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Review

Listeria: A foodborne pathogen that knows how to survive

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Abstract

The foodborne pathogen *Listeria* is the causative agent of listeriosis, a severe disease with high hospitalization and case fatality rates. *Listeria monocytogenes* can survive and grow over a wide range of environmental conditions such as refrigeration temperatures, low pH and high salt concentration. This allows the pathogen to overcome food preservation and safety barriers, and pose a potential risk to human health. This review focuses on the key issues such as survival of the pathogen in adverse environments, and the important adaptation and survival mechanisms such as biofilm formation, quorum sensing and antimicrobial resistance. Studies on the development of technologies to prevent and control *L. monocytogenes* contamination in foods and food processing facilities are also discussed.

Keywords: *Listeria monocytogenes*; Low temperature; Acid stress; Osmotic stress; Biofilm; Quorum sensing; Antimicrobial resistance; Food preservation

Contents

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1. Introduction

Illness caused due to the consumption of contaminated foods has a wide economic and public health impact worldwide. The Centers for Disease Control and Prevention (CDC) estimate that foodborne diseases are responsible for about 76 million illnesses, which result in 325,000 hospitalizations and 5000 deaths in the United States each year (Mead et al., 1999). The preliminary FoodNet data for 2004 has shown an overall decline in the incidence of infections caused by foodborne pathogens such as *Campylobacter*, *Yersinia*, *Salmonella* and *Listeria* (Anonymous, 2005). However, it is important to realize that many of the foodborne illnesses are sporadic and may not get accounted as part of an outbreak. Further, several factors such as consumer awareness, disease surveillance by the local and state public health departments, varying incubation periods and severity of a disease affect the degree of reporting of foodborne illnesses and outbreaks (Olsen et al., 2000). The Department of Health and Human Services in the United States has launched the "Healthy People 2010" initiative, aimed at health promotion and disease prevention with objectives for improving the health of all people in the first ten years of the 21st century. The primary focus areas of the initiative include improved food safety in the United States and a reduction in the incidence of foodborne diseases caused by *Campylobacter*, *E. coli* O157:H7, *Listeria* and *Salmonella* (www.cdc.gov/nchs/hphome.htm#Healthy%20People%202010, 2005).

Foodborne illnesses caused by pathogens such as *Campylobacter* and *Salmonella* are most commonly reported in the United States (Mead et al., 1999). However, pathogens such as *Listeria monocytogenes* can adapt to survive and grow in a wide range of environmental conditions and cause listeriosis, a severe disease with high hospitalization and case fatality rates (Mead et al., 1999). The elderly, pregnant, newborn and immunocompromised populations are more susceptible to listeriosis. The immunocompromised category includes people with AIDS or on immunosuppressive drugs such as corticosteroids for cancer treatment, which reduce their T-cell mediated immunity (Rocourt and Cossart, 1997). Listeriosis has a long incubation time, which makes it difficult to identify the pathogen and trace the contaminated food. Once the pathogen gains entry into mammalian cells by phagocytosis, they are released from the membrane-bound vacuole and begin to multiply. The pathogen uses actin polymerization for intracellular movement and cell-to-cell spread infecting a vast range of host tissues, with the liver being the main site of infection (Rocourt and Cossart, 1997). Meningitis, septicemia and other infections of the central nervous system are commonly seen in patients with listeriosis. In pregnant women, listeriosis may lead to spontaneous abortion, still birth or fetal death (Rocourt and Cossart, 1997).

L. monocytogenes can be found in a wide variety of raw and processed foods. Milk and dairy products, various meats and meat products such as beef, pork, fermented sausages, fresh produce such as radishes, cabbage, seafood and fish products have all been associated with *Listeria* contamination (Rocourt and Cossart, 1997). Foods such as soft cheeses, hot dogs and seafood have been implicated in several outbreaks of human listeriosis. *Listeria* is a Gram-positive pathogen, with the ability to adapt to a wide range of conditions such as refrigeration temperatures (2–4 °C), acidic foods, high salt foods and within the host immune system (Rocourt and Cossart, 1997).

Even though the statistics show that the incidence of listeriosis has declined, outbreaks and contaminated product recalls continue to occur (www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis_t.htm, 2005). A multi-state outbreak of *L. monocytogenes* occurred in the United States in 2002. Consumption of contaminated turkey deli meat resulted in 46 culture-confirmed cases, seven deaths and three fetal deaths in eight states (Anonymous, 2002). There are several factors that have influenced the contamination of foods by *Listeria* and the incidence of listeriosis. Advances in the field of medicine continue to affect the dynamic human population, increasing the average lifespan of people and survival of immunocompromised and elderly individuals. Recent modifications in food production and processing practices, globalization of the food industry and the growing demand for imported and ethnic foods are also important factors. Further, ever-changing food habits of the consumer, with a trend towards consumption of minimally processed, ready-to-eat convenience foods and refrigerated or

frozen food products have affected the incidence of listeriosis over the past years (Rocourt and Bille, 1997).

