA remained at $<10^4$ CFU/piece (<100 CFU/g) throughout the study (data not shown). Colonies from the two packages with counts had a homogeneous morphology on PCA and were identified, using standard biochemical tests, as enterococci. In contrast, a consistent background

microbiota developed on cold-packaged samples (Fig. 5.2). A total of 70 colonies were selected based on colony appearance from PCA plates of the highest dilution from samples that were stored at 3.5, 6.5, and 10°C. Gram-stain and cellular morphology showed 69 of 70 of the isolates to be gram-positive rods and coccobacilli, and one gram-negative rod which was not further characterized. Based on catalase and oxidase reactions, and growth on selective agar (streptomycin thallous acetate agar, Rogosa SL agar, and MRS agar adjusted to pH 8.5 to 9.0) gram-positive isolates were tentatively classified as *Brochothrix* spp. (10 isolates), *Carnobacterium* spp. (48 isolates), *Leuconostoc* spp. and *Lactococcus* spp. (11 isolates).

In all control trials, the background microbiota grew on CIN agar at low levels; however, none of the colonies had the typical appearance of yersiniae, and when selected colonies were identified using the Vitek Jr., none were *Y. enterocolitica*. Isolates were either identified as *Serratia* spp. or "not identified" gram-negative. Counts of the background microbiota growing on CIN agar were always two or more $\log_{10}$ cycles lower than counts on PCA. Although the dominant microbiota on cold-packed control samples consisted of lactic acid bacteria and *Brochothrix* spp., which are organisms commonly associated with MAP meats, there was a sub-population of gram-negative organisms.

### 5.3.3 Occurrence of pathogens on uninoculated controls

*Y. enterocolitica* or *Li. monocytogenes* were not recovered (<1000 CFU/150-g piece) from control samples during the course of the experiment as determined by surface plating initial dilutions onto selective media.

### 5.3.4 Influence of competitive microbiota on growth of *Li. monocytogenes and Y. enterocolitica* in MAP chicken meat

No significant difference ($p > 0.05$) was observed in the growth of pathogens in hot and cold-packed samples indicating that the presence of a competitive microbiota did not influence the growth of either *Li. monocytogenes* (Fig. 5.3) or *Y. enterocolitica* (Fig. 5.4) at any of the storage temperatures.

### 5.3.5 Influence of sodium lactate and ALTA 2341 on growth of pathogens

Addition of food-grade sodium lactate and ALTA 2341 to the brine did not prevent the growth of *Li. monocytogenes* (Fig. 5.6) and *Y. enterocolitica* (Fig. 5.7), but the presence of preservatives in the brine decreased the counts significantly ($p < 0.01$). The preservatives extended the lag phase of *Li. monocytogenes* by approximately 10 days in cold packed samples at 3.5°C and by 1 to 2 days in samples stored at 6.5 or at 10°C (Fig. 5.6). Numbers of *Li. monocytogenes* on samples injected with the test brine were 0.5- to 2 $\log_{10}$ units lower than counts on samples injected with the control brine during the log and stationary phase of growth at all storage temperatures.

The combined preservatives extended the lag phase of *Y. enterocolitica* by 3 days in samples stored at 3.5°C but little effect was seen at the higher storage temperatures (6.5 and 10°C). Counts on samples injected with the test brine were lower than those on control samples injected with the regular brine by <0.5 to 3 $\log_{10}$ units (Fig. 5.7).

### 5.3.6 Influence of storage temperature on growth rates of pathogens

Temperature had the greatest effect on growth of *Li. monocytogenes* and *Y. enterocolitica*. No significant difference was detected in the growth of *Li. monocytogenes* or *Y. enterocolitica* at 6.5°C and at 10°C (Fig. 5.6), but growth at 3.5°C was significantly different ($p < 0.01$) from 6.5 and 10°C. While 28 to 35 days at 3.5°C were required for levels of *Li. monocytogenes* to increase from 500-1000 CFU/piece to $10^9$ CFU/piece, only 9 to 12 days were needed to reach that level at 6.5 and 10°C.

Although growth of *Y. enterocolitica* at 3.5°C was significantly different from growth at 6.5 or 10°C, effects of temperature were not as pronounced. While 20 days were necessary to reach levels of $10^9$ CFU/piece at 3.5°C, less than 10 days were needed at either 6.5 or 10°C (Fig. 5.7).

No significant interactive effects of brine, package, and storage variables were observed for all treatment combinations.

### 5.3.7 Headspace analysis and pH measurements

The $CO_2$ concentration decreased from 43 to 44% to approximately 33 to 34% within 24 h of packaging. Oxygen concentrations, when detected, were <1%. The pH of the chicken was 6.3 ± 0.1 throughout the experiment and was not affected by brine treatments.

### 5.3.8 Inhibitory activity of ALTA 2341

*Li. monocytogenes* LI512 and LI514 were strongly inhibited at concentrations of 1, 2, and 3% ALTA 2341, but more weakly at 0.5%. *Li. monocytogenes* LI527 (Scott A) and LI549 were weakly inhibited at 2% and 3% ALTA, but not at lower ALTA concentrations. *Li. monocytogenes* LI 521(Murray B) was not affected by the agent at all concentrations tested. No inhibition was observed against *Y. enterocolitica.*

The antilisterial activity of ALTA 2341 was reported in arbitrary units/ml (AU/ml) as the inverse of the highest dilution spotted (5 µl) showing a zone of inhibition on a lawn of sensitive cells. Using the most sensitive strains of *Li. monocytogenes* (LI512 and LI514) as the reference organisms, the activity of ALTA 2341 in the 1% solution was 2000 AU/ml.

Reference cultures of *L. raffinolactis*, *L. garvieae*, and *L. lactis* ssp. *lactis* were not affected by ALTA 2341. *L. lactis* ssp. *cremoris* was very weakly inhibited by 3% ALTA 2341.

*C. divergens* ATCC 35586 was strongly inhibited by 2% and 3% ALTA 2341, but weakly inhibited at the 1% level. *C. divergens* LV13 was not affected by all concentrations of ALTA 2341. *C. piscicola* ATCC 35677 was inhibited by 2% and 3% ALTA; however, *C. piscicola* LV17 was not affected. *C. mobile* was not inhibited while *C. gallinarum* was strongly inhibited by 2% and 3% ALTA 2341. Type strains of *E. faecium* and *E. durans* were not affected at all concentrations of ALTA 2341. *E. faecalis* was weakly inhibited at the 3% level.

*C. divergens* poultry isolates were inhibited by 3% ALTA 2341, and weakly at 2%. Inhibition of *C. piscicola* isolates was strain dependent. One strain was not affected, while two other were inhibited at the highest concentration of ALTA (3%), and weakly inhibited at the 2% level. *L. raffinolactis* and *L. garvieae* poultry isolates were not affected by the agent. One *Enterococcus* isolate was not affected by ALTA 2341, however, another isolate was inhibited at the 3% level.

Heat-treated and unheated preparations of ALTA 2341 had the same inhibitory profile, indicating that heat treatment for 2 h at 85°C did not affect activity.

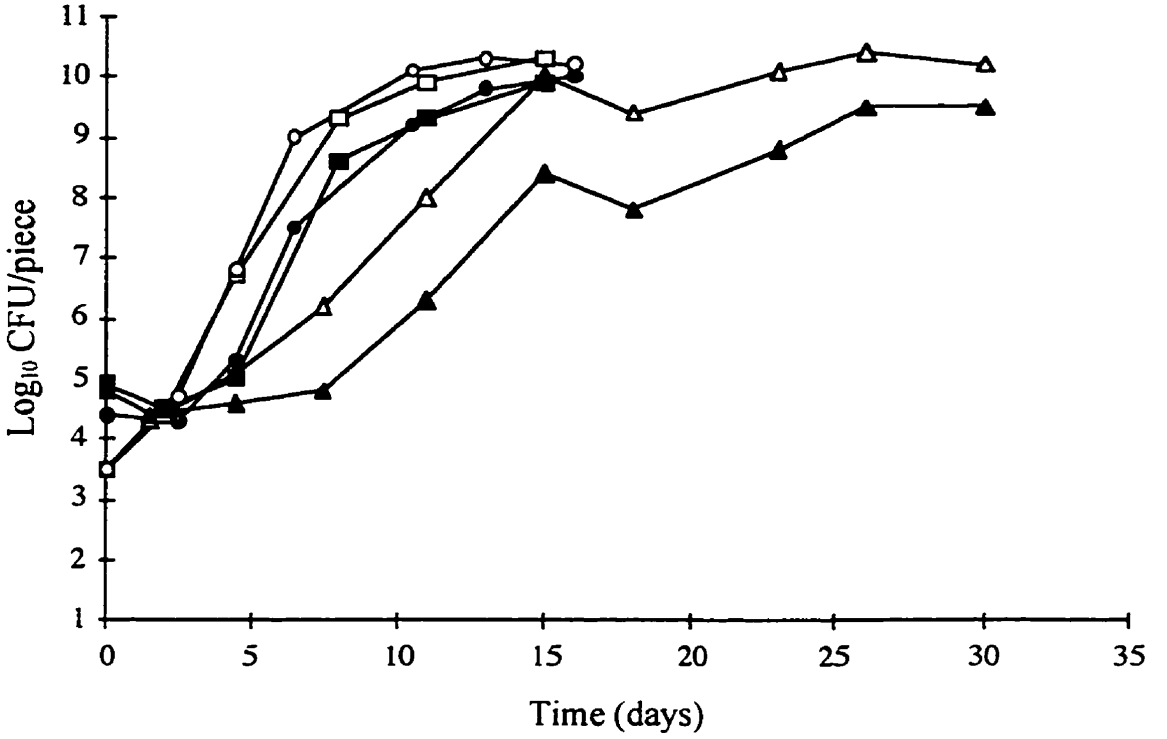



Figure 5.2 Aerobic plate counts of cooked MA, cold-packed poultry injected with regular brine (open symbols) or test brine containing lactate and ALTA™ 2341 (solid symbols) and stored at 3.5°C (▲), 6.5°C (●), and 10°C (■). Counts ≤ $2.5 \times 10^4$ CFU/piece are estimates.

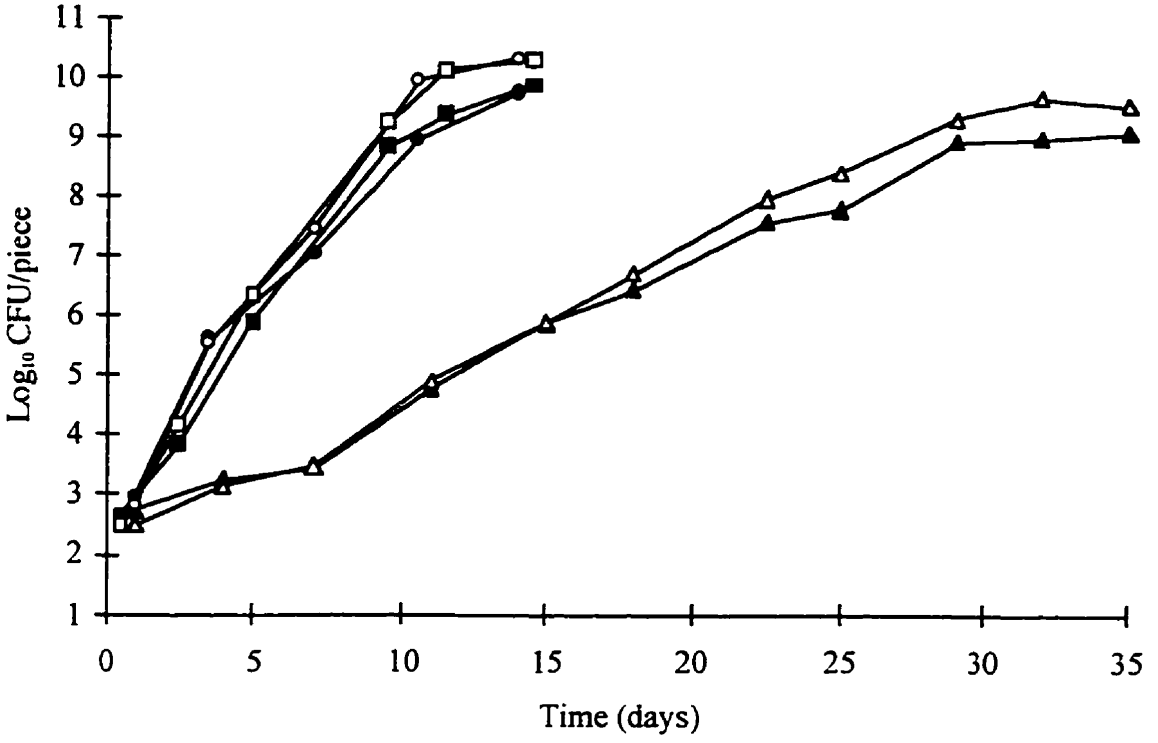



Figure 5.3 Growth of *Li. monocytogenes* on cooked, hot-packed (open symbols) or cold-packed (solid symbols) poultry injected with regular brine and stored at 3.5°C (▲), 6.5°C (●), and 10°C (■). Counts ≤ $2.5 \times 10^4$ CFU/piece are estimates or MPN/piece.

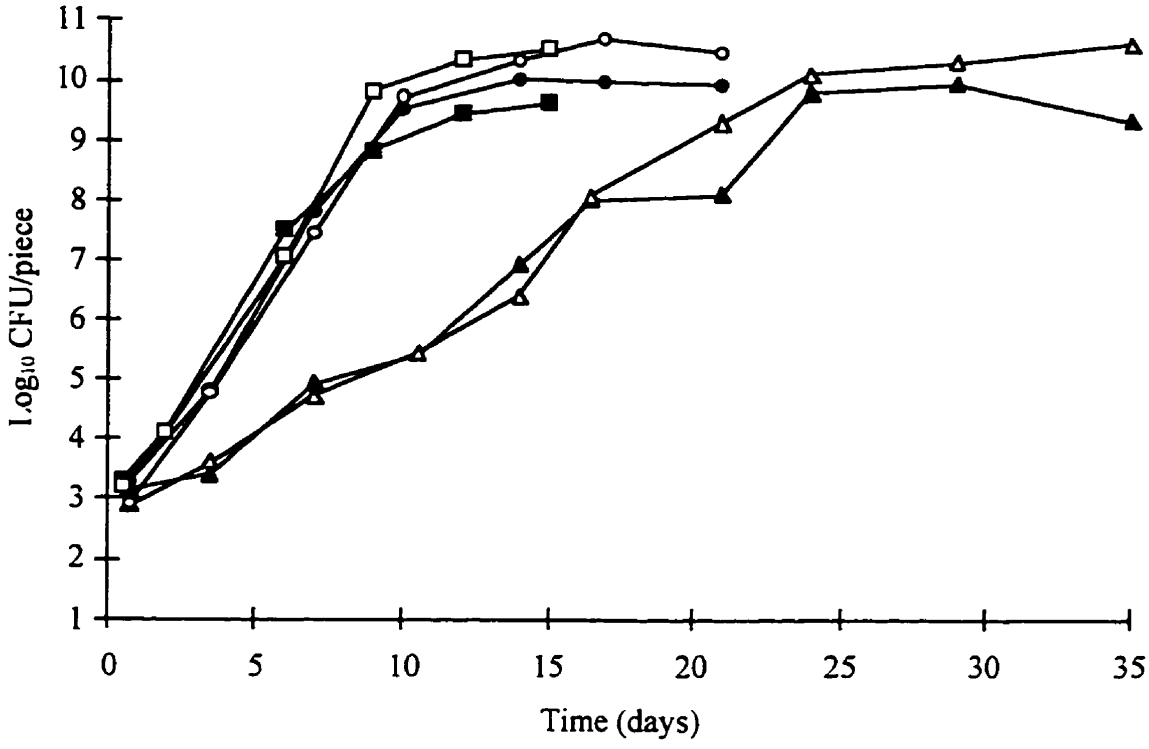



Figure 5.4 Growth of *Y. enterocolitica* on cooked, hot-packed (open symbols) or cold-packed (solid symbols) poultry injected with regular brine and stored at 3.5°C (▲), 6.5°C (●), and 10°C (■). Counts ≤ $2.5 \times 10^4$ CFU/piece are estimates or MPN/piece.

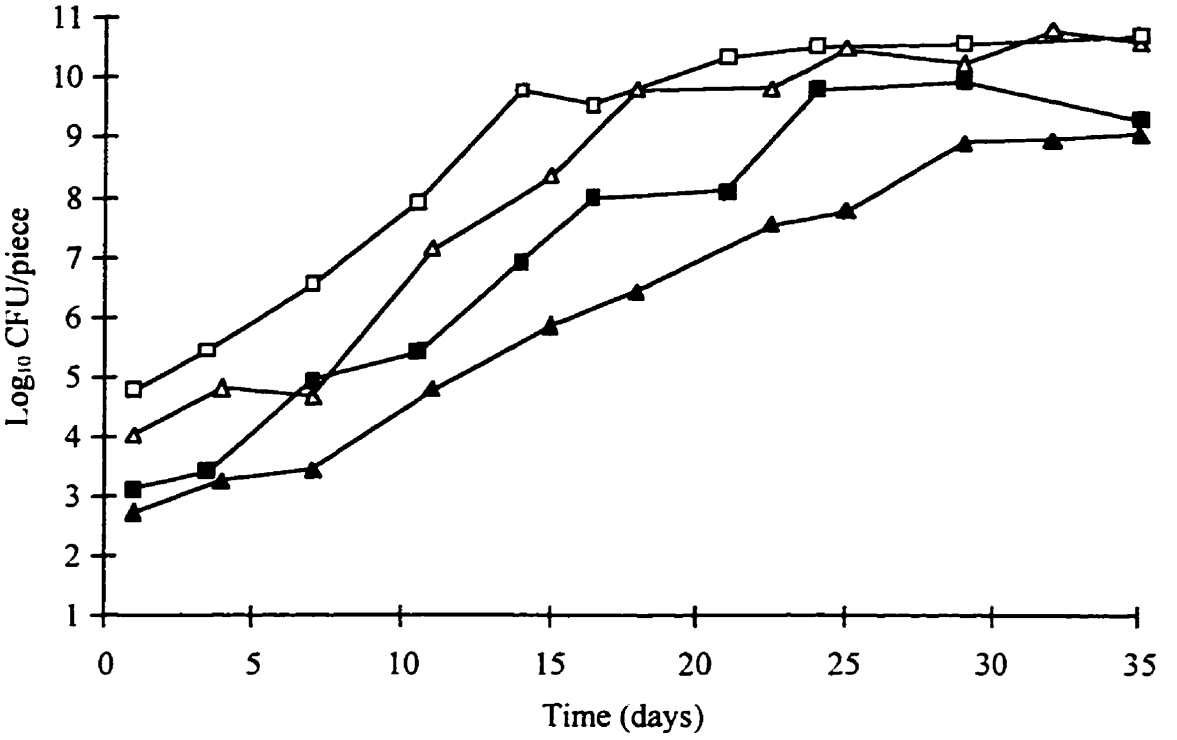



Figure 5.5 Aerobic plate counts (open symbols) and pathogen counts (solid symbols) in cold-packaged poultry cuts injected with regular brine, inoculated with either *Li. monocytogenes* (▲) or *Y. enterocolitica* (■), and stored at 3.5°C. Counts ≤ $2.5 \times 10^4$ CFU/piece are estimates or MPN/piece.