Collaborative efforts by the government, public health agencies, food industry and consumers are necessary to determine the risks associated with consumption of various foods, virulence mechanisms of *L. monocytogenes*, incidence and epidemiology of the disease. The United States Food and Drug Administration's (FDA) Center for Food Safety and Applied Nutrition (CFSAN) along with the Food Safety and Inspection Service (FSIS) and Centers for Disease Control and Prevention (CDC) published a quantitative assessment of relative risk to public health from the consumption of selected categories of ready-to-eat foods that may be contaminated with the foodborne pathogen *L. monocytogenes*. The assessment provides valuable information on the predicted risk of serious illness and death associated with consumption of ready-to-eat foods contaminated with *L. monocytogenes* (www.cfsan.fda.gov/~dms/lmr2-toc.html, 2003).

Currently, FDA has a zero-tolerance policy in place for *L. monocytogenes* in ready-to-eat foods. Based on this policy, the detection of any *L. monocytogenes* in the food makes the product adulterated. The FDA is reviewing a petition made by trade associations to change the zero-tolerance policy for *L. monocytogenes* in foods that do not support the growth of the organism. The petition has requested the establishment of a regulatory limit for *L. monocytogenes* of 100 CFU/g in foods that do not support the growth of the microorganism. Based on the risk assessment published by FDA and FSIS (www.cfsan.fda.gov/~dms/lmr2-toc.html, 2003), and research articles (Chen et al., 2003), the petition proposes that concentrating on the number of *L. monocytogenes* present in a food rather than just its presence alone may be more effective in improving food safety and promoting public health.

2. Mechanisms of survival under adverse environmental conditions

2.1. Survival at low temperatures

L. monocytogenes has the ability to grow over a wide range of temperatures (2–45 °C). Its survival and growth at refrigeration temperatures (2–4 °C) are two of the many factors that make the control of this foodborne pathogen difficult (Rocourt and Cossart, 1997). Since refrigeration is one of the most common ways to increase the shelf life of foods, understanding the mechanisms behind its survival and growth at low temperature could provide information to help develop more effective control methods for the pathogen.

2.1.1. Changes in membrane composition

The lipids in the membranes of bacterial cells are in a fluid, crystalline state and this physical state is important to maintain membrane fluidity and function. Changes in temperature lead to an alteration in the membrane lipid composition to maintain the ideal membrane fluidity required for proper enzyme activity and transport of solutes across the membrane. A high proportion of iso and anteiso, odd-numbered, branched-chain fatty acids characterize the cell membrane of *Listeria* (Annous et al., 1997). The changes that occur in the membrane fatty acid composition of *L. monocytogenes* in response to low temperature have been extensively studied. One of the main changes is an increase in the proportion of $C_{15:0}$ at the expense of $C_{17:0}$, when the temperature is reduced below optimum (7 °C). Growth at low temperatures also results in an increase in the degree of unsaturated fatty acids, which helps enhance the fluidity of the membrane (Beales, 2004). Annous et al. (1997) also showed that changing the growth temperature from 20 °C to 5 °C led to fatty acid shortening (a decrease in $C_{17:0}$) and a switch from iso to anteiso branching (i-$C_{15:0}$ to a-$C_{15:0}$). The shortening of fatty acid chain length decreases the carbon–carbon interaction between neighboring chains in the cell membrane and this helps maintain the optimum degree of membrane fluidity for growth at low temperatures (Beales, 2004).

2.1.2. Changes in gene expression and induction of proteins

L. monocytogenes produces cold shock proteins (Csps) in response to a temperature downshock and cold acclimation proteins (Caps) that are synthesized during balanced growth at low temperatures (Bayles et al., 1996). The authors subjected *L. monocytogenes* to a cold shock from 37 °C to 5 °C and used two-dimensional gel electrophoresis to identify the proteins induced as a result of the cold shock. About 12 proteins (Csps) were induced in cold-shocked cultures and about four proteins (Caps that were also identified as Csps) were produced during balanced growth at 5 °C compared to 37 °C (Bayles et al., 1996). Cold acclimation of a pathogen is accompanied by changes in microbial gene expression. Liu et al. (2002) identified RNAs that are synthesized at higher levels when *L. monocytogenes* is grown at 10 °C in comparison to 37 °C. Increased expression of mRNA for chaperone proteases such as GroEL, ClpP and ClpB indicates that these enzymes may be involved in the degradation of abnormal or damaged polypeptides that arise due to growth at low temperatures (Liu et al., 2002).

2.1.3. Compatible solutes as cryoprotectants

The ability of *L. monocytogenes* to accumulate compatible solutes such as glycine betaine and carnitine and the role of these compounds as cryoprotectants have been widely studied. Angelidis and Smith (2003) investigated the uptake and accumulation of compatible solutes in cells during growth at refrigeration temperatures. Mutants of *L. monocytogenes* were made to study the role of three compatible solute systems — glycine betaine porter I (BetL), glycine betaine porter II (Gbu) and the carnitine transporter (OpuC). The transport of betaine into the cell is mediated mainly through Gbu. The solute-mediated cryoprotection stimulates growth in cells subjected to cold stress. Low level of betaine was also transported via BetL and OpuC. Major uptake of carnitine is mediated through OpuC, with Gbu and BetL transporting low levels and thus providing only weak cryoprotection (Angelidis and Smith, 2003). Wemekamp-Kamphuis et al. (2004) also showed that deletions of these osmolyte transporters reduced the growth of *Listeria* at low temperatures (Wemekamp-Kamphuis et al., 2004).

2.1.4. Role of general stress sigma factor (σ^B)

The survival of bacteria under adverse environmental conditions involves changes in the transcription of genes that is made

possible by the association of alternative sigma factors with the core RNA polymerase. The alternative stress sigma factor, sigmaB (σ^B) has been identified in Gram-positive bacteria such as *Bacillus subtilis* and *L. monocytogenes*. The general stress sigma factor σ^B is stimulated in response to temperature downshift and the *sigB* mutant fails to accumulate solutes such as betaine and carnitine in *L. monocytogenes* (Becker et al., 2000). This suggests that accumulation of cryoprotectants is one of the functions of σ^B during growth at low temperature.

The varied responses of *L. monocytogenes* for survival and growth at low temperatures demonstrate the versatility of this emerging pathogen to adapt to a wide range of environmental conditions. Its ability to grow at refrigeration temperatures is of particular concern in refrigerated foods, which are consumed without any further processing such as soft cheeses, refrigerated smoked seafood and refrigerated meat spreads. Foods such as hot dogs and deli meats are also a concern, unless they are reheated thoroughly.

2.2. Survival under acid stress

L. monocytogenes encounters a low-pH environment in acidic foods, during gastric passage and in the phagosome of the macrophage (Cotter and Hill, 2003). The pathogen responds to and survives in these low-pH environments by utilizing a number of stress adaptation mechanisms. Exposure of *L. monocytogenes* to mild acidic pH of 5.5 (1 M lactic acid) induces the acid tolerance response (ATR), wherein the cells are resistant to severe acidic conditions (O'Driscoll et al., 1996).

2.2.1. Induction of proteins

The acid response on exposure of cells to an acidic pH involves several changes in the cell. Phan-Thanh and Mahouin (1999) studied the expression of proteins by exposing cells to a lethal acidic pH (acid stress) and a non-lethal acidic pH (acid adaptation). Even though more proteins were induced in cells exposed to the lethal pH, a majority of the proteins induced were common to both pH conditions used in the study. The protein GroEL that showed increased synthesis during the growth of *Listeria* at low temperature was also induced under acid stress. Other proteins induced were ATP synthase and various transcriptional regulators (Phan-Thanh and Mahouin, 1999). Acid-adapted *L. monocytogenes* (pH 5.2, 2 h) had increased resistance to heat shock (52 °C), osmotic shock (25–30% NaCl) and alcohol stress, suggesting that acid adaptation also provides cross-protection against other stress factors (Phan-Thanh et al., 2000). The cross-resistance of acid-adapted cells to other stresses has important implications for the food industry, particularly since foods commonly encounter sub-lethal acidic treatments during processing (van Schaik et al., 1999).

2.2.2. pH homeostasis

Microorganisms maintain their intracytoplasmic pH via the mechanism of pH homeostasis achieved by proton transport across the cell membrane. In aerobic organisms, the active transport of H^+ is coupled with electron transport in respiratory chains. On the other hand, anaerobic bacteria carry out H^+ transport via H^+-ATPase molecules using energy from ATP hydrolysis. *L. monocytogenes* is a facultative anaerobic bacterium that may use both processes for pH homeostasis (Shabala et al., 2002). The F_0F_1-ATPase is a multisubunit enzyme that serves as a channel for proton translocation across the cell membrane by utilizing ATP. The enzyme is highly conserved and has been extensively studied in *E. coli*. The F_1 portion of the enzyme consists of five subunits — α_3, β_3, γ, δ, ε, whereas the F_0 part is made up of a, b_2, c_{10}. The flow of protons (proton gradient) causes the rotary motion of F_0 and then F_1 subunit leading to the synthesis of ATP. In the reverse reaction, ATP hydrolysis causes the F_0 subunit to rotate in the opposite direction (Yoshida et al., 2001). Cotter et al. (2000) performed experiments using *N,N′*-dicyclohexylcarbodiimide (DCCD) that inhibits proton extrusion by F_0F_1-ATPase to determine the role of the enzyme in ATR of *Listeria*. The treatment of *L. monocytogenes* cells with the DCCD inhibitor before and during acid challenge resulted in enhanced acid sensitivity of acid-adapted cells. There was a three-log reduction in survival of the pathogen indicating the involvement of the F_0F_1-ATPase in acid adaptation of *Listeria* (Cotter et al., 2000).