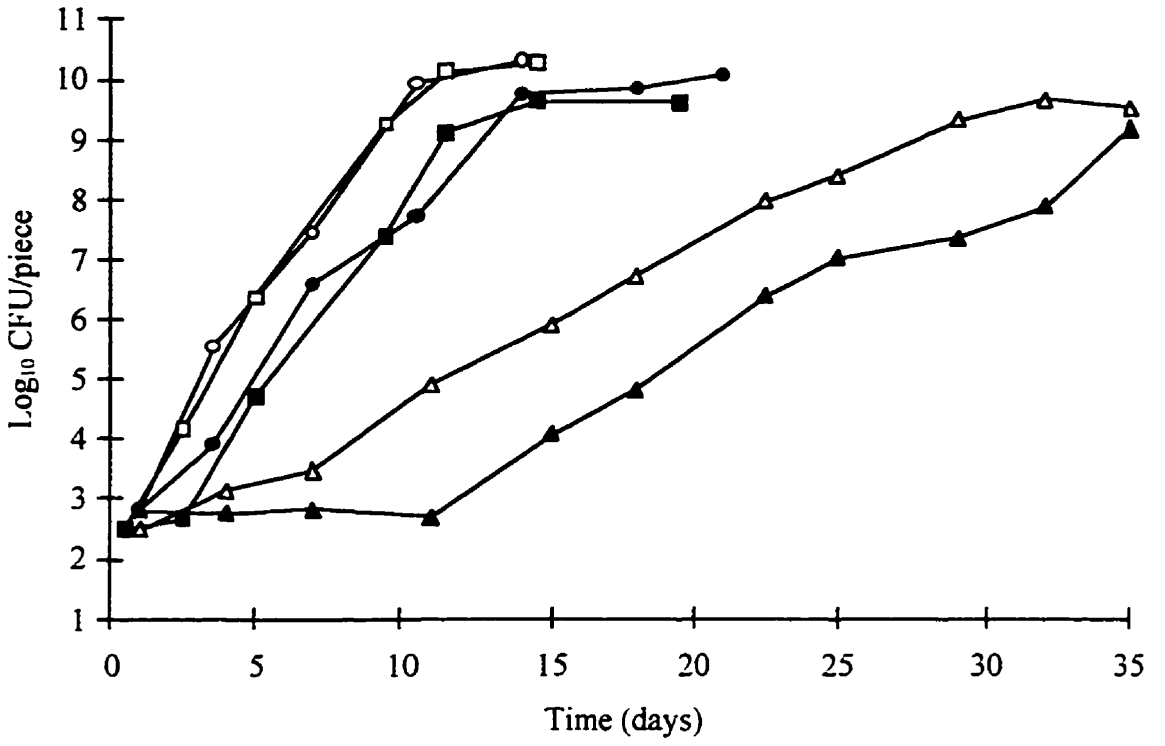



Figure 5.6 Growth of *Li. monocytogenes* on cooked MA hot-packed poultry injected with either regular brine (open symbols) or test brine (solid symbols) containing lactate and ALTA™ 2341 and stored at 3.5°C (▲), 6.5°C (●), and 10°C (■). Counts ≤ $2.5 \times 10^4$ CFU/piece are estimates or MPN/piece.

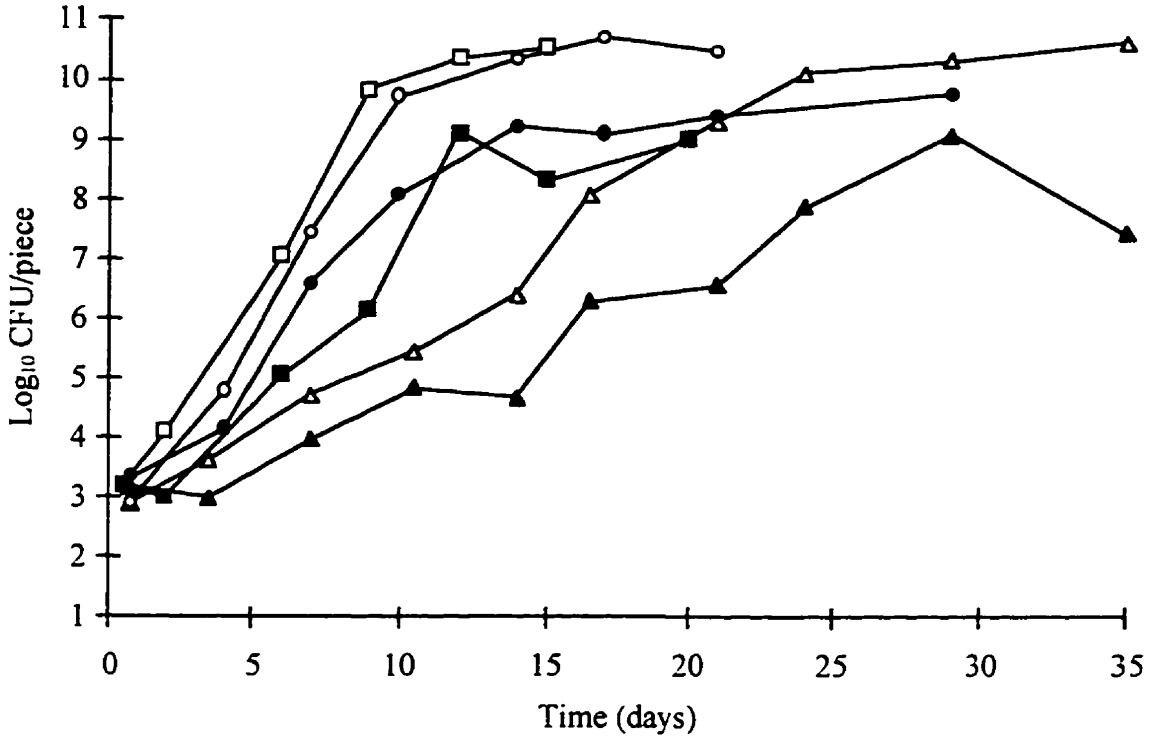



Figure 5.7 Growth of *Y. enterocolitica* on cooked MA hot-packed poultry injected with either regular brine (open symbols) or test brine (solid symbols) containing lactate and ALTA™ 2341 and stored at 3.5°C (▲), 6.5°C (●), and 10°C (■). Counts ≤ $2.5 \times 10^4$ CFU/piece are estimates or MPN/piece.

## 5.4 DISCUSSION

Although an extended shelf life for minimally processed foods is desirable from a production and distribution standpoint, a longer shelf life increases the opportunity for growth of psychrotrophic pathogens. The heat treatment applied to the poultry product used in this study was severe enough to eliminate vegetative organisms such as *Li. monocytogenes* and *Y. enterocolitica*, however, the potential for cross-contamination of the cooked product by personnel and equipment in the processing environment existed. For this reason, barriers to the growth of these pathogens were investigated.

The predominant spoilage microbiota in MAP products are lactic acid bacteria (LAB), also primarily responsible for the preservation of a variety of fermented products. This preservative action is largely due to the production of organic acids as metabolic by-products and a subsequent drop in pH (Gombas, 1989). Other mechanisms of inhibition may be decreased availability of nutrients, formation of hydrogen peroxide, or the production of bacteriocins (antimicrobial peptides or proteins). The competitive ability of lactic acid bacteria in non-fermented foods is much less clear. Leisner *et al.* (1996) demonstrated that bacteriocin-producing and bacteriocin-negative *Lc. gelidum* could effectively inhibit the spoilage organism *Lb. sake* in MAP raw meat. *Lc. gelidum* was selected based on a prior study (Leisner *et al.*, 1995) which indicated that out of four test strains of lactic acid bacteria (*C. piscicola* LV17 and UAL26, *Lb. sake* Lb 706, and *Lc. gelidum* UAL187-22) inoculated on sterile lean raw beef slices, the leuconostoc species was the best candidate as an antagonist since it did not decrease the storage life of the beef. The growth of bacteriocin-producing *Lb. sake* Lb 706 extended the lag phase of *Li.*

*monocytogenes* in raw minced meat, however, the bacteriocin-negative variant was not as effective (Schillinger *et al.*, 1991). Growth of *Y. enterocolitica* in raw minced meat was reduced by a natural competitive background flora (*Pseudomonadaceae* and *Enterobacteriaceae*) during aerobic but not anaerobic storage at 4°C, and under both anaerobic and aerobic conditions at 10 and 15°C (Kleinlein and Untermann, 1990).

The competitive action of LAB in cooked MAP chicken was investigated by inoculating meat collected in a processing plant, before (hot-packed) and after it acquired a natural microbiota (cold-packed). It was expected that the presence of LAB at levels higher than the pathogens would slow, or completely inhibit, the growth of *Li. monocytogenes* or *Y. enterocolitica*. However, high levels of LAB did not significantly influence the growth of either pathogen. This could be a reflection of the consistently high pH of the meat, or the type of organism that naturally predominated in the product. Cooked poultry meat and the standard brine solution do not have a readily fermentable carbohydrate source for the lactic acid bacteria to sufficiently reduce the pH. Bacteriocin production by a strain of LAB can be a significant factor in suppressing growth of pathogens when inoculated in large numbers into fermented or raw meat (Hugas *et al.*, 1995; Schillinger *et al.*, 1991) and the absence, or non-dominance, of an effective bacteriocin-producing strain in the MAP cooked poultry products tested in this study may explain the lack of effective pathogen inhibition. Additionally, levels of the background microbiota, although one to two $\log_{10}$ units higher than levels pathogenic organisms, may have been insufficient to exert an effective inhibitory action.

Most previous studies on the growth and survival of psychrotrophic pathogens in RTE or raw meats have used inoculated sterile or pasteurized, aseptically handled meat. Degnan *et al.* (1994) used steam-sterilized crabmeat to evaluate the ability of various lactic acid bacteria fermentation products and food grade chemicals to control the growth of *Li. monocytogenes*. Schlyter *et al.* (1993a) used pasteurized turkey slurries (68°C for 10 min) inoculated with *Li. monocytogenes* in the presence of ALTA 2341 and sodium diacetate. Hudson and Mott (1993b) subjected blocks of beef rumps to a simulated commercial cooking process in an autoclave, 80°C for 30 min, prior to inoculating the meat with one of three psychrotrophic pathogens, *Li. monocytogenes*, *Y. enterocolitica*, or *Aeromonas hydrophila*. When a competitive microbiota was present, it was usually a spoilage organism isolated from meat packaged in air (Ingham *et al.*, 1990a, 1990b; Marshall *et al.*, 1991, 1992), or a lactic acid bacterium selected for production of a bacteriocin (Kleinlein and Untermann, 1990; Schillinger *et al.*, 1991; Degnan *et al.*, 1992; Jeppesen and Huss, 1993; Winkowski *et al.*, 1993). These spoilage organisms were inoculated in the autoclaved/pasteurized meat along with the pathogen of interest. Meat products with a naturally occurring background microbiota were occasionally used for challenge studies (Gill and Reichel, 1989; Glass and Doyle, 1989; Wimpfheimer *et al.*, 1990; Manu-Tawiah *et al.*, 1993; Palumbo and Williams, 1994), however, the specific competitive action of the background spoilage organisms on inoculated pathogens could not be determined because a suitable bacteria-free control was not available.

Strict control of refrigeration temperatures (≤4°C) is recommended for the storage of extended shelf life RTE MAP products. However, these products often experience higher

temperatures during distribution, retail, or consumer handling. In this study, temperature was an important factor in inhibiting the growth of *Li. monocytogenes* and *Y. enterocolitica*. Preservatives, sodium lactate and ALTA 2341, provided an additional barrier, however, they were not a substitute for proper temperature control and their effectiveness diminished as storage temperature increased. The activity spectrum of ALTA 2341 indicates that it may not be effective against pathogenic organisms at the levels of use recommended by the manufacturer (0.5%; Anon., 1997). Only three out of the five *Li. monocytogenes* strains used in this study were marginally inhibited by 0.5% ALTA 2341. The zone of inhibition was slightly less turbid than the surrounding lawn, indicating a sub-population of resistant cells.

The development of a population of lactic acid bacteria had no effect on the growth of either pathogen. The presence of high levels of naturally-occurring lactic acid bacteria cannot be taken as an indication of the absence of pathogenic microorganisms. Additionally, the presence of a bacteriocin-containing agent such as ALTA 2341 may have an inhibitory effect on the background microbiota as demonstrated in the control samples injected with test brine.

The reason for the discrepancy in results between replicate one and replicate two for the *Y. enterocolitica* study could not be clearly identified. It was not possible to conduct an additional repetition of the experiment to determine which replicate is closer to reality.

## CHAPTER 6 CONCLUSION

Shelf life of a cooked poultry product was considerably extended by MAP and maintenance of proper refrigeration temperature. Signs of spoilage were not detected after two months at 3.5°C, and following achievement of maximal microbial population levels. The absence of obvious spoilage indicators may be attributed to the type of dominant microbiota present, *Carnobacterium* species and *Lactococcus raffinolactis*.

Although carnobacteria are commonly associated with meat products, this may be the first report of *L. raffinolactis* occurring in a cooked MAP meat product. Traditional, phenotypic-based classification methods were used to identify the dominant microbiota. These methods are time-consuming and may leave a doubt in the mind of the investigator as to the accuracy of the classification when atypical strains are encountered. The polymerase chain reaction using 16S rRNA-targeted, species-specific probes was a valuable tool for the identification of carnobacteria and lactococci rapidly and accurately, to the species level. Lactic acid bacteria are of major importance to the food industry due to their roles in food preservation and fermentation processes. The use of LAB as biopreservation agents to extend shelf life of MAP raw, cooked, and cured meat products, and to improve safety of food products is being actively investigated. It is thought that the early inclusion of a reliable background lactic acid microbiota, capable of growing actively at refrigeration temperatures while minimally affecting sensory properties, may extend the shelf life of meat products. Scientists are constantly on the lookout for new strains of LAB exhibiting desirable functional properties and the use of molecular-based techniques greatly speeds up screening and identification steps. The microbiota of MAP

meat products is highly variable and may include *Brochothrix thermosphacta*, and many species of carnobacteria, enterococci, lactobacilli, lactococci, leuconostocs, and *Weissella* spp. The use of species-specific primers for identification purposes requires a comprehensive primer database. It would be interesting to investigate the feasibility of using mixtures of genus-specific PCR primers for initial screening and classification, following which, species-specific primers could be used for identification to the species level if some isolates exhibit useful properties.

The presence of a competitive background microbiota has long been believed to be an indication of the microbiological safety of a product. Competitive organisms tested in different research laboratories were selected based on desirable properties such as bacteriocin or acid production. The effect of a naturally-occurring background microbiota on the growth of pathogens in meat products has not been as frequently investigated. When the cooked MAP poultry product was inoculated with either *Listeria monocytogenes* and *Yersinia enterocolitica*, presence or absence of a lactic acid microbiota did not have any significant effects on the survival and growth of the pathogens, regardless of the storage temperature. Additional hurdles in the form of sodium lactate and ALTA™ 2341 slowed but did not prevent the growth of the pathogens. It would be worthwhile to investigate the behaviour of pathogens in the presence of a competitive microbiota at levels higher than those used in this work, and in the presence of fermentable carbohydrate source.

## REFERENCES


Abee, T., L. Krockel, and C. Hill. 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int. J. Food Microbiol. 28:169-185.

Abeyta, C. Jr., C. A. Kaysner, M. M. Wekell, J. J. van Sullivan, and G. N. Stelma. 1986. Recovery of *Aeromonas hydrophila* from oysters implicated in an outbreak of foodborne illness. J. Food Prot. 49:643-646.

Adams, C. E. 1991. Applying HACCP to sous vide products. Food Technol. 45:148-149, 151.

Agriculture Canada. 1990a. Modified atmosphere packaging, an extended shelf life packaging technology. Food Development Division, Agriculture Development Branch, Agriculture Canada, Ottawa.

Agriculture Canada. 1990b. Canadian code of recommended manufacturing practices for pasteurized/modified atmosphere packaged/refrigerated food. Agri-Food Safety Division, Agriculture Canada, Ottawa.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403-410.

Ahn, C., and M. E. Stiles. 1990. Antimicrobial activity of lactic acid bacteria isolated from vacuum-packaged meats. J. Appl. Bacteriol. 69:302-310.

Ahrné, S., G. Molin, and S. Stahl. 1989. Plasmids in *Lactobacillus* strains isolated from meat and meat products. Syst. Appl. Microbiol. 11:320-325.

Andersen, L. 1995. Biopreservation with FloraCarn L-2. Fleischwirtsch. 75: 1327-1329.

Anonymous. 1997. Product information sheet for ALTA 2341. Quest International, Lachine, PQ.

Arihara, K., R. G. Cassens, and J. B. Luchansky. 1993. Characterization of bacteriocins produced by *Enterococcus faecium* strains with activity against *Listeria monocytogenes*. Int. J. Food Microbiol. 19:123-134.

Asplund, K., E. Nurmi, J. Hirn, T. Hirvi, and P. Hill. 1993. Survival of *Yersinia enterocolitica* in fermented sausage manufactured with different levels of nitrite and different starter cultures. J. Food Prot. 56:710-712.

Avery, S. M., J. A. Hudson, and N. Penney. 1994. Inhibition of *Listeria monocytogenes* on normal ultimate pH beef (pH 5.3-5.5) at abusive storage temperatures by saturated carbon dioxide controlled atmosphere packaging. J. Food Prot. 57:331-333.

Avery, S. M., A. R. Rogers, and R. Graham. 1995. Continued inhibitory effect of carbon dioxide packaging on *Listeria monocytogenes* and other microorganisms on normal pH beef during abusive retail display. Int. J. Food Sci. Technol. 30:725-735.

Avery, S. M., and S. Buncic. 1997. Antilisterial effectiveness of a sorbate-nisin combination in vitro and on packaged beef at refrigeration temperatures. J. Food Prot. 60:1075-1080.

Baccus-Taylor, G., K. A. Glass, J. B. Luchansky, and A. J. Maurer. 1993. Fate of *Listeria monocytogenes* and pediococcal starter cultures during the manufacture of chicken summer sausage. Poult. Sci. 72:1772-1778.

Baker, R. C., J. H. Hotchkiss, and R. A. Qureshi. 1985. Elevated carbon dioxide atmospheres for packaging poultry. I. Effects on ground chicken. Poultry Sci. 64:328-332.