2.2.3. Glutamate decarboxylase system

L. monocytogenes utilizes the glutamate decarboxylase (GAD) system to survive acid stress. The GAD system is composed of three genes *gadA*, *gadB* and *gadC* genes. The *gadA* and *gadB* genes encode two glutamate decarboxylases and the *gadC* gene codes for a glutamate/ϒ-aminobutyrate antiporter (Cotter et al., 2001). It has been proposed that glutamate is taken up by the cell via a specific transporter followed by its decarboxylation in the cytoplasm, producing ϒ-aminobutyrate and resulting in the utilization of an intracellular proton. The ϒ-aminobutyrate is then exported from the cell via an antiporter located in the cell membrane. The proton loss from the cell results in an increase in the pH of the cytoplasm and the release of alkaline ϒ-aminobutyrate into the environment raises the external pH slightly (Small and Waterman, 1998). Cotter et al. (2001) studied the role of the GAD system in the acid resistance of *L. monocytogenes* during gastric transit by using synthetic human and porcine gastric fluid. They found that addition of glutamate increased the survival of the wild type strain in gastric fluid and this is of concern in foods containing glutamate. Further, deletions of *gadA*, *gadB* and *gadC* genes resulted in enhanced sensitivity of the strain to low pH. The *gadAB* mutant also showed reduced survival rate in gastric fluid. This shows that a functional GAD system is vital for the acid resistance of *L. monocytogenes* and to successfully pass through the gastric environment and infect the small intestine (Cotter et al., 2001).

2.2.4. Role of general stress sigma factor (σ^B)

Wiedmann et al. (1998) conducted studies to determine the role of general stress transcription factor σ^B on the acid resistance of *L. monocytogenes*. The *sigB* mutant showed a lower level of acid resistance in stationary phase compared to the wild type. Their findings suggested that the survival of *L. monocytogenes* upon exposure to an acidic environment is dependent on the expression of σ^B-dependent proteins (Wiedmann et al., 1998). Investigating the role of σ^B in growth phase-dependent acid resistance and adaptive ATR in the wild type strain and *sigB*

mutant, Ferreira et al. (2003) showed the presence of σ^B-dependent and σ^B-independent mechanisms of acid resistance through various phases of growth. The survival and increased resistance of log-phase *L. monocytogenes* cells to gastric fluid following exposure to mild acidic conditions were partially dependent on σ^B (Ferreira et al., 2003). Further evidence on the regulation of expression of *L. monocytogenes* genes transcribed from σ^B-dependent promoters was provided by Kazmierczak et al. (2003). The alternative stress sigma factor regulates the expression of the *gadB* gene involved in acid stress survival and OpuC, which is a chill-activated transporter for carnitine. These studies shed light on the diverse role of σ^B in the survival of *L. monocytogenes* under acid stress conditions. In addition to the regulation of genes for survival under acid stress conditions, the stress-responsive factor σ^B also regulates virulence gene expression in this foodborne pathogen (Kazmierczak et al., 2003).

2.2.5. Two-component regulatory systems

A two-component regulatory system, consisting of *lisR* and *lisK*, which encode the response regulator and histidine kinase respectively has been identified in *L. monocytogenes*. These two-component signal transductions systems can sense changes in the environment such as low pH, oxidative and ethanol stresses via a membrane-associated histidine kinase, and the response regulator enables the cell to respond by altering gene expression (Cotter et al., 1999). The study by Cotter et al. (1999) has shown that LisRK signal transduction system is involved in response to stresses such as low pH and regulation of virulence gene expression in *Listeria*.

The acidic pH of many foods is one of the many factors that help to prevent the growth of foodborne pathogens. Therefore, the acid tolerance response observed in *L. monocytogenes* is of particular concern during food processing, because an exposure of the pathogen to mild acidic conditions could confer resistance to more severe acidic conditions. Gahan et al. (1996) used acid-adapted and non-adapted *L. monocytogenes* to compare their survival in a variety of acidic food products. A constitutive acid tolerant mutant isolated by prolonged exposure to pH 3.5 (3 M lactic acid) was used to investigate survival of the pathogen in acidic foods (O'Driscoll et al., 1996). The acid-adapted *L. monocytogenes* and the acid tolerant mutant showed better survival in commercial natural yogurt and cottage cheese made in the laboratory. The acid tolerant mutant showed enhanced resistance during the ripening of hard cheeses such as cheddar cheese and the authors recovered a significant number of cells after the 70-day ripening period. Cheese manufacturers should take the survival of acid tolerant strains of *L. monocytogenes* after the ripening period into consideration. The authors investigated the survival of these *L. monocytogenes* strains during milk fermentation by *Streptococcus thermophilus*. The acid-adapted strain demonstrated enhanced survival compared to the non-adapted culture. The acid tolerant mutant also survived similar to the acid-adapted cells during the first 4 h of fermentation (Gahan et al., 1996).

2.3. Survival under osmotic stress

Microorganisms can 'sense' and adapt to constantly changing environments, and this is an essential part of their growth and survival. The response of microorganisms to osmotic stress involves both physiological changes and variations of gene expression patterns and is called osmoadaptation (Hill et al., 2002). The use of salt to lower the water activity is one of the methods of food preservation used by the food industry; however, the ability of *Listeria* to adapt and survive in high concentrations of salt makes it difficult to control the pathogen in foods.

2.3.1. Induction of proteins

One of the mechanisms used by *Listeria* to tolerate salt stress is a change in its gene expression leading to an increased or decreased synthesis of various proteins. Duche et al. (2002a) studied the expression pattern of proteins using 2-D gel electrophoresis after inducing salt stress in *L. monocytogenes*. About twelve proteins induced by salt stress were identified by microsequencing and mass spectrometry. Similar to the two groups of proteins induced in response to cold shock mentioned earlier (Csp and Cap) (Bayles et al., 1996), Duche et al. (2002a) identified salt shock proteins (Ssp) induced rapidly but overexpressed for a short period and the stress acclimation proteins (Sap), that are rapidly induced and continue to be overexpressed several hours after conditions return to normal. Two general stress proteins (DnaK and Ctc) were identified among the Ssps induced in *L. monocytogenes*. DnaK functions as a heat shock protein, stabilizing cellular proteins. Among the eleven Saps identified, GbuA, which functions as an osmoprotectant transporter for glycine betaine was induced in response to salt stress. Since the Ctc protein was induced in response to salt stress (Duche et al., 2002a,b), Gardan et al. (2003) investigated the role of this protein in osmotic stress. The *ctc* gene is involved in the resistance of *L. monocytogenes* to high osmolarity in the absence of osmoprotectants such as glycine betaine and carnitine in the medium (Gardan et al., 2003).

2.3.2. Compatible solutes as osmoprotectants

Several studies have been conducted on the role of compatible solutes in osmoadaptation. These are highly soluble compounds that have no net charge at physiological pH and can be accumulated at high concentrations within a cell without affecting cellular functions. Bayles and Wilkinson (2000) showed that glycine betaine, proline betaine, acetyl carnitine, carnitine, ϒ-butyrobetaine and 3-dimethylsulphoniopropionate function as osmoprotectants in *L. monocytogenes*. The presence of these compounds resulted in an up to 2.6-fold increase in growth rate of salt-stressed cells compared to stressed cells without any osmoprotectants. The cells take up osmolytes from the external environment as a response to osmotic stress, which helps to regain the osmotic balance within cells (Bayles and Wilkinson, 2000).

2.3.3. Role of general stress sigma factor (σ^B)

The general stress sigma factor σ^B in *L. monocytogenes* is important for the utilization of betaine and carnitine as osmoprotectants (Becker et al., 1998). Later studies by Kazmierczak et al. (2003) identified the genes regulated by σ^B. The expression of the *ctc* gene that was shown to contribute to the osmotic stress response by Gardan et al. (2003) is dependent on σ^B in *L. monocytogenes*. While the sigma factor is an important part of the general stress response of *L. monocytogenes* to adverse

environmental conditions, it should be noted that the extent to which an organism depends on σ^B for its stress response varies between serotypes (Moorhead and Dykes, 2003).

2.3.4. Two-component regulatory systems

Kallipolitis and Ingmer (2001) identified response regulators that are a part of the two-component signal transduction system and involved in the osmotic stress response. One of the proteins identified was homologous to KdpE proteins that are a part of the Kdp two-component system. The Kdp uptake system is involved in the transport of potassium (K^+) into *L. monocytogenes* cells (Kallipolitis and Ingmer, 2001). Further studies by Brøndsted et al. (2003) investigated the role of *kdpE*, which encodes the response regulator and the downstream gene (*orfX*) in adaptation to salt stress. Their results indicate that adaptation to high osmotic stress requires expression of both *kdpE* and *orfX* genes and this effect depends on the potassium level in the medium. Thus, the uptake of potassium from the environment via the Kdp system has a protective effect on *L. monocytogenes* against salt stress. The *orfX* gene is responsible for triggering the activation of σ^B (Brøndsted et al., 2003).