Baker, R. C., R. A. Qureshi, and J. H. Hotchkiss. 1986. Effect of an elevated level of carbon dioxide on the growth of spoilage and pathogenic bacteria at 2, 7, and 13°C. Poultry Sci. 65:729-737.

Baker, D. A., and C. Genigeorgis. 1990. Predicting the safe storage of fresh fish under modified atmospheres with respect to *Clostridium botulinum* toxigenesis by modeling length of the lag phase of growth. J. Food Prot. 53:131-140.

Barnhart, H. M., O. C. Pancorbo, D. W. Dreesen, and E. B. Shotts, Jr. 1989. Recovery of *Aeromonas hydrophila* from carcasses and processing water in a broiler processing operation. J. Food Prot. 52:646-649.

Baya, A. M., A. E. Toranzo, B. Lupiani, T. Li, B. S. Roberson, and F. M. Hetrick. 1991. Biochemical and serological characterization of *Carnobacterium* spp. isolated from farmed and natural populations of striped bass and catfish. Appl. Environ. Microbiol. 57:3114-3120.

Beimfohr, C., A. Krause, R. Amann, W. Ludwig, and K.-H. Schleifer. 1993. *In situ* identification of lactococci, enterococci and streptococci. Syst. Appl. Microbiol. 16:450-456.

Bell, R. G., and K. M. De Lacy. 1986. Factors influencing the determination of nisin in meat products. J. Food Technol. 21:1-7.

Bell, R. G., N. Penney, and S. M. Moorhead. 1995. Growth of the psychrotrophic pathogens *Aeromonas hydrophila*, *Listeria monocytogenes* and *Yersinia enterocolitica* on smoked blue cod (*Parapercis colias*) packed under vacuum or carbon dioxide. Int. J. Food Sci. Technol. 30:515-521.

Ben Embarek, P. K., V. F. Jeppesen, and H. H. Huss. 1994. Antibacterial potential of *Enterococcus faecium* strains isolated from sous-vide cooked fish. Food Microbiol. 11:525-536.

Benkerroum, N., and W. E. Sandine. 1988. Inhibitory activity of nisin against *Listeria monocytogenes*. J. Dairy Sci. 71: 3237-3245.

Bennett, R. W., and W. T. Amos. 1982. *Staphylococcus aureus* growth and toxin production in nitrogen-packed sandwiches. J. Food Prot. 45:157-161.

Bennik, M. H. J., E. J. Smid, F. M. Rombouts, and L. G. M. Gorris. 1995. Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. Food Microbiol. 12:509-519.

Bergey's Manual of Determinative Bacteriology. 1994a. Facultatively anaerobic gram-negative rods, p. 175-289. *In* J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams (ed.), Williams & Wilkins, Baltimore, MD.

Bergey's Manual of Determinative Bacteriology. 1994b. Gram-positive cocci, p. 527-558. *In* J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams (ed.), Williams & Wilkins, Baltimore, MD.

Bergey's Manual of Determinative Bacteriology. 1994c. Regular, nonsporing gram-positive rods. p. 565-570. *In* J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams (ed.), Williams & Wilkins, Baltimore, MD.

Berry, E. D., M. B. Liewen, R. W. Mandigo, and R. W. Hutkins. 1990. Inhibition of *Listeria monocytogenes* by bacteriocin producing *Pediococcus* during the manufacture of fermented semidry sausage. J. Food Prot. 53: 194-197.

Berry, E. D., R. W. Hutkins, and R. W. Mandigo. 1991. The use of bacteriocin-producing *Pediococcus acidilactici* to control postprocessing *Listeria monocytogenes* contamination of frankfurters. J. Food Prot. 54: 681-686.

Betzl, D., W. Ludwig, and Schleifer, K.-H. 1990. Identification of lactococci and enterococci by colony hybridization with 23S-rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 56:2927-2929.

Beuchat, L. R. 1996. *Listeria monocytogenes*: incidence on vegetables. Food Control 7:223-228.

Beumer, R. R., M. C. te Giffel, E. de Boer, and F. M. Rombouts. 1996. Growth of *Listeria monocytogenes* on sliced cooked meat products. Food Microbiol. 13:333-340.

Björkroth, K. J., and H. J. Korkeala. 1996a. Evaluation of *Lactobacillus sake* contamination in vacuum-packaged sliced cooked meat products by ribotyping. J. Food Prot. 59:398-401.

Björkroth, K. J., and H. J. Korkeala. 1996b. rRNA restriction patterns as a characterization tool for *Lactobacillus sake* strains producing ropy slime. Int. J. Food Microbiol. 30:293-302.

Björkroth, K. J., and H. J. Korkeala. 1996c. Characterization of *Lactobacillus sake* strains associated with production of ropy slime by randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) patterns. Int. J. Food Microbiol. 31:59-68.

Björkroth, K. J., and H. J. Korkeala. 1997. Use of rRNA restriction patterns to evaluate lactic acid bacterium contamination of vacuum-packaged sliced cooked whole-meat product in a meat processing plant. Appl. Environ. Microbiol. 63:448-453.

Blickstad, E., and G. Molin. 1983a. Carbon dioxide as a controller of the spoilage flora of pork, with special reference to temperature and sodium chloride. J. Food Prot. 46:756-763.

Blickstad, E., and G. Molin. 1983b. The microbial flora of smoked pork loin and frankfurter sausage stored in different gas atmospheres at 4°C. J. Appl. Bacteriol. 54:45-56.

Borch, E., and G. Molin. 1988. Numerical taxonomy of psychrotrophic lactic acid bacteria from prepacked meat and meat products. Antonie van Leeuwenhoek 54:301-323.

Borch, E., and H. Agerhem. 1992. Chemical, microbial and sensory changes during the anaerobic cold storage of beef inoculated with a homofermentative *Lactobacillus* sp. or a *Leuconostoc* sp. Int. J. Food Microbiol. 15:99-108.

Borch, E., M.-L. Kant-Muermans, and Y. Blixt. 1996. Bacterial spoilage of meat and cured meat products. Int. J. Food Microbiol. 33:103-120.

Brackett, R. E. 1992. Microbiological safety of chilled foods: current issues. Trends Food Sci. Technol. 3:81-85.

Breidt, F., K. A. Crowley, and H. P. Fleming. 1995. Controlling cabbage fermentations with nisin and nisin-resistant *Leuconostoc mesenteroides*. Food Microbiol. 12:109-116.

Breidt, F., and H. P. Fleming. 1997. Using lactic acid bacteria to improve the safety of minimally processed fruits and vegetables. Food Technol. 51:44-46, 48, 51.

Brody, A. L. 1989. Modified atmosphere/vacuum packaging of meat. p. 17-37. *In* A. L. Brody (ed.), Controlled/modified atmosphere/vacuum packaging of foods, Food and Nutrition Press, Inc., Trumbull, CT.

Brody, A. L. 1993. The market. p. 19-40. *In* R. T. Parry (ed.), Principles and applications of modified atmosphere packaging of food, Blackie Academic & Professional, Glasgow.

Brody, A. L. 1996. Integrating aseptic and modified atmosphere packaging to fulfill a vision of tomorrow. Food Technol. 56-66.

Brooks, J. L., A. S. Moore, R. S. Patchett, M. D. Collins, and R. G. Kroll. 1992. Use of the polymerase chain reaction and oligonucleotide probes for the rapid detection and identification of *Carnobacterium* species from meat. J. Appl. Bacteriol. 72:294-301.

Buchanan, R. L., and L. A. Klawitter. 1992a. Characterization of a lactic acid bacterium, *Carnobacterium piscicola* LK5, with activity against *Listeria monocytogenes* at refrigeration temperatures. J. Food Safety 12:199-217.

Buchanan, R. L., and L. A. Klawitter. 1992b. Effectiveness of *Carnobacterium piscicola* LK5 for controlling the growth of *Listeria monocytogenes* Scott A in refrigerated foods. J. Food Safety 12:219-236.

Buchanan, R. L., and S. A. Palumbo. 1985. *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: A review. J. Food Safety 7:15-29.

Bula, C., J. Bille, and M. P. Glauser. 1995. An epidemic of foodborne listeriosis in western Switzerland: Description of 57 cases involving adults. Clin. Infect. Dis. 20:66-72.

Buncic, S., L. Paunovic, and D. Radisic. 1991. The fate of *Listeria monocytogenes* in fermented sausages and in vacuum-packaged frankfurters. J. Food Prot. 54:413-417.

Buncic, S., C. M. Fitzgerald, R. G. Bell, and J. A. Hudson. 1995. Individual and combined listericidal effect of sodium lactate, potassium sorbate, nisin and curing salts at refrigeration temperature. J. Food Safety 15:247-264.

Burke, V., M. Gracey, J. Robinson, D. Peck, J. Beaman, and E. Blundell. 1983. The microbiology of childhood gastroenteritis; *Aeromonas* species and other infective agents. J. Infect. Dis. 148:68-74.

Callister, S. M., and W. A. Agger. 1987. Enumeration and characterization *of Aeromonas hydrophila* and *Aeromonas caviae* isolated from grocery store produce. Appl. Environ. Microbiol. 53:249-253.

Campanini, M., I. Pedrazzoni, S. Barbuti, and P. Baldini. 1993. Behaviour of *Listeria monocytogenes* during the maturation of naturally and artificially contaminated salami: effect of lactic-acid starter cultures. Int. J. Food Microbiol. 20:169-175.

Cancilla, M. R., I. B. Powell, A. J. Hillier, and B. E. Davidson. 1992. Rapid genomic fingerprinting of *Lactococcus lactis* strains by arbitrarily primed polymerase chain reaction with 32P and fluorescent labels. Appl. Environ. Microbiol. 58:1772-1775.

Carlin, F., and M. W. Peck. 1995. Growth and toxin production by non-proteolytic and proteolytic *Clostridium botulinum* in cooked vegetables. Lett. Appl. Microbiol. 20:152-156.

Carlin, F., and M. W. Peck. 1996. Growth and toxin production by non-proteolytic *Clostridium botulinum* in cooked vegetables at refrigeration temperatures. Appl. Environ. Microbiol. 62:3069-3072.

Carlin, F., C. Nguyen-The, and C. E. Morris. 1996. Influence of background microflora on *Listeria monocytogenes* on minimally processed fresh broad-leaved endive (*Cichorium endivia* var. *latifolia*). J. Food Prot. 59:698-703.

Carpenter, S. L., and M. A. Harrison. 1989. Fate of small populations of *Listeria monocytogenes* on poultry processed using moist heat. J. Food Prot. 52:768-770.

Carr, T. P., and J. A. Marchello. 1986. Microbial changes of precooked beef slices as affected by packaging procedure. J. Food Prot. 49:534-536.

Carr, T. P., and J. A. Marchello. 1987. Growth of aerobic psychrotrophs and color changes of precooked beef slices as affected by packaging procedure. J. Food Prot. 50:733-736.

Charteris, W. P., P. M. Kelly, L. Morelli, J. K. Collins. 1997. Selective detection, enumeration and identification of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in mixed bacterial populations. Int. J. Food Microbiol. 35:1-27.

Choi, S.-Y., and L. R. Beuchat. 1994. Growth inhibition of *Listeria monocytogenes* by a bacteriocin of *Pediococcus acidilactici* M during fermentation of kimchi. Food Microbiol. 11:301-307.

Christopher, F. M., S. C. Seideman, Z. L. Carpenter, G. C. Smith, and C. Vanderzant. 1979. Microbiology of beef packaged in various gas atmospheres. J. Food Prot. 42:240-244.

Chung, K.-T., J. S. Dickson, and J. D. Crouse. 1989. Effects of nisin on growth of bacteria attached to meat. Appl. Environ. Microbiol. 55:1329-1333.

Church, P. N. 1993. Meat products. p. 229-268. *In* R. T. Parry (ed.), Principles and applications of modified atmosphere packaging of food, Blackie Academic & Professional, Glasgow.

Church, I. J., and A. L. Parsons. 1995. Modified atmosphere packaging technology: a review. J. Sci. Food Agric. 67:143-152.

Clark, D. S., and C. P. Lentz. 1969. The effect of carbon dioxide on the growth of slime producing bacteria in fresh beef. Can. Inst. Food Sci. Technol. J. 2:72-75.

Clark, D. S., and C. P. Lentz. 1972. Use of carbon dioxide for extending shelf-life of prepackaged beef. Can. Inst. Food Sci. Technol. J. 5:175-178.

Collins, M. D., J. A. E. Farrow, B. A. Phillips, S. Ferusu, and D. Jones. 1987. Classification of *Lactobacillus divergens*, *Lactobacillus piscicola*, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, *Carnobacterium*. Int. J. Syst. Bacteriol. 37:310-316.

Collins, M. D., J. Samelis, J. Metaxopoulos, and S. Wallbanks. 1993. Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. J. Appl. Bacteriol. 75:595-603.

Collins-Thompson, D. L., and G. R. Lopez. 1982. Control of *Brochothrix thermosphacta* by *Lactobacillus* species in vacuum packed bologna. Can. Inst. Food Sci. Technol. J. 15:307-309.

Conner, D. E., V. N. Scott, D. T. Bernard, and D. A. Kautter. 1989. Potential *Clostridium botulinum* hazards associated with extended shelf-life refrigerated foods: a review. J. Food Safety 10:131-153.

Cooksey, K., B. P. Klein, F. K. McKeith, and H. P. Blaschek. 1993a. Post-packaging pasteurization reduces *Clostridium perfringens* and other bacteria in precooked vacuum-packaged beef loin chunks. J. Food Sci. 58:239-241.

Cooksey, K., B. P. Klein, F. K. McKeith, and H. P. Blaschek. 1993b. Reduction of *Listeria monocytogenes* in precooked vacuum-packaged beef using postpackaging pasteurization. J. Food Prot. 56:1034-1038.

Coventry, M. J., K. Muirhead, and M. W. Hickey. 1995. Partial characterization of pediocin PO2 and comparison with nisin for biopreservation of meat products. Int. J. Food Microbiol. 26: 133-145.

Coventry, M. J., J. B. Gordon, A. Wilcock, K. Harmack, B. E. Davidson, M. W. Hickey, A. J. Hillier, and J. Wan. 1997. Detection of bacteriocins of lactic acid bacteria isolated from foods and comparison with pediocin and nisin. J. Appl. Microbiol. 83:248-258.

Cox, L. J. 1989. A perspective on listeriosis. Food Technol. 43:52-59.

Cox, L. J., T. Kleiss, J. L. Cordier, C. Cordellana, P. Konkel, C. Pedrazzini, R. Beumer, and A. Siebenga. 1989. *Listeria* spp. in food processing, non-food, and domestic environments. Food Microbiol. 6:49.

Crandall, A. D., and T. J. Montville. 1993. Inhibition of *Clostridium botulinum* growth and toxigenesis in a model gravy system by coinoculation with bacteriocin-producing lactic acid bacteria. J. Food Prot. 56: 485-488.

Crandall, A. D., K. Winkowski, and T. J. Montville. 1994. Inability of *Pediococcus pentosaceus* to inhibit *Clostridium botulinum* in sous vide beef with gravy at 4 and 10°C. J. Food Prot. 57: 104-107.

Cutter, C. N., and G. R. Siragusa. 1994. Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiol. 11:481-489.

Cutter, C. N., and G. R. Siragusa. 1996a. Reduction of *Brochothrix thermosphacta* on beef surfaces following immobilization of nisin in calcium alginate gels. Lett. Appl. Microbiol. 23:9-12.

Cutter, C. N., and G. R. Siragusa. 1996b. Reductions of *Listeria innocua* and *Brochothrix thermosphacta* on beef following nisin spray treatments and vacuum packaging. Food Microbiol. 13:23-33.

Daeschel, M. A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol. 43:164-167.

Daeschel, M. A. 1990. Applications and interactions of bacteriocins from lactic acid bacteria in foods and beverages. p. 63-91. *In* D. G. Hoover and L. R. Steenson (ed.), Bacteriocins of lactic acid bacteria, Academic Press, Inc., San Diego, CA.

Dainty, R. H., C. M. Hibbard, and R. A. Edwards. 1984. Cellular fatty acids of streptobacteria isolated from vacuum packaged meats. Syst. Appl. Microbiol. 5:233-240.

Daniels, J. A., R. Krishnamurthi, and S. S. H. Rizvi. 1985. A review of the effects of carbon dioxide on microbial growth and food quality. J. Food Prot. 48:532-537.

Davidson, W. D.. 1987. Retail store handling conditions for refrigerated foods. Presented at the 80th Annual Convention of the National Processors Assn., Jan. 26, Chicago, IL.

Davidson, P. M., and D. G. Hoover. 1993. Antimicrobial components from lactic acid bacteria. p. 127-159. *In* S. Salminen and A. von Wright (ed.), Lactic acid bacteria, Marcel Dekker, Inc., New York.

Davies, A. R. 1996. Advances in modified-atmosphere packaging. p. 304-320. *In* G. W. Gould (ed.), New methods of food preservation, Blackie Academic & Professional, London.

Davies, E. A., H. E. Bevis, and J. Delves-Broughton. 1997. The use of the bacteriocin, nisin, as a preservative in ricotta-type cheeses to control the food-borne pathogen *Listeria monocytogenes*. Lett. Appl. Microbiol. 24:343-346.

Davis, H. K. 1993. Fish. p. 189-228. *In* R. T. Parry (ed.), Principles and applications of modified atmosphere packaging of food, Blackie Academic & Professional, Glasgow.

Decallone, J., M. Delmee, P. Wauthoz, M. El Lioui, and R. Lambert. 1991. A rapid procedure for the identification of lactic acid bacteria based on the gas chromatographic analysis of the cellular fatty acids. J. Food Prot. 54:217-224.

Degnan, A. J., and J. B. Luchansky. 1992. Influence of beef tallow and muscle on the antilisterial activity of pediocin AcH and liposome-encapsulated pediocin AcH. J. Food Prot. 55:552-554.

Degnan, A. J., A. E. Yousef, and J. B. Luchansky. 1992. Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. J. Food Prot. 55: 98-103.

Degnan, A. J., N, Buyong, and J. B. Luchansky. 1993. Antilisterial activity of pediocin AcH in model food systems in the presence of an emulsifier or encapsulated within liposomes. Int. J. Food Microbiol. 18: 127-138.

Degnan, A. J., C. W. Kaspar, W. S. Otwell, M. L. Tamplin, and J. B. Luchansky. 1994. Evaluation of lactic acid bacterium fermentation products and food-grade chemicals to control *Listeria monocytogenes* in blue crab (*Callinectes sapidus*) meat. Appl. Environ. Microbiol. 60: 3198-3203.

Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. Food Technol. 44:100, 102, 104, 106, 108, 111, 112, 117.

De Man, J. C., M. Rogosa, and M. E. Sharpe. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23:130-135.

De Vos, W. M., J. W. M. Mulders, R. J. Siezen, J. Hugenholtz, and O. P. Kuipers. 1993. Properties of nisin Z and distribution of its gene, *nisZ*, in *Lactococcus lactis*. Appl. Environ. Microbiol. 59:213-218.

Devriese, L. A., M. D. Collins, and R. Wirth. 1991. The genus *Enterococcus*. p.1535-1594. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes: A handbook on habitats, isolation, and identification of bacteria, 2nd ed. Springer-Verlag, Heidelberg.

Dicks, L. M. T., B. Janssen, and F. Dellaglio. 1995. Differentiation of *Carnobacterium divergens* and *Carnobacterium piscicola* by numerical analysis of total soluble cell protein patterns and DNA-DNA hybridization. Curr. Microbiol. 31:77-79.

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

Dodd, H. M., N. Horn, Z. Hao, and M. J. Gasson. 1992. A lactococcal expression system for engineered nisin. Appl. Environ. Microbiol. 58:3683-3693.

Donnelly, C. W., R. E. Brackett, S. Doores, W. H. Lee, and J. Lovett. 1992. *Listeria*, p. 637-663. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, 3rd ed., Washington, D.C.

Döring, B., S. Erhardt, and F.-K. Lücke. 1988. Computer-assisted identification of lactic acid bacteria from meats. Syst. Appl. Bacteriol. 11:67-74.

Duffy, L. L., P. B. Vanderlinde, and F. H. Grau. 1994. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, $a_w$, nitrite and ascorbate. Int. J. Food Microbiol. 23:377-390.

Duxbury, D. D. 1990. Sodium lactate extends shelf-life, improves flavour of cooked beef. Food Process., April pp. 46-47.

Dykes, G. A., T. E. Cloete, and A. von Holy. 1995. Taxonomy of lactic acid bacteria associated with vacuum-packaged processed meat spoilage by multivariate analysis of cellular fatty acids. Int. J. Food Microbiol. 28:89-100.

Egan, A. F., A. L. Ford, and B. J. Shay. 1980. A comparison of *Microbacterium thermosphactum* and lactobacilli as spoilage organisms of vacuum-packaged sliced luncheon meats. J. Food Sci. 45:1745-1748.

Egan, A. F., and B. J. Shay. 1982. Significance of lactobacilli and film permeability in the spoilage of vacuum-packaged beef. J. Food Sci. 47:1119-1122, 1126.

Egan, A. F., B. J. Shay, and P. J. Rogers. 1989. Factors affecting the production of hydrogen sulphide by *Lactobacillus sake* L13 growing in vacuum-packed beef. J. Appl. Bacteriol. 67:255-262.

Ehrmann, M., Ludwig, W. and Schleifer, K.-H. 1994. Reverse dot blot hybridization: A useful method for the direct identification of lactic acid bacteria in fermented food. FEMS Microbiol. Lett. 117:143-150.

Einarsson, H., and H. L. Lauzon. 1995. Biopreservation of brined shrimp (*Pandalus borealis*) by bacteriocins from lactic acid bacteria. Appl. Environ. Microbiol. 61: 669-676.

Eklund, T., and T. Jarmund. 1983. Microculture studies on the effect of various gas atmospheres on microbial growth at different temperatures. J. App. Bacteriol. 55:119-125.

El-Khateib, T., A. E. Yousef, and H. W. Ockerman. 1993. Inactivation and attachment of *Listeria monocytogenes* on beef muscle treated with lactic acid and selected bacteriocins. J. Food Prot. 56: 29-33.

Enfors, S.-O., and G. Molin. 1978. The influence of high concentrations of carbon dioxide on the germination of bacterial spores. J. Appl. Bacteriol. 45:279-285.

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

Enfors, S. O., and G. Molin. 1980. Effect of high concentration of carbon dioxide on growth rate of *Pseudomonas fragi*, *Bacillus cereus*, and *Streptococcus cremoris*. J. Appl. Bacteriol. 48:409-416.

Enfors, S.-O., and G. Molin. 1981a. The effect of different gases on the activity of microorganisms. p. 335-343. *In* T. A. Roberts, G. Hobbs, J. H. B. Christian, and N. Skovgaard (ed.), Psychrotrophic microorganisms in spoilage and pathogenicity, Academic Press, London.

Enfors, S.-O., and G. Molin. 1981b. The influence of temperature on the growth inhibitory effect of carbon dioxide on *Pseudomonas fragi* and *Bacillus cereus*. Can. J. Microbiol. 27:15-19.

Erichsen, L., and G. Molin. 1981. Microbial flora of normal and high pH beef stored at 4°C in different gas environments. J. Food Prot. 44:866-869.

Eyles, M. 1994. Australian perspective on *Listeria monocytogenes*. Dairy Food Environ. Sanit. 14:208-209.

Fang, T. J., and L.-W. Lin. 1994. Growth of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked pork in a modified atmosphere packaging/nisin combination system. J. Food Prot. 57:479-485.

Fang, T. J., and C.-Y. Chen. 1997. Inhibition of *Staphylococcus aureus* and *Bacillus cereus* on a vegetarian food treated with nisin combined with either potassium sorbate or sodium benzoate. J. Food Safety 17:69-87.

Farber, J. M., D. W. Warburton, L. Gour, and M. Milling. 1990. Microbiological quality of foods packaged under modified atmospheres. Food Microbiol. 7:327-334.

Farber, J. M. 1991. Microbiological aspects of modified atmosphere packaging technology - A review. J. Food Prot. 54:58-70.

Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55:476-511.

Farber, J. M., and E. Daley. 1994. Fate of *Listeria monocytogenes* on modified-atmosphere packaged turkey roll slices. J. Food Prot. 57:1098-1100.

Farber, J. M., and J. Harwig. 1996. The Canadian position on *Listeria monocytogenes* in ready-to-eat foods. Food Control 7:253-258.

Farber, J. M., W. H. Ross, and J. Harwig. 1996. Health risk assessment of *Listeria monocytogenes* in Canada. Int. J. Food Microbiol. 30:145-156.

Ferreira, M. A. S. S., and B. M. Lund. 1996. The effect of nisin on *Listeria monocytogenes* in culture medium and long-life cottage cheese. Lett. Appl. Microbiol. 22:433-438.

Fey, M. S., and J. M. Regenstein. 1982. Extending shelf-life of fresh wet red hake and salmon using $CO_2$-$O_2$ modified atmosphere and potassium sorbate ice at 1°C. J. Food Sci. 47:1048-1054.

Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. New Engl. J. Med. 312:404-407.

Foegeding, P. M., and F. F. Busta. 1983. Effect of carbon dioxide, nitrogen and hydrogen gases on germination of *Clostridium botulinum* spores. J. Food Prot. 46:987-989.

Foegeding, P. M., A. B. Thomas, D. H. Pilkington, and T. R. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. 58: 884-890.

Fricker, C. R., and S. Tompsett. 1989. *Aeromonas* species in foods: a significant cause of food poisoning? Int. J. Food Microbiol. 9:17-23.

Gardner, G. A., A. W. Carson, and J. Patton. 1967. Bacteriology of prepacked pork with reference to the gas composition within the pack. J. Appl. Bacteriol. 30:321-333.

Gardner, G. A., J. H. Denton, and S. E. Hartley. 1977. Effects of carbon dioxide environments on the shelf life of broiler carcasses. Poultry Sci. 56:1715-1716.

Garriga, M., M. Hugas, T. Aymerich, and J. M. Montfort. 1993. Bacteriocinogenic activity of lactobacilli isolated from fermented sausages. J. Appl. Bacteriol. 75:142-148.

Garver, K. I., and P. M. Muriana. 1993. Detection, identification and characterization of bacteriocin-producing lactic acid bacteria from retail food-products. Int. J. Food Microbiol. 19:241-258.

Garvie, E. I. 1953. Some group N streptococci isolated from raw milk. J. Dairy Res. 20:41-44.

Garvie, E. I. 1978. *Streptococcus raffinolactis* Orla-Jensen and Hansen, a group N streptococcus found in raw milk. Int. J. Syst. Bacteriol. 28:190-193.

Garvie, E. I. 1979. Proposal of National Collection of Dairy Organisms strain 617 as the neotype strain of *Streptococcus raffinolactis* Orla-Jensen and Hansen. Int. J. Syst. Bacteriol. 29:152.

Gellin, B. G., C. V. Broome, W. F. Bibb, R. E. Weaver, S. Gaventa, and L. Mascola. 1991. The epidemiology of listeriosis in the US - 1986. Am. J. Epidem. 133:392-401.

Genigeorgis, C. A., J. Meng, and D. A. Baker. 1991. Behavior of nonproteolytic *Clostridium botulinum* type B and E spores in cooked turkey and modeling lag phase and probability of toxigenesis. J. Food Sci. 56:373-379.

Gilbert, R. J., K. L. Miller, and D. Roberts. 1989. *Listeria monocytogenes* and chilled foods. Lancet i:383-384.

Gill, C. O., and K. H. Tan. 1980. Effect of carbon dioxide on the growth of meat spoilage bacteria. Appl. Environ. Microbiol. 39:317-319.

Gill, C. O., and J. C. L. Harrison. 1989. The storage life of chilled pork packaged under carbon dioxide. 26:313-324.

Gill, C. O., and M. P. Reichel. 1989. Growth of the cold-tolerant pathogens *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. Food Microbiol. 6:223-230.

Gill, C. O., and K. M. DeLacy. 1991. Growth of *Escherichia coli* and *Salmonella typhimurium* on high-pH beef packed under vacuum or carbon dioxide. Int. J. Food Microbiol. 13:21-30.

Giraffa, G. 1995. Enterococcal bacteriocins: their potential as anti-*Listeria* factors in dairy technology. Food Microbiol. 12:291-299.

Giraffa, G., D. Carminati, and G. Torri Tarelli. 1995. Inhibition of *Listeria innocua* by bacteriocin-producing *Enterococcus faecium* 7C5. J. Food Prot. 58:621-623.

Giraffa, G. 1996. Lactic and non-lactic acid bacteria as a tool for improving the safety of dairy products. Industrie Alimentari 35:244-248.

Glass, K. A., and M. P. Doyle. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl. Environ. Microbiol. 55:1565-1569.

Goff, J. H., A. K. Bhunia, and M. G. Johnson. 1996. Complete inhibition of low levels of *Listeria monocytogenes* on refrigerated chicken meat with pediocin AcH bound to heat-killed *Pediococcus acidilactici* cells. J. Food Prot. 59: 1187-1192.

Gombas, D. E. 1989. Biological competition as a preserving mechanism. J. Food Safety 10:107-117.

Gonzales, C. F. and B. S. Kunka. 1987. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici.* Appl. Environ. Microbiol. 53:2534-2538.

Gorris, L. G. M. and T. Abee. 1996. Biopreservation of ready-to-eat salads. Zeit. Lebensm. Technol. 47: 79-81.

Gracey, M., V. Burke, and J. Robinson. 1982. *Aeromonas* associated gastroenteritis. Lancet ii:1304-1306.

Grant, I. R., and M. F. Patterson. 1991a. Effect of irradiation and modified atmosphere packaging on the microbiological safety of minced pork stored under temperature abuse conditions. Int. J. Food Sci. Technol. 26:521-533

Grant, I. R., and M. F. Patterson. 1991b. A numerical taxonomy of lactic acid bacteria isolated from irradiated pork and chicken packaged under various gas atmospheres. J. Appl. Bacteriol. 70:302-307.

Grant, K. A., J. H. Dickinson, M. J. Payne, S. Campbell, M. D. Collins, and R. G. Kroll. 1993. Use of the polymerase chain reaction and 16S rRNA sequences for the rapid detection of *Brochothrix thermosphacta* in foods. J. Appl. Bacteriol. 74:260-267.

Grau, F. H., and P. B. Vanderlinde. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. 55:4-7.

Green, S. S. 1990. *Listeria monocytogenes* in meat and poultry products. Interim Rept. to Nat'l Adv. Comm. Microbiol. Spec. Foods, FSIS/USDA, Nov.27.

Hague, M. A., C. L. Kastner, D. Y. C. Fung, K. Kone, and J. R. Schwenke. 1997. Use of nisin and microwave treatment reduces *Clostridium sporogenes* outgrowth in precooked vacuum-packaged beef. J. Food Prot. 60:1072-1074.

Hammes, W. P., A. Bantleon, and S. Min. 1990. Lactic acid bacteria in meat fermentations. FEMS Microbiol. Rev. 87:165-174.

Hammes, W. P., N. Weiss, and W. Holzapfel. 1991. The genera *Lactobacillus* and *Carnobacterium*, p. 1535-1594. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows,

and H. G. Schlegel (ed.), The prokaryotes: A handbook on habitats, isolation, and identification of bacteria , 2nd ed. Springer-Verlag, Heidelberg.

Hammes, W. P., and C. Hertel. 1996. Selection and improvement of lactic acid bacteria used in meat and sausage fermentation. Lait 76:159-168.

Hammes, W. P., and P. S. Tichaczek. 1994. The potential of lactic acid bacteria for the production of safe and wholesome food. Zeit. Lebensm. Unter. Forsch. 198:193-201.

Hanlin, M. B., N. Kalchayanand, P. Ray, and B. Ray. 1993. Bacteriocins of lactic acid bacteria in combination have greater antibacterial activity. J. Food Prot. 56:252-255.

Hanna, M. O., D. L. Zink, Z. L. Carpenter, and C. Vanderzant. 1976. *Yersinia enterocolitica*-like organisms from vacuum-packaged beef and lamb. J. Food Sci. 41:1254-1256.

Hanna, M. O., L. C. Hall, G. C. Smith, and C. Vanderzant. 1980. Inoculation of beef steaks with *Lactobacillus* species before vacuum packaging. I. Microbiological considerations. J. Food Prot. 43:837-841.

Hardin, M. D., S. E. Williams, and M. A. Harrison. 1993. Survival of *Listeria monocytogenes* in postpasteurized precooked beef roasts. J. Food Prot. 56:655-660.

Harmon, M. C., B. Swaminathan, and J. C. Forrest. 1984. Isolation of *Yersinia enterocolitica* and related species from porcine samples obtained from an abattoir. J. Appl. Bacteriol. 56:421-427.

Harris, R. D. 1989. Kraft builds safety into next generation refrigerated foods. Food Process. 50:111-112, 114.

Harris, L. J., M. A. Daeschel, M. E. Stiles, and T. R. Klaenhammer. 1989. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. J. Food Prot. 52:384-387.

Harris, L. J., H. P. Fleming, and T. R. Klaenhammer. 1992. Novel paired starter culture system for sauerkraut, consisting of a nisin-resistant *Leuconostoc mesenteroides* strain and a nisin-producing *Lactococcus lactis* strain. Appl. Environ. Microbiol. 58:1484-1489.

Harrison, M. A., and S. L. Carpenter. 1989a. Survival of large populations *Listeria monocytogenes* on chicken breasts processed using moist heat. J. Food Prot. 52:376-378.

Harrison, M. A., and S. L. Carpenter. 1989b. Survival of *Listeria monocytogenes* on microwave cooked poultry. Food Microbiol. 6:153-157.

Hart, C. D., G. C. Mead, and A. P. Norris. 1991. Effects of gaseous environment and temperature on the storage behavior of *Listeria monocytogenes* on chicken breast meat. J. Appl. Bacteriol. 70:40-46.

Hastings, J. W., and M. E. Stiles. 1991. Antibiosis of *Leuconostoc gelidum* isolated from meat. J. App. Bacteriol. 70:127-134.

Hauschild, A. H. W. 1989. *Clostridium botulinum.* p. 111-189. *In* M. P. Doyle (ed.), Foodborne Bacterial Pathogens, Marcel Dekker, Inc., New York

Héchard, Y., B. Dérijard, F. Letellier, and Y. Cenatiempo. 1992. Characterization and purification of mesentericin Y105, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*. J. Gen. Microbiol. 138:2725-2731

Helander, I. M., A. von Wright, and T.-M. Mattila-Sandholm. 1997. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends Food Sci. Technol. 8:146-150.

Hertel, C., W. Ludwig, M. Obst, R. F. Vogel, W. P. Hammes, and K.-H. Schleifer. 1991. 23S rRNA-targeted oligonucleotide probes for the rapid identification of lactobacilli. Syst. Appl. Microbiol. 14:173-177.

Hertel, C., W. Ludwig, B. Pot, K. Kersters, and K.-H. Schleifer. 1993. Differentiation of lactobacilli occurring in fermented milk products by using oligonucleotide probes and electrophoretic protein profiles. Syst. Appl. Microbiol. 16:463-467.

Hintlian, C. B., and J. H. Hotchkiss. 1987a. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. J. Food Prot. 50:218-223.

Hintlian, C. B., and J. H. Hotchkiss. 1987b. Microbiological and sensory evaluation of cooked roast beef packaged in a modified atmosphere. J. Food Process. Preserv. 11:171-179.

Hitchener, B. J., A. F. Egan, and P. J. Rogers. 1982. Characteristics of lactic acid bacteria isolated from vacuum-packaged beef. J. Appl. Bacteriol. 52:331-337.

Hiu, S. F., R. A. Holt, N. Sriranganathan, R. J. Seidler, and J. L. Fryer. 1984. *Lactobacillus piscicola,* a new species from salmonid fish. Int. J. Syst. Bacteriol. 34:393-400

Holzapfel, W. H., R. Geisen, and U. Schillinger. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol. 24:343-362.

Hood, D. E., and G. C. Mead. 1993. Modified atmosphere storage of fresh meat and poultry. p. 269-298. *In* R. T. Parry (ed.), Principles and applications of modified atmosphere packaging of food, Blackie Academic & Professional, Glasgow.

Hotchkiss, J. H. 1988. Experimental approaches for determining the safety of food packaged in modified atmospheres. Food Technol. 42:55-64.

Hudson, J. A., S. J. Mott, K. M. DeLacy, and A. L. Edridge. 1992. Incidence and coincidence of *Listeria* spp., motile aeromonads and *Yersinia enterocolitica* on ready-to-eat fleshfoods. 16:99-108.

Hudson, J. A., and S. J. Mott. 1993a. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cold-smoked salmon under refrigeration and mild temperature abuse. Food Microbiol. 10:61-68.

Hudson, J. A., and S. J. Mott. 1993b. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cooked beef under refrigeration and mild temperature abuse. Food Microbiol. 10:429-437.

Hudson, J. A., and S. M. Avery. 1994. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cooked mussel tissue under refrigeration and mild temperature abuse. J. Food Safety 14:41-52.

Hudson, J. A., S. J. Mott, and N. Penney. 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.

Hugas, M., M. Garriga, M. T. Aymerich, and J. M. Monfort. 1995. Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC494. J. Appl. Bacteriol. 79:322-330.

Huss, H. H., V. F. Jeppesen, C. Johansen, and L. Gram. 1995. Biopreservation of fish products - a review of recent approaches and results. J. Aquatic Food Product Technol. 4:5-26.