In addition to individual stress factors, it is important to keep in mind that cross-protection to environmental stresses is commonly seen as part of the stress response of *Listeria* in foods. This is crucial when deciding on food processing and preservation parameters, since exposure of the pathogen to one kind of sub-lethal stress can confer cross-protection to other lethal stresses. For example, making of cheese involves exposure of the product initially to an acidic environment followed by salting, where the product is exposed to sodium chloride (Faleiro et al., 2003). Faleiro et al. (2003) showed that acid-adapted strains of *L. monocytogenes* isolated from cheese showed enhanced survival in salt stress (20% NaCl) compared to the non-adapted strains. The reverse, where induction of an acid tolerance response occurs following osmoadaptation was also investigated. The results showed that osmoadaptation resulted in the induction of ATR and the cells were able to survive lethal acidic conditions, but there were strain differences in the acid response (Faleiro et al., 2003).

3. Biofilms and quorum sensing

3.1. Formation of biofilms

Microorganisms can exist in the environment either as planktonic cells or as communities in biofilms, where they are attached to a surface and enclosed in a matrix predominantly made up of polysaccharide material. Microbial biofilms demonstrate a decreased growth rate, variation in the genes transcribed (Donlan, 2002), higher rate of gene transfer by conjugation (Hausner and Wuertz, 1999), increased production of exopolysaccharide (Sutherland, 2001) and more importantly an enhanced resistance to sanitizers, disinfectants and antimicrobial agents (Robbins et al., 2005). Biofilms can form on a wide range of surfaces such as medical devices, water system piping, industrial equipment, as well as in food processing facilities (Donlan, 2002). Biofilms in food processing environments occur on food handling surfaces or areas where food is stored or on food processing surfaces such as conveyer belts and stainless steel equipment (Wong, 1998; Kumar and Anand, 1998). The formation of biofilms in food manufacturing and processing facilities is of concern because bacteria from biofilms can be transferred to food products. *L. monocytogenes* is commonly isolated from food processing environments, especially in the meat (Midelet and Carpentier, 2002) and dairy (Pritchard et al., 1995) industries. Biofilms of *Listeria* are of particular concern, since they are more resistant to disinfectants and sanitizing agents compared to planktonic cells and this makes their elimination from food processing facilities a big challenge (Mah and O'Toole, 2001; Lewis, 2001).

3.2. Characteristics of biofilms

Cells attached to surfaces in a biofilm differ in many aspects from freely suspended planktonic cells. Hefford et al. (2005) studied the differences in the physiology of cells in biofilms and planktonic cells from the same culture by comparing their protein expression. Out of the nineteen proteins that showed higher expression in biofilm-grown cells, many of them were involved in stress response, protein synthesis and several regulatory functions of the cell (Hefford et al., 2005). *Listeria* can either exist in monoculture biofilms or be a part of mixed culture biofilms with bacteria such as *Flavobacterium* (Bremer et al., 2001). Bremer et al. (2001) conducted a study to determine the level of attachment of *L. monocytogenes* to stainless steel surfaces in a pure culture biofilm and a mixed culture biofilm with *Flavobacterium*. They found that a significantly higher number of *L. monocytogenes* cells attached to stainless steel surfaces in a mixed culture biofilm compared to the single culture biofilm. Further, cells of *L. monocytogenes* from the mixed culture biofilm could survive for a longer period of time (Bremer et al., 2001). The results of this study are important because pathogens such as *Listeria* are found with different microorganisms in food processing facilities and this increases the possibility for mixed culture biofilms to develop. Carpentier and Chassaing (2004) isolated twenty-nine bacterial strains from food processing environments after cleaning and disinfection, and grew them in binary culture biofilms along with *L. monocytogenes*. The aim of the study was to investigate the effect of these isolates on the biofilm-forming capacity of *L. monocytogenes* on stainless steel coupons. Their data showed that sixteen of the strains led to a decrease in the biofilm colony forming units counts, whereas four of the strains had a positive effect on the settlement of *L. monocytogenes* on stainless steel surfaces (Carpentier and Chassaing, 2004). Thus, it is important to keep in mind the impact of resident microorganisms on the biofilm-forming capacity of *L. monocytogenes* in food processing facilities.

3.3. Strain-specific biofilm-forming capacity

Several studies have shown that *L. monocytogenes* strains vary in their ability to adhere to surfaces and form biofilms (Chae and Schraft, 2000; Kalmokoff et al., 2001; Borucki et al., 2003). Chae and Schraft (2000) grew thirteen *L. monocytogenes* strains on glass surfaces to study the differences in adherence of cells and biofilm growth among various strains. There was a significant

difference in the adherence of various strains to the glass surface. Biofilm growth for 24 h also showed significant differences in cell numbers among *L. monocytogenes* strains (Chae and Schraft, 2000). Kalmokoff et al. (2001) investigated the differences in adsorption, attachment and biofilm formation among *L. monocytogenes* isolates on stainless steel surfaces. Their results showed no relation between the level of adsorption and serotype of the strain. Among the isolates studied, only one strain formed a biofilm, but there were significant differences in the adherence of cells from various strains (Kalmokoff et al., 2001). A recent study examined the relationship between *L. monocytogenes* biofilm formation, phylogeny and persistence in the environment (Borucki et al., 2003). They observed increased biofilm formation in serotypes 1/2a and 1/2c and in persistent strains isolated from milk samples compared to non-persistent strains. However, the serotypes mentioned above with increased biofilm ability are not commonly involved in foodborne disease outbreaks (Borucki et al., 2003).

3.4. Role of general stress sigma factor (σ^B) in biofilm formation

To investigate whether the alternative sigma factor σ^B that plays an important role in the various stress responses of *L. monocytogenes* affects the surface attachment of this foodborne pathogen, Schwab et al. (2005) conducted studies that looked at the attachment of wild type *L. monocytogenes* and a *sigB* mutant to stainless steel. The data suggested that initial attachment of both wild type and mutant to the surface was the same; however, the number of cells of *sigB* mutant attached was significantly lower than the wild type after 48 or 72 h of incubation (Schwab et al., 2005).

3.5. Quorum sensing

The formation of biofilms as discussed above is a complex, step-wise process involving adsorption, attachment of cells to a surface and differentiation. Each of these stages is controlled by a wide range of factors such as the properties of the surface, characteristics of the organism such as the genetics and cell surface properties and environmental parameters like temperature and pH (Donlan, 2002). Among the many factors, cell-to-cell signaling commonly known as quorum sensing in bacteria has been an area of science that is extensively studied and its role in biofilm formation has been a subject of much debate in recent years (Kjelleberg and Molin, 2002). A review on biofilm formation and quorum sensing discusses the two social behaviors of microorganisms and sheds some light on the relationship between the two phenomena (Parsek and Greenberg, 2005).(Xavier and Bassler 2003). defined quorum sensing as "a process of bacterial cell-to-cell communication involving the production and detection of extracellular signaling molecules called autoinducers." An important criterion for this phenomenon to occur is the presence of a threshold cell density, such that a sufficient quantity of the signaling molecule can be produced (Bassler, 2002). The Gram-positive bacteria typically produce oligopeptides as autoinducers, and signaling between cells is via a two-component phosphorelay system. On the other hand, acylated homoserine lactones (HSLs) function as autoinducers in Gram-negative organisms (Winans and Bassler, 2002). Studies have shown that this type of cell-to-cell signaling can regulate virulence, bioluminescence, sporulation and biofilm formation in bacteria (Bassler, 2002).

3.6. Role of quorum sensing in biofilm formation

The discussion on quorum sensing in this paper is limited to its role in biofilm formation and growth of pathogens in foods. Studies on the role of quorum sensing in a biofilm environment and its effect on the pathogenicity or antimicrobial resistance of a biofilm have been published. Davies et al. (1998) showed that cell-to-cell communication in bacteria via a synthetic signaling molecule was involved in the development of biofilms of *Pseudomonas aeruginosa*. This organism has two cell-to-cell signaling systems — the *lasR–lasI* and *rhlR–rhlI* systems. The authors used the wild type strain and a double mutant that lacked both quorum sensing systems to study the role of cell-to-cell signals in biofilm differentiation. Both strains were similar with respect to initial attachment and growth on the glass surface; however, the biofilm produced by the mutant was much thinner and the cells were more densely packed in comparison to the biofilm made by the wild type strain. Further, treatment with the detergent sodium dodecyl sulfate (SDS) removed the mutant biofilm much more easily from the surface compared to the wild type biofilm (Davies et al., 1998). The enhanced susceptibility of the mutant biofilm to the detergent is encouraging because disabling the quorum sensing system in a pathogen could serve as a way of controlling biofilms in food processing environments. On one hand, several papers have shown that signaling molecules are involved in cell-to-cell communication in biofilm development, while on the other, papers such as the one by Van Houdt et al. (2004) suggest that *N*-acyl-homoserine lactone-based quorum sensing does not play a role in biofilm formation by Gram-negative bacteria isolated from a raw vegetable processing line. However, the authors mention that this does not rule out the possibility that quorum sensing systems could be involved in other aspects such as biofilm resistance (Van Houdt et al., 2004). There has been a plethora of studies on the formation of *L. monocytogenes* biofilms in food processing environments, but there have been no significant studies investigating the presence of a quorum sensing system in this organism. Recently, Ermolaeva et al. (2004) discussed the presence of a diffusible, low molecular weight autorepressor that restricts the expression of the PrfA virulence regulon in *L. monocytogenes* via a quorum sensing mechanism (Ermolaeva et al., 2004). This finding of an autorepressor that plays a role in the virulence and pathogenicity may serve as a lead for further studies to uncover any other quorum sensing systems that might be present in *L. monocytogenes*. A good understanding of the cell-to-cell signaling phenomenon of microorganisms such as *L. monocytogenes* can be used to control the growth or virulence of foodborne pathogens by identification of compounds that can function as quorum sensing antagonists (Smith et al., 2004). Lu et al. (2004) investigated if fresh produce and processed foods can have autoinducer-2-like activity and whether food additives could behave as autoinducers and show similar activity. The data