Hutton, M. T., P. A. Chehak, and J. L. Hanlin. 1991. Inhibition of botulinum toxin production by *Pediococcus acidilactici* in temperature abused refrigerated foods. J. Food Safety 11:255-267.

Ibrahim, A., and I. C. Mac Rae. 1991. Isolation of *Yersinia enterocolitica* and related species from red meat and milk. J. Food Sci. 56:1524-1526.

Ikawa, J. Y. 1991. *Clostridium botulinum* growth and toxigenesis in shelf stable noodles. J. Food Sci. 56:264-265.

Ingham, S. 1990. Growth of *Aeromonas hydrophila* and *Plesiomonas shigelloides* on cooked crayfish tails during cold storage under air, vacuum, and a modified atmosphere. J. Food Prot. 53:665-667.

Ingham, S. C., and N. N. Potter. 1988. Growth of *Aeromonas hydrophila* and *Pseudomonas fragi* on mince and surimis made from Atlantic pollock and stored under air or modified atmosphere. J. Food Prot. 51:966-970.

Ingham, S. C., J. M. Escude, and P. McCown. 1990a. Comparative growth rates of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked chicken loaf stored under air and two modified atmospheres. J. Food Prot. 53:289-291.

Ingham, S. C., R. A. Alford, and A. P. McCown. 1990b. Comparative growth rates of *Salmonella typhimurium* and *Pseudomonas fragi* on cooked crab meat stored under air and modified atmosphere. J. Food Prot. 53:566-567.

Ingham, S. C., and C. L. Tautorus. 1991. Survival of *Salmonella typhimurium*, *Listeria monocytogenes* and indicator bacteria on cooked turkey loaf stored under vacuum at 3°C. J. Food Safety 11:285-292.

Jack, R. W., J. Wan, J. Gordon, K. Harmark, B. E. Davidson, A. J. Hillier, R. E. H. Wettenhall, M. W. Hickey, and M. J. Coventry. 1996. Characterization of the chemical and antimicrobial properties of piscicolin 126, a bacteriocin produced by *Carnobacterium piscicola* JG126. Appl. Environ. Microbiol. 62:2897-2903.

Jacquet, C., B. Catimel, R. Brosch, C. Buchrieser, P. Dehaumont, V. Goulet, A. Lepoutre, P. Veit, and J. Rocourt. 1995. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. Appl. Environ. Microbiol. 61:2242-2246.

Jay, J. M. 1996a. Prevalence of *Listeria* spp. in meat and poultry products. Food Control 7:209-214.

Jay, J. M. 1996b. Modern food microbiology, p. 478-506. 5th ed., Chapman & Hall, Ltd., New York.

Jay, J. M. 1997. Do background microorganisms play a role in the safety of fresh foods? Trends Food Sci. Technol. 8:421-424.

Jemmi, T., and A. Keusch. 1992. Behavior of *Listeria monocytogenes* during the processing and storage of experimentally contaminated hot-smoked trout. Int. J. Food Microbiol. 15:339-346.

Jemmi, T., and A. Keusch. 1994. Occurrence of *Listeria monocytogenes* in freshwater fish farms and fish-smoking plants. Food Microbiol. 11:309-316.

Jeppesen, V. F., and H. H. Huss. 1993. Antagonistic activity of two strains of lactic acid bacteria against *Listeria monocytogenes* and *Yersinia enterocolitica* in a model fish product. Int. J. Food Microbiol. 19: 179-186.

Johnson, J. L., M. P. Doyle, and R. G. Cassens. 1988. Survival of *Listeria monocytogenes* in ground beef. Int. J. Food Microbiol. 6:243-247.

Johnson, J. L., M. P. Doyle, and R. G. Cassens. 1990. *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products: a review. J. Food Prot. 53:81-91.

Jujena, V. K., J. E. Call, B. S. Marmer, and A. J. Miller. 1994a. The effect of temperature abuse on *Clostridium perfringens* in cooked turkey stored under air and vacuum. Food Microbiol. 11:187-193.

Jujena, V. K., B. S. Marmer, and A. J. Miller. 1994b. Growth and sporulation potential of *Clostridium perfringens* in aerobic and vacuum-packaged cooked beef. J. Food Prot. 57:393-398.

Jujena, V. K., and W. M. Majka. 1995. Outgrowth of *Clostridium perfringens* spores in cook-in-bag beef products. J. Food Safety 15:21-34.

Jujena, V. K., and B. S. Marmer. 1996. Growth of *Clostridium perfringens* from spore inocula in *sous-vide* turkey products. Int. J. Food Microbiol. 32:115-123.

Jujena, V. K., B. S. Marmer, and J. E. Call. 1996. Influence of modified atmosphere packaging on growth of *Clostridium perfringens* in cooked turkey. J. Food Safety 16:141-150.

Jung, D.-S., F. W. Bodyfelt, and M. A. Daeschel. 1992. Influence of fat and emulsifiers on the efficacy of nisin in inhibiting *Listeria monocytogenes* in fluid milk. J. Dairy Sci. 75:387-393.

Kandler, O., and N. Weiss. 1986. Genus *Lactobacillus*. p. 1209-1234. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2, Williams & Wilkins Co., Baltimore, MD.

Kautter, D. A., R. K. Lynt, T. Lilly, Jr., and H. M. Solomon. 1981. Evaluation of the botulism hazard from nitrogen-packed sandwiches. J. Food Prot. 44:59-61.

Kelly, W. J., R. V. Asmundson, and C. M. Huang. 1996. Isolation and characterization of bacteriocin-producing lactic acid bacteria from ready-to-eat food products. Int. J. Food Microbiol. 33:209-218.

Kempton, A. G., and S. R. Bobier. 1970. Bacterial growth in refrigerated, vacuum-packed luncheon meats. Can. J. Microbiol. 16:287-297.

Kerr, K. G., N. A. Rotowa, P. M. Hawkey, and R. W. Lacey. 1990. Incidence of *Listeria* spp. in pre-cooked, chilled chicken products as determined by culture and enzyme-linked immunoassay (ELISA). J. Food Prot. 53:606-607.

Kerr, K. G., D. Birkenhead, K. Seale, J. Major, and P. M. Hawkey. 1993. Prevalence of *Listeria* spp. on the hands of food workers. J. Food Prot. 56:525-527.

Kilpper-Bälz, R., G. Fischer, and K. H. Schleifer. 1982. Nucleic acid hybridization of group N and group D streptococci. Curr. Microbiol. 7:245-250.

Kim, K.-T., E. A. Murano, and D. G. Olson. 1994. Heating and storage conditions affect survival and recovery of *Listeria monocytogenes* in ground pork. J. Food Sci. 59:30-32, 59.

Kim, W. J. 1993. Bacteriocins of lactic acid bacteria: their potential as food biopreservatives. Food Rev. Int. 9:299-313.

King, A. D., and C. W. Nagel. 1967. Growth inhibition of a *Pseudomonas* by carbon dioxide. J. Food Sci. 32:575-579.

Kleinlein, N., and F. Untermann. 1990. Growth of pathogenic *Yersinia enterocolitica* strains in minced meat with and without protective gas with consideration of the competitive background flora. Int. J. Food Microbiol. 10:65-72.

Klijn, N., A. H. Weerkamp, and W. M. De Vos. 1991. Identification of mesophilic lactic acid bacteria by using polymerase chain reaction-amplified variable regions of 16S rRNA and specific DNA probes. Appl. Environ. Microbiol. 57:3390-3393.

Klijn, N., A. H. Weerkamp, and W. M. De Vos. 1995. Detection and characterization of lactose-utilizing *Lactococcus* spp. in natural ecosystems. Appl. Environ. Microbiol. 61:788-792.

Köhler, G., W. Ludwig, and K.-H. Schleifer. 1991. Differentiation of lactococci by rRNA gene restriction analysis. FEMS Microbiol. Lett. 84:307-312.

Kotzekidou, P., and J. G. Bloukas. 1996. Effect of protective cultures and packaging film permeability on shelf-life of sliced vacuum-packed cooked ham. Meat Sci. 42:333-345.

Kozak, J., T. Balmer, R. Byrne, and K. Fisher. 1996. Prevalence of *Listeria monocytogenes* in foods: Incidence in dairy products. Food Control 7:215-221.

Kraft, A. A. 1986. Meat microbiology, p. 239-278. *In* P. J. Bechtel (ed.), Muscle as food, Academic Press, Inc., New York.

Krovacek, K., A. Faris, and I. Månsson. 1991. Growth and toxin production by *Aeromonas hydrophila* and *Aeromonas sobria* at low temperatures. Int. J. Food Microbiol. 13:165-176.

Lambert, A. D., J. P. Smith, and K. L. Dodds. 1991. Shelf-life extension and microbiological safety of fresh meat - a review. Food Microbiol. 8:267-297.

Lamkey, J. W., F. W. Leak, W. B. Tuley, D. D. Johnson, and R. L. West. 1991. Assessment of sodium lactate addition to fresh pork sausage. J. Food Sci. 56:220-223.

Le Bourgeois, P., M. Mata, and P. Ritzenthaler. 1989. Genome comparison of *Lactococcus* strains by pulsed-field gel electrophoresis. FEMS Microbiol. Lett. 59:65-70.

Le Bourgeois, P., M. Laurier, and P. Ritzenthaler. 1993. Chromosome mapping in lactic acid bacteria. FEMS Microbiol. Rev. 12:109-124.

Leisner, J. J., G. G. Greer, B. D. Dilts, and M. E. Stiles. 1995. Effects of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and in air. Int. J. Food Microbiol. 26:231-243.

Leisner, J. J., G. G. Greer, and M. E. Stiles. 1996. Control of beef spoilage by a sulfide-producing *Lactobacillus sake* strain with bacteriocinogenic *Leuconostoc gelidum* UAL 187 during anaerobic storage at 2°C. Appl. Environ. Microbiol. 62:2610-2614.

Leistner, L. 1994. Further developments in the utilization of hurdle technology for food preservation. J. Food Eng. 22:421-432.

Leistner, L. 1995. Principles and applications of hurdle technology. p. 1-21. *In* G. W. Gould (ed.), New methods of food preservation. Blackie Academic & professional, London.

Leistner, L., and W. Rodel. 1976. The stability of intermediate moisture foods with respect to microorganisms, p. 120-134. *In* R. Davies, G. Birch and K. J. Parker (ed.), Intermediate moisture foods, Applied Science Publishers, London.

Lewus, C. B., A. Kaiser, and T. J. Montville. 1991. Inhibition of foodborne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. 57:1683-1688.

Light, N., P. Hudson, R. Williams, J. Barrett, and J. Schafheitle. 1988. A pilot study on the use of *sous-vide* vacuum cooking as a production system for high quality foods in catering. Int. J. Hosp. Management 7:21-27.

Lindgren, S. E., and W. J. Dobrogosz. 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. 87:149-164.

Lindroth, S. E., and C. Genigeorgis. 1986. Probability of growth and toxin production by nonproteolytic *Clostridium botulinum* in rockfish stored under modified atmospheres. Int. J. Food Microbiol. 3:167-181.

Line, J. E., and M. A. Harrison. 1992. *Listeria monocytogenes* inactivation in turkey rolls and battered chicken nuggets subjected to simulated commercial cooking. J. Food Sci. 57:787-788, 793.

Linnan, M. J., L. M. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. Y. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. New Engl. J. Med. 319:823-828.

Lovett, J. 1989. *Listeria monocytogenes*. p. 283-325. *In* M. P. Doyle (ed.), Foodborne bacterial pathogens, Marcel Dekker, Inc., New York.

Luchansky, J. B., K. A. Glass, K. D. Harsono, A. J. Degnan, N. G. Faith, B. Cauvin, G. Baccus-Taylor, K. Arihara, B. Bater, A. J. Maurer, and R. G. Cassens. 1992. Genomic analysis of *Pediococcus* starter cultures used to control *Listeria monocytogenes* in turkey summer sausage. Appl. Environ. Microbiol. 58:3053-3059.

Luiten, L. S., J. A. Marchello, and F. D. Dryden. 1982a. Growth of *Salmonella typhimurium* and mesophilic organisms on beef steaks as influenced by type of packaging. J. Food Prot. 45:263-267.

Luiten, L. S., J. A. Marchello, and F. D. Dryden. 1982b. Growth of *Staphylococcus aureus* on beef steaks as influenced by type of packaging. J. Food Prot. 45:268-270.

Maas, M. R., K. A. Glass, and M. P. Doyle. 1989. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag turkey products. Appl. Environ. Microbiol. 55:2226-2229.

Mahadeo, M., and S. R. Tatini. 1994. The potential use of nisin to control *Listeria monocytogenes* in poultry. Lett. Appl. Microbiol. 18:323-326.

Maisnier-Patin, S., N. Deschamps, S. R. Tatini, and J. Richard. 1992. inhibition of *Li. monocytogenes* in Camembert cheese made with a nisin-producing starter. Lait 72:249-263.

Mano, S. B., G. D. G. de Fernando, D. Lopez-Galvez, M. D. Selgas, M. L. Garcia, M. I. Cambero, and J. A. Ordoñez. 1995. Growth/survival of *Listeria monocytogenes* on refrigerated uncooked pork and turkey packaged under modified atmospheres. J. Food Safety 15:305-319.

Manu-Tawiah, W., D. J. Myers, D. G. Olson, and R. A. Molins. 1993. Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops packaged under modified gas atmospheres. J. Food Sci. 58:475-479.

Marshall, D. L., and R. H. Schmidt. 1988. Growth of *Listeria monocytogenes* at 10°C in milk preincubated with selected pseudomonads. J. Food Prot. 51:277-282.

Marshall, D. L., P. L. Wiese-Lehigh, J. H. Wells, and A. J. Farr. 1991. Comparative growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken nuggets stored under modified atmospheres. J. Food Prot. 54:841-843.

Marshall, D. L., L. S. Andrews, J. H. Wells, and A. J. Farr. 1992. Influence of modified atmosphere packaging on the competitive growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken. Food Microbiol. 9:303-309.

Mathieu, F., M. Michel, and G. Lefebvre. 1993. Properties of a bacteriocin produced by *Carnobacterium piscicola* CP5. Biotechnol. Lett. 15:587-590.

Mathieu, F., M. Michel, A. Lehibri, and G. Lefebvre. 1994. Effect of the bacteriocin carnocin CP5 and of the producing strain *Carnobacterium piscicola* CP5 on the viability of *Listeria monocytogenes* ATCC 15313 in salt solution, broth and skimmed milk at various incubation temperatures. Int. J. Food Microbiol. 22:155-172.

Mattila-Sandholm, T., and E. Skÿtta. 1991. The effect of spoilage flora on the growth of pathogens in minced meat stored at chilled temperatures. Lebensm. Wiss. Technol. 24:116-120.

Mattila-Sandholm, T., A. Haikara, and E. Skÿtta. 1991. The effect of *Pediococcus damnosus* and *Pediococcus pentosaceus* on the growth of pathogens in minced meat. Int. J. Food Microbiol. 13:87-94.

Mauguin S., and G. Novel. 1994. Characterization of lactic acid bacteria isolated from seafood. J. Appl. Bacteriol. 76:616-625.

McDaniel, M. C., J. A. Marchello, and A. M. Tinsley. 1984. Effect of different packaging treatments on microbiological and sensory evaluation of precooked beef roasts. J. Food Prot. 47:23-26.

McKellar, R. C., R. Moir, and M. Kalab. 1994. Factors influencing the survival and growth of *Listeria monocytogenes* on the surface of Canadian retail wieners. J. Food Prot. 57:387-392.

McMullen, L. M., and M. E. Stiles. 1989. Storage life of selected meat sandwiches at 4°C in modified gas atmospheres. J. Food Prot. 52:792-798.

McMullen, L. M., and M. E. Stiles. 1996. Potential for use of bacteriocin-producing lactic acid bacteria in the preservation of meats. J. Food Prot. Suppl. 64-71.

Mead, G. C. 1983. Effects of packaging and gaseous environments on the microbiology and shelf-life of processed poultry products. p. 203-216. *In* T. A. Roberts, and F. A. Skinner (ed.), Food microbiology: advances and prospects., Academic Press, London.

Mead, G. C., and B. W. Adams. 1977. A selective medium for the rapid isolation of pseudomonads associated with poultry meat storage. Br. Poult. Sci. 18:661-670.

Meng, J., and C. A. Genigeorgis. 1993. modeling lag phase of nonproteolytic *Clostridium botulinum* toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, sodium chloride and spore inoculum. Int. J. Food Microbiol. 19:109-122.

Meng, J., and C. A. Genigeorgis. 1994. Delaying toxigenesis of *Clostridium botulinum* by sodium lactate in 'sous-vide' products. Lett. Appl. Microbiol. 19:20-23.

Michel, M. E., T. J. Keeton, and G. R. Acuff. 1991. Pathogen survival in precooked beef products and determination of critical control points in processing. J. Food Prot. 54:767-772.

Miller, R. K., and G. R. Acuff. 1994. Sodium lactate affects pathogens in cooked beef. J. Food Sci. 59:15-19.

Millière, J. B., M. Michel, F. Mathieu, and G. Lefebvre. 1994. Presence of *Carnobacterium* spp. in French surface mould-ripened soft-cheese. J. Appl. Bacteriol. 76:264-269.

Mokhele, K., A. R. Johnson, E. Barrett, and D. M. Ogrydziak. 1983. Microbial analysis of rock cod (*Sebastes* spp.) stored under elevated carbon dioxide atmospheres. Appl. Environ. Microbiol. 45:878-883.

Montel, M. C., R. Talon, J. Fournaud, and M. C. Champomier. 1991. A simplified key for identifying homofermentative *Lactobacillus* and *Carnobacterium* from meat. J. Appl. Bacteriol. 70:469-472.

Mossel, D. A. A. 1983. Essentials and perspectives of the microbial ecology of foods. p. 1-45. *In* T. A. Roberts and D. F. Skinner (ed.). Food microbiology: Advances and prospects. Academic Press, London.

Motlagh, A. M., S. Holla, M. C. Johnson, B. Ray, and R. A. Field. 1992. Inhibition of *Listeria* spp. in sterile food systems by pediocin AcH, a bacteriocin produced by *Pediococcus acidilactici* H. J. Food Prot. 55:337-343.

Mulders, J. W. M., I. J. Boerrigter, H. S. Rollema, R. J. Siezen, and W. M. De Vos. 1991. Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. Eur. J. Biochem. 201:581-584.

Muriana, P. M. 1996. Bacteriocins for control of *Listeria* spp. in food. J. Food Prot. Suppl. pp. 54-63.

Myers, B. R., R. T. Marshall, J. E. Edmondson, and W. C. Stringer. 1982. Isolation of pectinolytic *Aeromonas hydrophila* and *Yersinia enterocolitica* from vacuum-packaged pork. J. Food Prot. 45:33-37.

Newton, K. G., J. C. L. Harrison, and K. M. Smith. 1977. The effect of storage in various gaseous atmospheres on the microflora of lamb chops held at -1°C. J. Appl. Bacteriol. 43:53-59.

Nielsen, H.-J. S., and P. Zeuthen. 1985. Influence of lactic acid bacteria and the overall flora on development of pathogenic bacteria in vacuum-packed, cooked emulsion-style sausage. J. Food Prot. 48:28-34.

Nielsen, J. W., J. S. Dickson, and J. D. Crouse. 1990. Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. Appl. Environ. Microbiol. 56: 2142-2145.

Nissen, H., A. Holck, and R. H. Dainty. 1994. Identification of *Carnobacterium* spp. and *Leuconostoc* spp. in meat by genus-specific 16S rRNA probes. Lett. Appl. Microbiol. 19:165-168.

Nissen, H., and R. Dainty. 1995. Comparison of the use of rRNA probes and conventional methods in identifying strains of *Lactobacillus sake* and *L. curvatus* isolated from meat. Int. J. Food Microbiol. 25:311-315.

Nissen, H., O. Sørheim, and R. Dainty. 1996. Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. Food Microbiol. 13:183-191.

Niven, C. F., Jr., K. L. Smiley, and J. M. Sherman. 1942. The hydrolysis of arginine by streptococci. J. Bacteriol. 43:651-660.

Notermans, S., J. Dufrenne, and M. J. H. Keijbets. 1981. Vacuum-packed cooked potatoes: Toxin production by *Clostridium botulinum* and shelf life. J. Food Prot. 44:572-575.

Oh, D.-H., and D. L. Marshall. 1995. Influence of packaging method, lactic acid and monolaurin on *Listeria monocytogenes* in crawfish tail meat homogenate. Food Microbiol. 12:159-163.

Okereke, A., and T. J. Montville. 1991a. Bacteriocin inhibition of *Clostridium botulinum* spores by lactic acid bacteria. J. Food Prot. 54:349-353.

Okereke, A., and T. J. Montville. 1991b. Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration and abuse temperatures. Appl. Environ. Microbiol. 57:3423-3428.

Okrend, A. J. G., B. E. Rose, and B. Bennett. 1987. Incidence and toxigenicity of *Aeromonas* species in retail poultry, beef and pork. J. Food Prot. 50:509-513.

Olasupo, N. A., U. Schillinger, C. M. A. P. Franz, and W. H. Holzapfel. 1994. Bacteriocin production by *Enterococcus faecium* NA01 from "wara"- a fermented skimmed cow milk product from West Africa. Lett. Appl. Microbiol. 19:438-441.

Orla-Jensen, A. D., and P. A. Hansen. 1932. The bacteriological flora of spontaneously soured milk and of commercial starters for butter making. Zentralblatt für Bakteriologie, Parasitenkd. Infektionskr und Hygiene., Abt. 2. 88:6-29.

Palumbo, S. A., F. Maxino, A. C. Williams, R. L. Buchanan, and D. W. Thayer. 1985a. Starch ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl. Environ. Microbiol. 50:1027-1030.

Palumbo, S. A., D. R. Morgan, and R. L. Buchanan. 1985b. Influence of temperature, NaCl, and pH on the growth of *Aeromonas hydrophila*. J. Food Sci. 50:1417-1421.

Palumbo, S. A., and A. C. Williams. 1994. Control of *Listeria monocytogenes* on the surface of frankfurters by acid treatment. Food Microbiol. 11:293-300.

Papadopoulos, L. S., R. K. Miller, G. R. Acuff, L. M. Lucia, C. Vanderzant, and H. R. Cross. 1991. Consumer and trained sensory comparisons of cooked beef top rounds treated with sodium lactate. J. Food Sci. 56:1141-1147, 1153.

Parente, E., and C. Hill. 1991. Characterization of enterocin 1146, a bacteriocin from *Enterococcus faecium* inhibitory to *Listeria monocytogenes*. J. Food Prot. 55:497-502.

Parente, E., and C. Hill. 1992. Inhibition of *Listeria* in buffer, broth, and milk by enterocin 1146, a bacteriocin produced by *Enterococcus faecium*. J. Food Prot. 55:503-508.

Partman, W., M. T. Bomar, M. Hajek, H. Bohling, and H. Schlazsus. 1975. Application of controlled atmospheres containing 20% $CO_2$ to the storage of beef. Fleischwirtschaft 55:1441-1442, 1445-1446, 1449-1451.

Peck, M. W. 1997. *Clostridium botulinum* and the safety of refrigerated processed foods of extended durability. Trends Food Sci. Technol. 8:186-192.

Peeler, J. T., G. A. Houghtby, and A. P. Rainosek. 1992. The most probable number technique, p. 105-120. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, 3rd ed., Washington, D.C.

Pelroy, G., M. Peterson, R. Paranjpye, J. Almond, and M. Eklund. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. J. Food Prot. 57:114-119.

Penney, N., C. J. Hagyard, and R. G. Bell. 1993. Extension of shelf life of chilled sliced roast beef by carbon dioxide packaging. Int. J. Food Microbiol. 28:181-191.

Post, L. S., D. A. Lee, M. Solberg, D. Furgang, J. Specchio, and C. Graham. 1985. Development of botulinal toxin and sensory deterioration during storage of vacuum and modified atmosphere packaged fish fillets. J. Food Sci. 50:990-996.

Pot, B., L. A. Devriese, D. Ursi, P. Vandamme, F. Haesebrouck, and K. Kersters. 1996. Phenotypic identification and differentiation of *Lactococcus* strains isolated from animals. Syst. Appl. Microbiol. 19:213-222.

Pucci, M. J., E. R. Vedamuthu, B. S. Kunka, and P. A. Vandenbergh. 1988. Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. Appl. Environ. Microbiol. 54: 2349-2353.

Qvist, S., K. Sehested, and P. Zeuthen. 1994. Growth suppression of *Listeria monocytogenes* in a meat product. Int. J. Food Microbiol. 24:283-293.

Raccach, M., R. C. Baker, J. M. Regenstein and E. J. Mulnix. 1979. Potential application of microbial antagonism to extended storage stability of a flesh type food. J. Food Sci. 44:43-46.

Raccach, M., and E. C. Henningsen. 1984. Role of lactic acid bacteria, curing salts, spices and temperature in controlling the growth of *Yersinia enterocolitica*. J. Food Prot. 47:354-358.

Raccach, M., and D. J. Geshell. 1993. The inhibition of *Listeria monocytogenes* in milk by pediococci. Food Microbiol. 10:181-186.

Rayman, M. K., B. Aris, and A. Hurst. 1981. Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. Appl. Environ. Microbiol. 41:375-380.

Rayman, K., N. Malik, and A. Hurst. 1983. Failure of nisin to inhibit outgrowth of *Clostridium botulinum* in a model cured meat system. Appl. Environ. Microbiol. 46:1450-1452.

Reddy, N. R., D. J. Armstrong, E. J. Rhodehamel, and D. A. Kautter. 1992. Shelf-life extension and safety concerns about fishery products packaged under modified atmospheres: a review. J. Food Safety 12:87-118.

Richard, J. 1996. Utilisation de bactériocines pour la production d'aliments plus sûrs: mythe ou réalité? Lait 76:179-189.

Riemann, H., W. H. Lee, and C. Genigeorgis. 1972. Control of *Clostridium botulinum* and *Staphylococcus aureus* in semi-preserved meat products. J. Milk Food Technol. 35:514-523.

Rodriguez, J. M., L. M. Cintas, P. Casaus, N. Horn, H. M. Dodd, P. E. Hernandez, and M. J. Gasson. 1995. Isolation of nisin-producing *Lactococcus lactis* strains from dry fermented sausages. J. Appl. Bacteriol. 78:109-115.

Rogers, A. M., and T. J. Montville. 1994. Quantification of factors which influence nisin's inhibition of *Clostridium botulinum* 56A in a model food system. J. Food Sci. 59:663-668, 686.

Rozbeh, M., N. Kalchayanand, R. A. Field, M. C. Johnson, and B. Ray. 1993. The influence of biopreservatives on the bacterial level of refrigerated vacuum packaged beef. J. Food Safety 13: 99-111.

Ruiz-Barba, J. L., D. P. Cathcart, P. J. Warner, and R. Jiménez-Díaz. 1994. Use of *Lactobacillus plantarum* LPCO10, a bacteriocin producer, as a starter culture in Spanish-style green olive fermentation. Appl. Environ. Microbiol. 60:2059-2064.

Salama, M., W. Sandine, and S. Giovannoni. 1991. Development and application of oligonucleotide probes for identification of *Lactococcus lactis* subsp. *cremoris*. Appl. Environ. Microbiol. 57:1313-1318.

Sander, E. H., and H.-M. Soo. 1978. Increasing shelf life by carbon dioxide treatment and low temperature storage of bulk pack fresh chickens packaged in nylon/surlyn film. J. Food Sci. 43:1519-1523.

Savell, J. W., M. O. Hanna, C. Vanderzant, and G. C. Smith. 1981. An incident of predominance of *Leuconostoc* sp. in vacuum-packaged beefstrip loins--Sensory and microbial profile of steaks stored in $O_2$-$CO_2$-$N_2$ atmospheres. J. Food Prot. 44:742-745.

Schellekens, M. 1996. New research issues in sous-vide cooking. Trends Food Sci. Technol. 7:256-262.

Scherer, S. 1995. Biological control of pathogens in foods: option or fiction? Lebensm. Milchwir. 116:432-439.

Schiemann, D. A. 1989. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. p. 601-672. *In* M. P. Doyle, Foodborne bacterial pathogens, Marcel Dekker, Inc., New York.

Schiemann, D. A., and G. Wauters. 1992. *Yersinia*, p. 433-450. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, 3rd ed., Washington, D.C.

Schillinger, U. 1990. Bacteriocins of lactic acid bacteria. p. 55-74. *In* D. Bills and S. Kung (ed.), Biotechnology and food safety, Butterworths, London.

Schillinger, U., and F.-K. Lücke. 1987a. Identification of lactobacilli from meat and meat products. Food Microbiol. 4:199-208.

Schillinger, U., and F.-K Lücke. 1987b. Lactic acid bacteria on vacuum-packaged meat and their influence on shelf-life. Fleischwirtsch. 67:1244-1248.

Schillinger, U., and F.-K. Lücke. 1989a. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 55:1901-106.

Schillinger, U., and F.-K. Lücke. 1989b. Use of lactic acid bacteria as protective cultures in meat products. Mitteilungsblatt Bundesanstalt Fleischforschung 104:200-207.

Schillinger, U., and W. H. Holzapfel. 1990. Antibacterial activity of carnobacteria. Food Microbiol. 4:305-310.

Schillinger, U., M. Kaya, and F.-K. Lücke. 1991. Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. 70:473-478.

Schillinger, U., R. Geisen, and W. H. Holzapfel. 1996. Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. Trends Food Sci. Technol. 7: 158-164.

Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, C. A. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nichols, and C. V. Broome. 1983. Epidemic listeriosis - Evidence for transmission by food. New Engl. J. Med. 308:203-206.

Schleifer, K. H., and R. Kilpper-Bälz. 1984. Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. Int. J. Syst. Bacteriol. 34:31-34.

Schleifer, K. H., J. Kraus, C. Dvorak, R. Kilpper-Bälz, M. D. Collins, and W. Fischer. 1985. Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen. nov. Syst. Appl. Microbiol. 6:183-195.

Schleifer, K. H., and R. Kilpper-Bälz. 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. 10:1-19.

Schlyter, J. H., A. J. Degnan, J. Loeffelholz, K. A. Glass, and J. B. Luchansky. 1993a. Evaluation of sodium diacetate and ALTA™2341 on viability of *Listeria monocytogenes* in turkey slurries. J. Food Prot. 56:808-810.

Schlyter, J. H., K. A. Glass, J. Loeffelholz, A. J. Degnan, and J. B. Luchansky. 1993b. The effects of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. Int. J. Food Microbiol. 19:271-281.

Schofield, G. M. 1992. Emerging food-borne pathogens and their significance in chilled foods. J. Appl. Bacteriol. 72:267-273.

Schwartz, B., C. A. Ciecielski, C. V. Broome, S. Gaventa, G. R. Brown, B. G. Gellin, A. W. Hightower, L. Mascola, and the Listeriosis Study Group. 1988. Association of sporadic listeriosis with consumption of undercooked hot dogs and undercooked chicken. Lancet II:779-782.

Scott, V. N. 1989. Interaction of factors to control microbial spoilage of refrigerated foods. J. Food Prot. 52:431-435.

Seideman, S. C., and P. R. Durland. 1984a. The utilization of modified gas atmosphere packaging for fresh meat: a review. J. Food Quality 6:239-252.

Seideman, S. C., and P. R. Durland. 1984b. Vacuum packaging of fresh beef: a review. J. Food Quality 6:26-47.

Seideman, S. C., G. C. Smith, Z. L. Carpenter, T. R. Dutson, and C. W. Dill. 1979. Modified gas atmospheres and changes in beef during storage. J. Food Sci. 44:1036-1040.

Shamsuzzaman, K., N. Chuaqui-Offermanns, L. Lucht, T. McDougall, and J. Borsa. 1992. Microbiological and other characteristics of chicken breast meat following electron-beam and sous-vide treatment. J. Food Prot. 55:528-533.

Shank, F. R., E. L. Elliott, I. K. Wachsmuth, and M. E. Losikoff. 1996. US position on *Listeria monocytogenes* in foods. Food Control 7:229-234.

Shaw, B. G., and C. D. Harding. 1984. A numerical taxonomic study of lactic acid bacteria from vacuum-packed beef, pork, lamb, and bacon. J. Appl. Bacteriol. 56:25-40.

Shaw, B. G., and C. D. Harding. 1985. Atypical lactobacilli from vacuum-packaged meats: comparison by DNA hybridization, cell composition and biochemical tests with a description of *Lactobacillus carnis* sp. nov. Syst. Appl. Microbiol. 6:291-297.

Shaw, B. G., and C. D. Harding. 1989. *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. from chill-stored meats. Int. J. Syst. Bacteriol. 39:217-233.

Shelef, L. A., and Q. Yang. 1991. Growth suppression of *Listeria monocytogenes* by lactates in broth, chicken, and beef. J. Food Prot. 54:283-287.

Shelef, L. 1994. Antimicrobial effects of lactates: a review. J. Food Prot. 57:445-450.

Shelef, L. A., and V. Potluri. 1995. Behaviour of foodborne pathogens in cooked liver sausage containing lactates. Food Microbiol. 12:221-227.

Silliker, J. H., R. E. Woodruff, J. R. Lugg, S. K. Wolfe, and W. D. Brown. 1977. Preservation of refrigerated meats with controlled atmospheres: treatments and post-treatment effects of carbon dioxide on pork and beef. Meat Sci. 1:195-204.

Silliker, J. H., and S. K. Wolfe. 1980. Microbiological safety considerations in controlled-atmosphere storage of meats. Food Technol. 34: 59-63.

Simpson, M. V., J. P. Smith, B. K. Simpson, H. Ramaswamy, and K. L. Dodds. 1994. Storage studies on a *sous vide* spaghetti and meat sauce product. Food Microbiol. 11:5-14.

Simpson, M. V. *et al.* 1995. Challenge studies with *Clostridium botulinum* in a sous-vide spaghetti and meat sauce. J. Food Prot. 58:229-234.

Sizmur, K., and C. W. Walker. 1988. *Listeria* in prepacked salads. Lancet 1:1167

Skura, B. J. 1991. Modified atmosphere packaging of fish and fish products. p.148-167. *In* B. Ooraikul and M. E. Stiles (ed.), Modified atmosphere packaging of food, Ellis Horwood, New York.

Skÿtta, E., W. Hereijgers, and T. Mattila-Sandholm. 1991. Broad spectrum antibacterial activity of *Pediococcus damnosus* and *Pediococcus pentosaceus* in minced meat. Food Microbiol. 8:231-237.

Smith, A. K. 1995. Preparation of microorganisms for SEM. Technical tips. Microscopical Society of Canada Bulletin, May 1995. 23:19.

Smith, G. C., L. C. Hall, and C. Vanderzant. 1980. Inoculation of beef steaks with *Lactobacillus* species before vacuum packaging. II. Effect on meat quality characteristics. J. Food Prot. 43:842-849.

Smith, D. M., and V. B. Alvarez. 1988. Stability of vacuum cook-in-bag turkey breast rolls during refrigerated storage. J. Food Sci. 53:46-48, 61.

Smith, J. P., C. Toupin, B. Gagnon, R. Voyer, P. P. Fiset, and M. V. Simpson. 1990. A hazard analysis critical control point approach (HACCP) to ensure the microbiological safety of sous vide processed meat/pasta product. Int. J. Food Microbiol. 7:177-198.

Solomon, H. M., D. A. Kautter, and R. K. Lynt. 1982. Effect of low temperatures on growth on nonproteolytic *Clostridium botulinum* types B and F and proteolytic type G in crabmeat and broth. J. Food Prot. 45:516-518.

Solomon, H. M., D. A. Kautter, T. Lilly, and E. J. Rhodehamel. 1990. Outgrowth of *Clostridium botulinum* in shredded cabbage at room temperature under a modified atmosphere. J. Food Prot. 53:831-833.

Sooltan, J. R. A., G. C. Mead, and A. P. Norris. 1987. Incidence and growth potential of *Bacillus cereus* in poultrymeat products. Food Microbiol. 4:347-351.

Stammen, K., D. Gerdes, and F. Caporaso. 1990. Modified atmosphere packaging of seafood. Crit. Rev. Food Sci. Nutrit. 29:301-331.

Stecchini, M. L., V. Aquili, and I. Sarais. 1995. Behavior of *Listeria monocytogenes* in Mozzarella cheese in presence of *Lactococcus lactis*. Int. J. Food Microbiol. 25:301-310.

Steele, J. E., and M. E. Stiles. 1981. Food poisoning potential of artificially contaminated vacuum packaged sliced ham in sandwiches. J. Food Prot. 44:430-434.

Stier, R. F., L. Bell, K. A. Ito, B. D. Shafer, L. A. Brown, M. L. Seeger, B. H. Allen, M. N. Porcuna, and P. A. Lerke. 1981. Effect of modified atmosphere on *Clostridium botulinum* toxigenesis and the spoilage microflora of salmon fillets. J. Food Sci. 46:1639-1642.

Stiles, M. E. 1991. Modified atmosphere packaging of meat, poultry and their products. p. 118-147. *In* B. Ooraikul and M. E. Stiles (ed.), Modified atmosphere packaging of food, Ellis Horwood, New York.

Stiles, M. E. 1994. Potential for biological control of agents of foodborne disease. Food Res. Int. 27:245-250.

Stiles, M. E. 1996. Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek. 70:331-345.

Stiles, M. E., and L.-K. Ng. 1979. Fate of pathogens inoculated onto vacuum-packaged sliced hams to simulate contamination during packaging. J. Food Prot. 42:464-469.

Stiles, M. E., and J. W. Hastings. 1991. Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. Trends Food Sci. Technol. 2:247-251.

Stiles, M. E., and W. H. Holzapfel. 1997. Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol. 36:1-29.

Stillmunkes, A. A., G. A. Prabhu, J. G. Sebranek, and R. A. Molins. 1993. Microbiological safety of cooked beef roasts treated with lactate, monolaurin or gluconate. J. Food Sci. 58:953-958.

Sulzer, G., and M. Busse. 1991. Growth inhibition of *Listeria* spp. on Camembert cheese by bacteria producing inhibitory substances. Int. J. Food Microbiol. 14:287-296.

Sutherland, J. P., J. T. Patterson, P. A. Gibbs, and J. G. Murray. 1977. The effect of several gaseous environments on the multiplication of organisms isolated from vacuum-packaged beef. J. Food Technol. 12:249-255.

Swanson, K. M. J., F. F. Busta, E. H. Peterson, and M. G. Johnson. 1992. Colony count method, p. 75-95. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, 3rd ed., Washington, D.C.

Tanaka, N., E. Traisman, M. H. Lee, R. G. Cassens, and E. M. Foster. 1980. Inhibition of botulinum toxin formation in bacon by acid development. J. Food Prot. 43:450-457.

Tanaka, N., N. M. Gordon, R. C. Lindsay, L. M. Meske, M. P. Doyle, and E. Traisman. 1985a. Sensory characteristics of reduced nitrite bacon manufactured by the Wisconsin Process. J. Food Prot. 48:687-692.

Tanaka, N., L. Meske, M. P. Doyle, E. Traisman, D. W. Thayer, and R. W. Johnston. 1985b. Plant trials of bacon made with lactic acid bacteria, sucrose and lowered sodium nitrite. J. Food Prot. 48:679-686.

Taylor, S. L., and E. B. Somers. 1985. Evaluation of the antibotulinal effectiveness of nisin in bacon. J. Food Prot. 48:949-952.

Taylor, S. L., E. B. Somers, and L. A. Krueger. 1985. Antibotulinal effectiveness of nisin-nitrite combinations in culture medium and chicken frankfurter emulsions. J. Food Prot. 48:234-239.

Ternström, A., and G. Molin. 1987. Incidence of potential pathogens on raw pork, beef and chicken in Sweden, with special reference to *Erysipelothrix rhusiopathiae*. J. Food Prot. 50:141-146.

Teufel, P. 1994. European perspective on *Listeria monocytogenes*. Dairy Food Environ. Sanit. 14:212-214.

Thatcher, F. S., J. Robinson, and I. Erdmann. 1962. The "vacuum pack" method of packaging foods in relation to the formation of the botulinum and staphylococcal toxins. J. Appl. Bacteriol. 25:120-124.

Tilsala-Timisjärvi, A., and T. Alatossava. 1997. Development of oligonucleotide primers from the 16S-23S rRNA intergenic sequence for identifying different dairy and probiotic lactic acid bacteria by PCR. Int. J. Food Microbiol. 35:49-56.

Todd, E. C. D. 1992. Foodborne disease in Canada - a 10-year summary from 1975 to 1984. J. Food Prot. 55:123-132.

Tolstoy, A. 1991. Practical monitoring of the chill chain. Int. J. Food Microbiol. 13:225-230.

Toora, S., E. Budu-Amoako, R. F. Ablett, and J. Smith. 1994. Isolation of *Yersinia enterocolitica* from ready-to-eat foods by a simple two step procedure. Food Microbiol. 11:369-374.

Torri Tarelli, G., D. Carminati, and G. Giraffa. 1994. Production of bacteriocins active against *Listeria monocytogenes* and *Listeria innocua* from dairy enterococci. Food Microbiol. 11:243-252.

Turner, B. E., P. M. Foegeding, D. K. Larick, and A. H. Murphy. 1996. Control of *Bacillus cereus* spores and spoilage microflora in sous-vide chicken breast. J. Food Sci. 61:217-219, 234.

Uhlman, L., U. Schillinger, J. R. Rupnow, and W. H. Holzapfel. 1992. Identification and characterization of two bacteriocin-producing strains of *Lactococcus lactis* isolated from vegetables. Int. J. Food Microbiol. 16:141-151.

Unda, J. R., R. A. Molins, and H. W. Walker. 1991. *Clostridium sporogenes* and *Listeria monocytogenes*: survival and inhibition in microwave-ready beef roasts containing selected antimicrobials. J. Food Sci. 56:198-205, 219.

Van Garde, S. J., and M. J. Woodburn. 1987. Food discard practices of householders. J. Am. Diet. Assoc. 87:322-329.

Vaughan, E. E., E. Caplice, R. Looney, N. O'Rourke, H. Coveney, C. Daly, and G. F. Fitzgerald. 1994. Isolation from food sources, of lactic acid bacteria that produce antimicrobials. J. Appl. Bacteriol. 76:118-123.

Vescovo, M., C. Orsi, G. Scolari, and S. Torriani. 1995. Inhibitory effect of selected lactic acid bacteria on microflora associated with ready-to-use vegetables. Lett. Appl. Microbiol. 21:121-125.

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

Vignolo, G., S. Fadda, M. N. de Kairuz, A. A. P. de Ruiz Holgado, and G. Oliver. 1996. Control of *Listeria monocytogenes* in ground beef by 'Lactocin 705', a bacteriocin produced by *Lactobacillus casei* CRL 705. Int. J. Food Microbiol. 29:397-402.

Vogel, R. F., B. S. Pohle, P. S. Tichaczek, and W. P. Hammes. 1993. The competitive advantage of *Lactobacillus curvatus* LTH 1174 in sausage fermentations is caused by formation of curvacin A. Syst. Appl. Microbiol. 16:457-462.

Von Holy, A., W. H. Holzapfel, and G. A. Dykes. 1992. Bacterial populations associated with Vienna sausage packaging. Food Microbiol. 9:45-53.

Wan, J., J. B. Gordon, K. Muirhead, M. W. Hickey, and M. J. Coventry. 1997. Incorporation of nisin in micro-particles of calcium alginate. Lett. Appl. Microbiol. 24:153-158.

Wan, J., K. Harmark, B. E. Davidson, A. J. Hillier, J. B. Gordon, A. Wilcock, M. W. Hickey, and M. J. Coventry. 1997. Inhibition of *Listeria monocytogenes* by piscicolin 126 in milk and Camembert cheese manufactured with a thermophilic starter. J. Appl. Microbiol. 82:273-280.

Wang, M. Y., and W. D. Brown. 1983. Effects of elevated $CO_2$ atmosphere on storage of freshwater crayfish (*Pacifastacus leniusculus*). J. Food Sci. 48:158-162.

Wang, M. Y., and D. M. Ogrydziak. 1986. Residual effect of storage in an elevated carbon dioxide atmosphere on the microbial flora of rock cod (*Sebastes* spp.). Appl. Environ. Microbiol. 52:727-732.

Wang, C., and P. M. Muriana. 1994. Incidence of *Listeria monocytogenes* in packages of retail franks. J. Food Prot. 57:382-386.

Weaver, R. A., and L. A. Shelef. 1993. Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. J. Food Safety 13:133-146.

Wederquist, H. J., J. N. Sofos, and G. R. Schmidt. 1994. *Listeria monocytogenes* inhibition in refrigerated vacuum packaged turkey bologna by chemical additives. J. Food Sci. 59:498-500, 516.

Weiss, J., and A. P. R. Seeliger. 1975. Incidence of *Listeria monocytogenes* in nature. Appl. Microbiol. 30:29-32.

Wessels, S., and H. H. Huss. 1996. Suitability of *Lactococcus lactis* subsp. *lactis* ATCC 11454 as a protective culture for lightly preserved fish products. Food Microbiol. 13:323-332.

Williams, A. M., J. L. Fryer, and M. D. Collins. 1990. *Lactococcus piscium* sp. nov. a new *Lactococcus* species from salmonid fish. FEMS Microbiol. Lett. 68:109-114.

Wimpfheimer, L., A. S. Altman, and J. H. Hotchkiss. 1990. Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. Int. J. Food Microbiol. 11:205-214

Winkowski, K., and T. J. Montville. 1992. Use of meat isolate, *Lactobacillus bavaricus* MN, to inhibit *Listeria monocytogenes* growth in a model meat gravy system. J. Food Safety 13:19-31.

Winkowski, K., A. D. Crandall, and T. J. Montville. 1993. Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. Appl. Environ. Microbiol. 59:2552-2557.

Wyatt, C. Y., and V. Guy. 1980. Relationships of microbial quality of retail meat samples and sanitary conditions. J. Food Prot. 43:385-389.

Young, H., A. Youngs, and N. Light. 1987. The effects of packaging on the growth of naturally occurring microflora in cooked, chilled foods used in the catering industry. Food Microbiol. 4:317-327.

Young, H., H. J. H. MacFie, and N. Light. 1989. Effect of packaging and storage on the sensory quality of cooked chicken menu items served from chilled vending machines. J. Sci. Food Agric. 48:323-338.

Yousef, A. E., J. B. Luchansky, A. J. Degnan, and M. P. Doyle. 1991. Behavior of *Listeria monocytogenes* in wiener exudates in the presence of *Pediococcus acidilactici* H or pediocin AcH during storage at 4 or 25°C. Appl. Environ. Microbiol. 57: 1461-1467.

Zeitoun, A. A. M., and J. M. Debevere. 1991. Inhibition, survival and growth of *Listeria monocytogenes* on poultry as influenced by buffered lactic acid treatment and modified atmosphere packaging. Int. J. Food Microbiol. 14:161-169.

Zottola, E. A., T. L. Yezzi, D. B. Ajao, and R. F. Roberts. 1994. Utilization of cheddar cheese containing nisin as an antimicrobial agent in other foods. Int. J. Food Microbiol. 24:227-238.

# APPENDIX

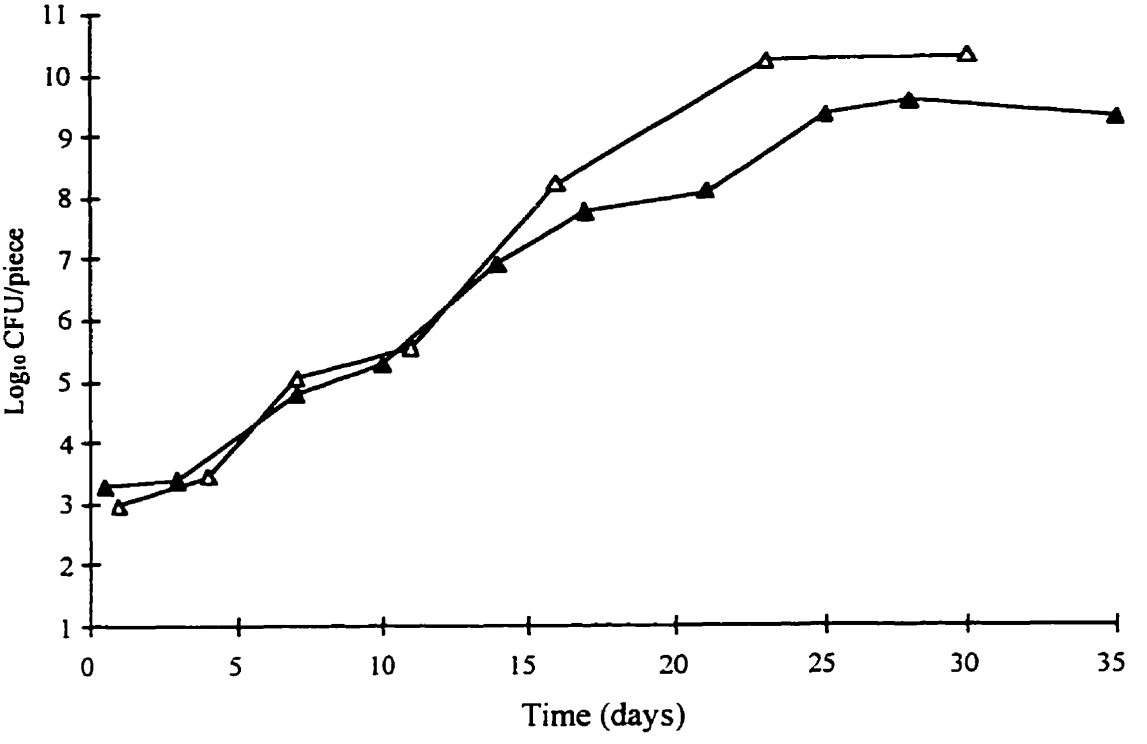



Figure 1 Growth of *Y. enterocolitica* on cooked, cold-packed poultry injected with regular brine and stored at 3.5°C; replicate 1 (Δ) and replicate 2 (▲).

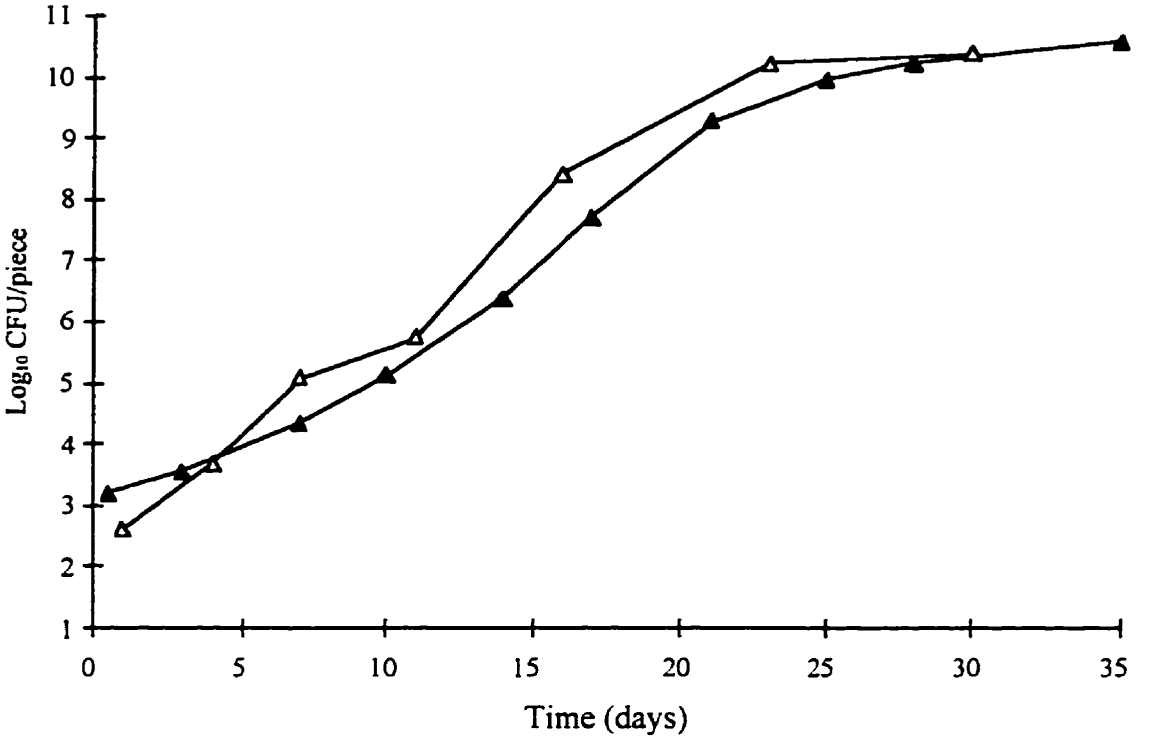



Figure 2 Growth of *Y. enterocolitica* on cooked, hot-packed poultry injected with regular brine and stored at 3.5°C; replicate 1 (Δ) and replicate 2 (▲).

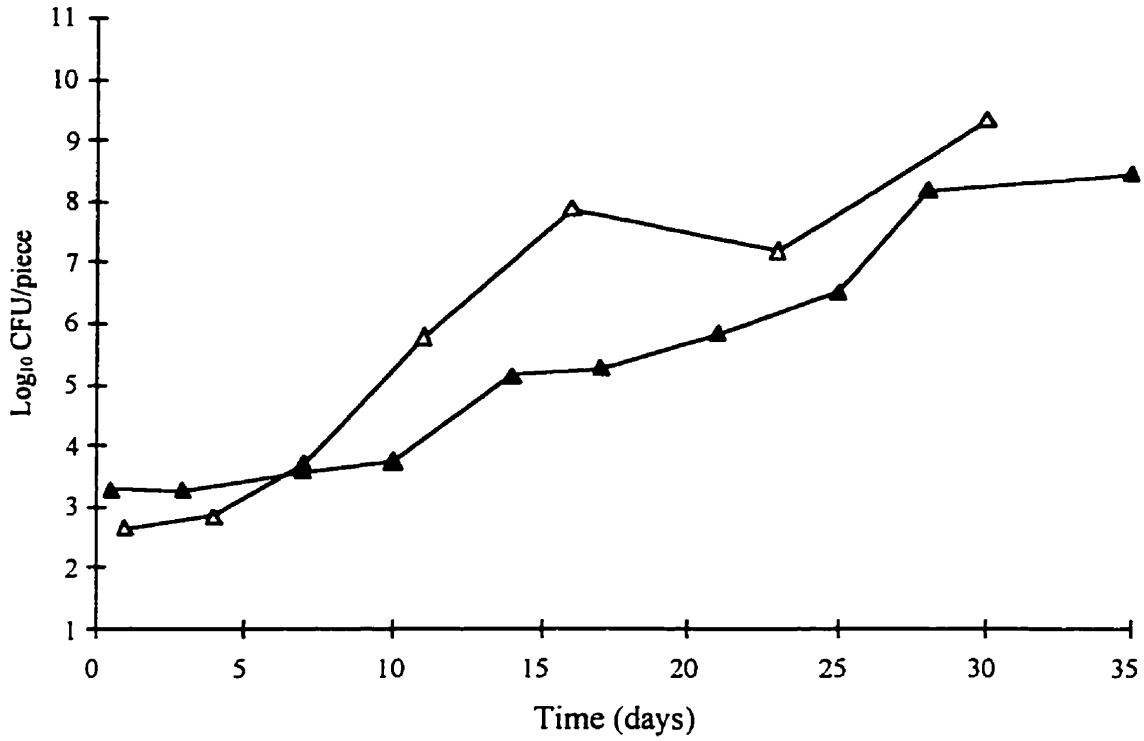



Figure 3 Growth of *Y. enterocolitica* on cooked, cold-packed poultry injected with test brine and stored at 3.5°C; replicate 1 (Δ) and replicate 2 (▲).

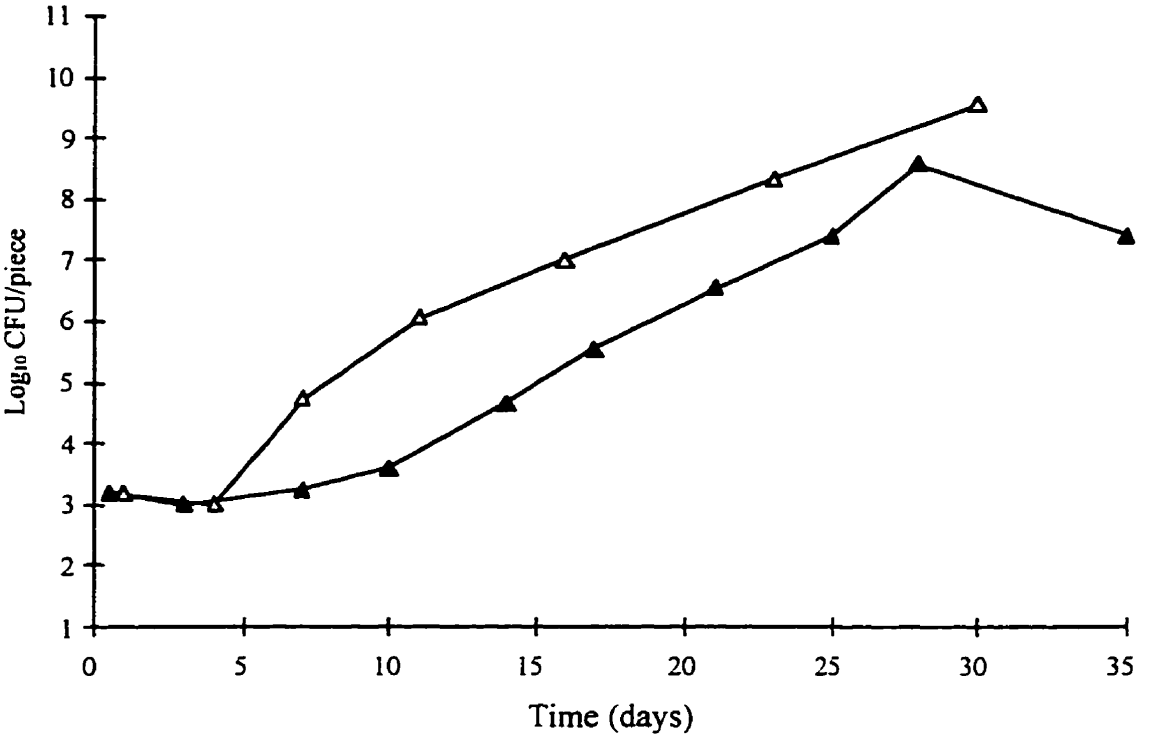



Figure 4 Growth of *Y. enterocolitica* on cooked, hot-packed poultry injected with test brine and stored at 3.5°C; replicate 1 (Δ) and replicate 2 (▲).

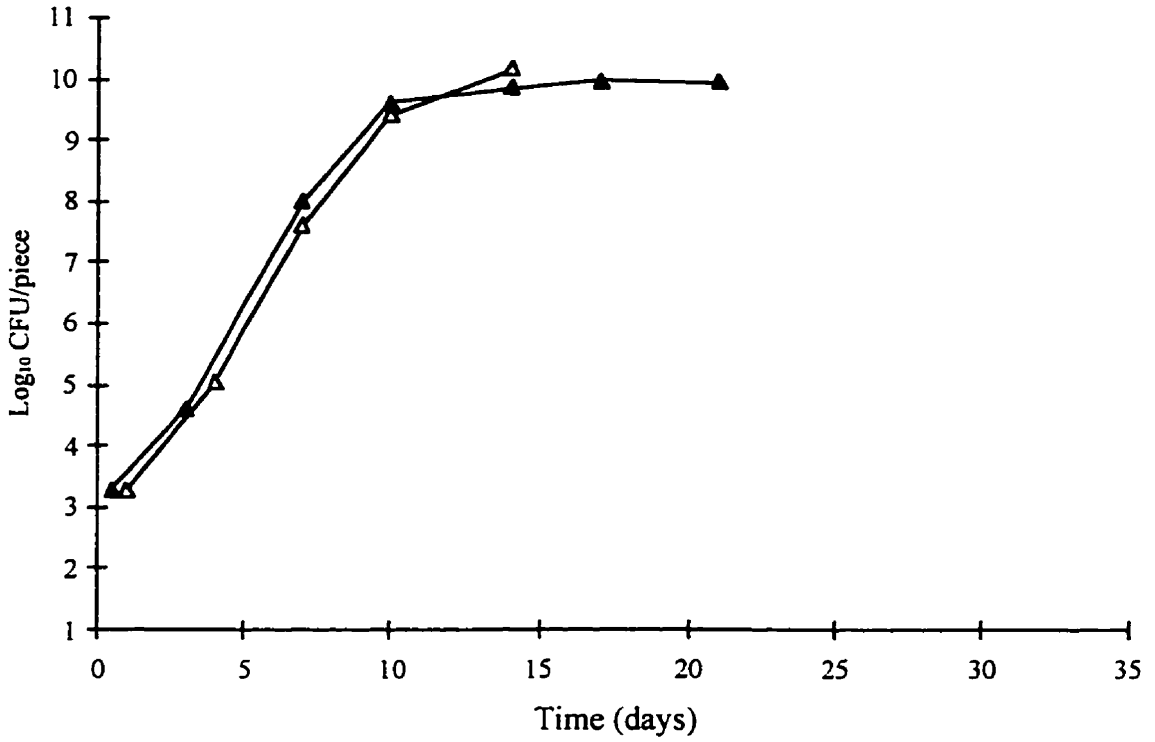



Figure 5 Growth of *Y. enterocolitica* on cooked, cold-packed poultry injected with regular brine and stored at 6.5°C; replicate 1 (Δ) and replicate 2 (▲).

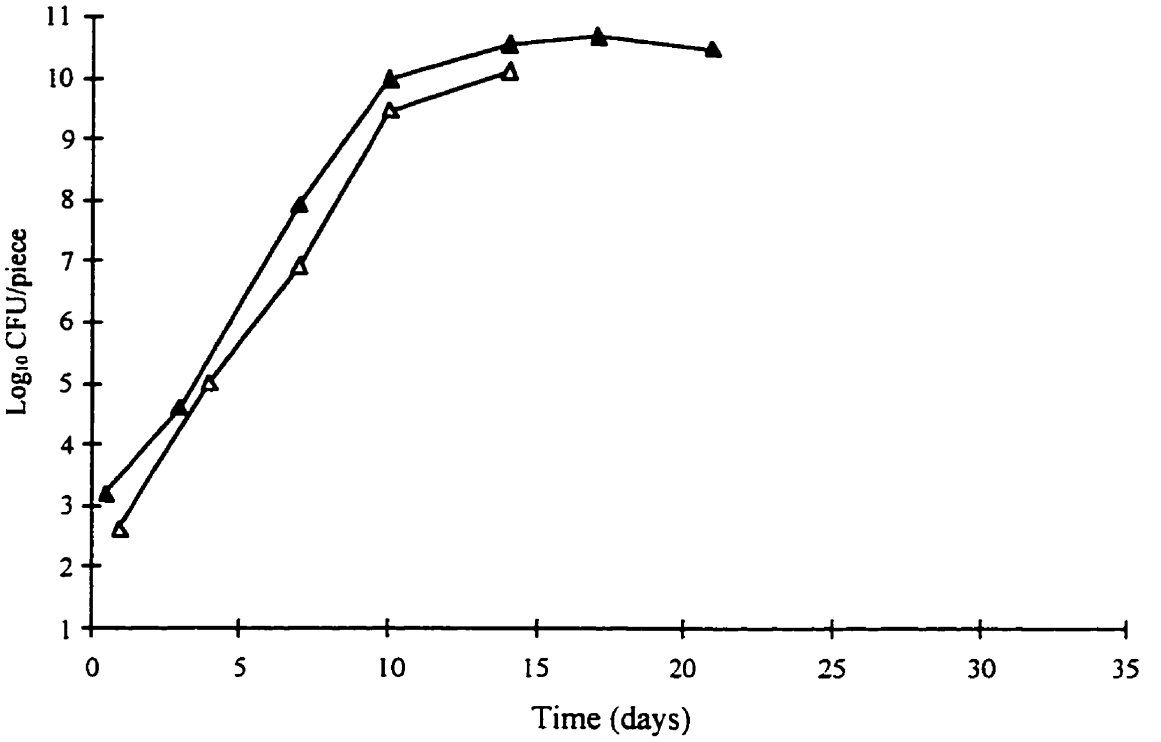



Figure 6 Growth of *Y. enterocolitica* on cooked, hot-packed poultry injected with regular brine and stored at 6.5°C; replicate 1 (Δ) and replicate 2 (▲).

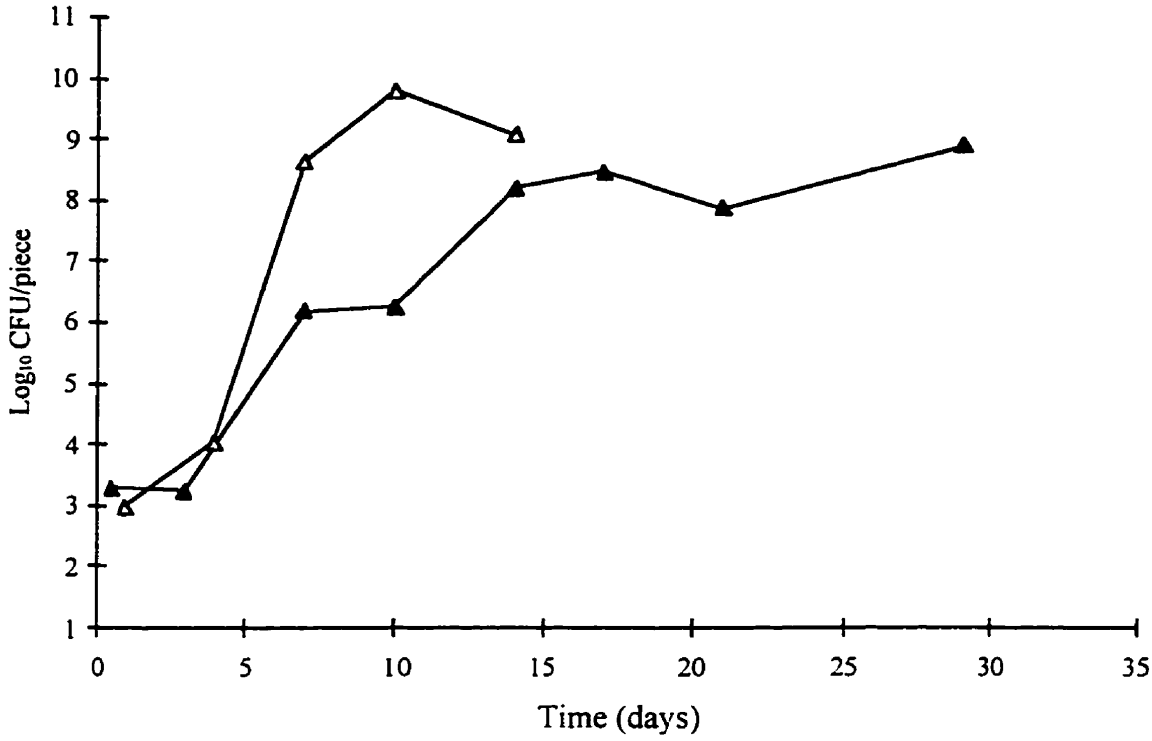



Figure 7 Growth of *Y. enterocolitica* on cooked, cold-packed poultry injected with test brine and stored at 6.5°C; replicate 1 (Δ) and replicate 2 (▲).

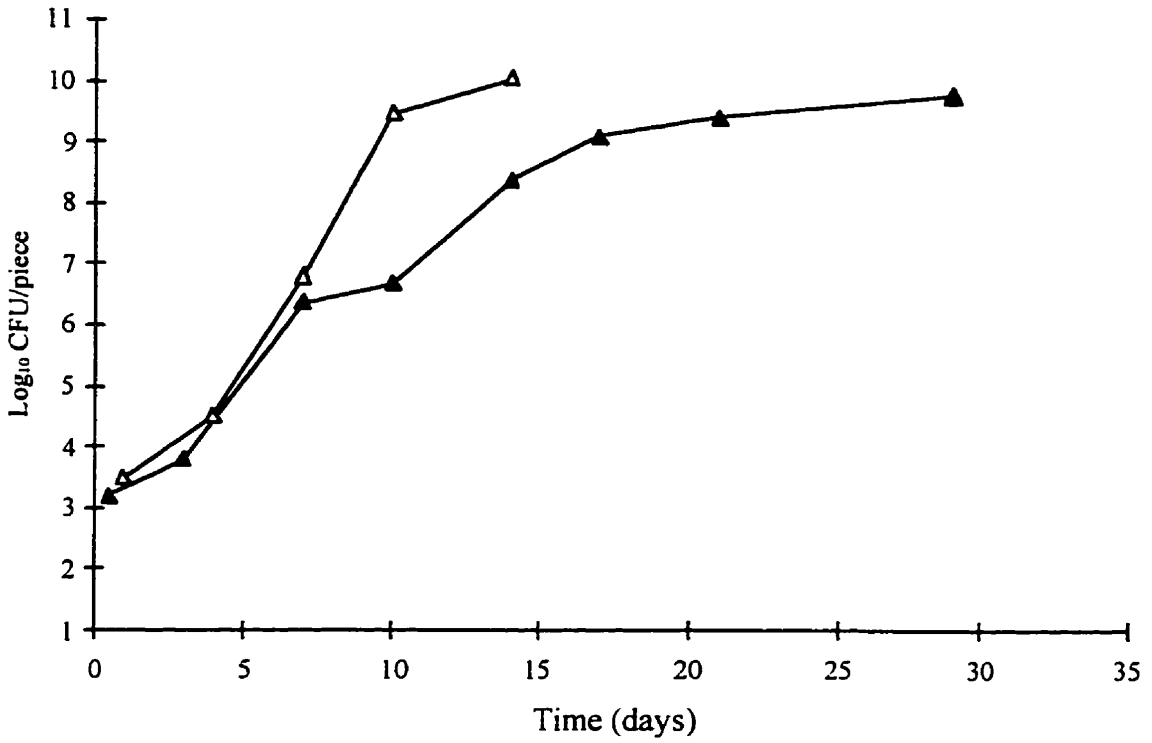
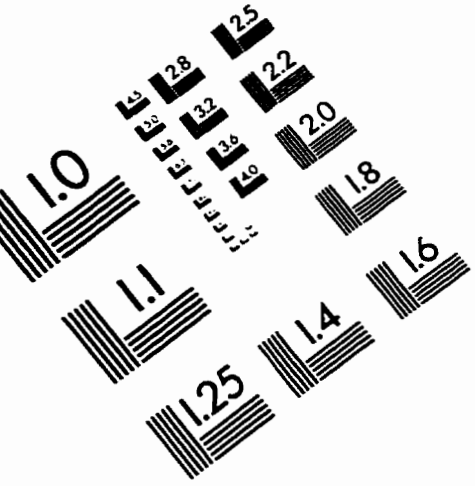
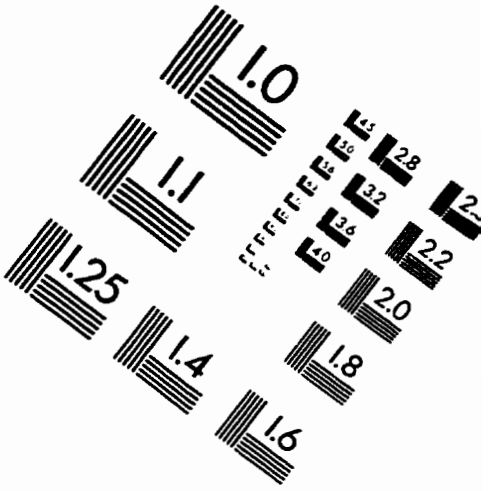
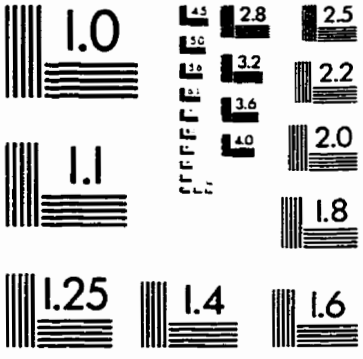


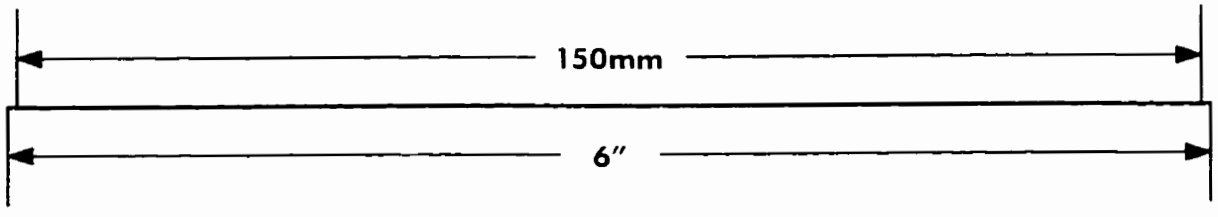

Figure 8 Growth of *Y. enterocolitica* on cooked, hot-packed poultry injected with test brine and stored at 6.5°C; replicate 1 (Δ) and replicate 2 (▲).

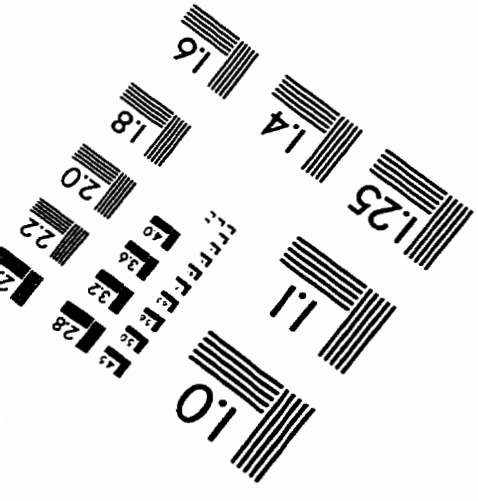
# IMAGE EVALUATION
# TEST TARGET (QA-3)

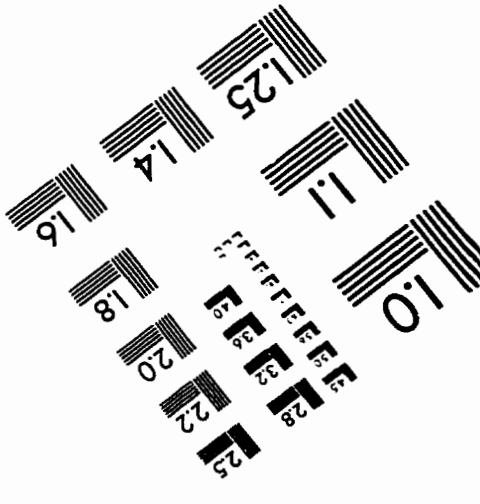



APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989