showed maximum autoinducer-2-like activity in the frozen fish sample, whereas some samples like turkey patties showed highest inhibition of autoinducer-2-like activity. Studies of this kind can help in determining factors useful for controlling the growth of microorganisms in foods (Lu et al., 2004).

3.7. Significance of biofilms in the food industry

Biofilms are a major concern in the meat industry, where the surviving microflora from carcasses can contaminate surfaces of equipment and this in turn can lead to contamination of products placed on these surfaces. Conveyer belts and stainless steel surfaces of equipment are commonly found to be contaminated even after sanitizing treatments (Midelet and Carpentier, 2002). A study by Midelet and Carpentier (2002) investigated the transfer of microorganisms to beef from conveyer belt and stainless steel surfaces, which were conditioned with meat exudates and then contaminated with different microorganisms including *L. monocytogenes* to simulate a meat-processing environment. The conveyer belt surfaces were made of either polyvinylchloride (PVC) or polyurethane, which is the material frequently used by the food industry. *L. monocytogenes* attached more strongly to the polymers in comparison to other microorganisms on the surface. In general, attachment strengths of all strains were higher for polymer surfaces than for stainless steel. The data revealed that the number of microorganisms transferred is dependent on the density of the microflora on the food contact surfaces and their respective attachment strengths. Under the conditions used in the study, *L. monocytogenes* seemed to be the most difficult to remove by sanitizing treatments from polymer surfaces compared to other strains used (Midelet and Carpentier, 2002).

3.8. Control of biofilms

A number of studies aimed at finding effective strategies to eliminate biofilms from food processing environments have been published. A study by Norwood and Gilmour (2000) tested the efficacy of sodium hypochlorite in eliminating a steady-state mixed culture biofilm of *L. monocytogenes*, *Pseudomonas fragi* and *Staphylococcus xylosus*. Exposure to 1000 ppm free chlorine for 20 min was required to reduce *L. monocytogenes* biofilm by a two-log cycle; however, planktonic cells of all three organisms were eliminated by an exposure to 10 ppm free chlorine for 30 s. The paper also discusses the protective effects of microorganisms by enhanced production of extracellular polysaccharide in multispecies biofilms, which could lead to a greater resistance to antimicrobial agents in comparison to a monoculture biofilm (Norwood and Gilmour, 2000). Somers and Wong (2004) conducted studies to determine the inactivation of *L. monocytogenes* by two detergent and sanitizer combinations. The first combination used a chlorinated-alkaline, low-phosphate detergent and dual peracid sanitizer and the second combination contained a solvated-alkaline environmental sanitation product and hypochlorite sanitizer. For the study, biofilms were developed in the presence or absence of meat residue on various materials commonly used in the food industry such as conveyer belts, rubber and stainless steel surfaces. Over time, the presence of meat residue increased the biofilm-containing cell numbers. Both the detergents brought about a significant reduction in cell numbers. Among the sanitizers, dual peracid was not effective in eliminating the organism significantly. The first combination was effective only on 50% of the samples, whereas the second one was effective on 86.1% of the samples tested (Somers and Wong, 2004). While a large proportion of research is focused on testing various antimicrobial agents to eliminate biofilms, Chmielewski and Frank (2004) used predictive modeling to determine the effect of heat inactivation of *L. monocytogenes* in monoculture and in mixed biofilms with *Pseudomonas* species and *Pantoea agglomerans*. Their data suggested that with proper control of time and temperature, hot water sanitation of stainless steel surfaces could serve as an efficient method for elimination of *L. monocytogenes* biofilms (Chmielewski and Frank, 2004). Zhao et al. (2004) studied the competitive-exclusion of *L. monocytogenes* by microorganisms isolated from biofilms in drains of food processing facilities. The organisms with anti-listerial activity isolated were tested further for their effectiveness to eliminate *L. monocytogenes* biofilms on stainless steel coupons. *Enterococcus durans* and *Lactococcus lactis* were the two isolates that brought about a reduction of more than 5 $\log_{10}$ CFU of *L. monocytogenes*/cm^2 (Zhao et al., 2004). Similar studies could help develop the competitive-exclusion strategy to effectively control *L. monocytogenes* biofilms.

4. Resistance to antimicrobial agents

Many of the foodborne diseases that result in diarrhea are usually not severe and the patient recovers over a short period of time. However, more severe and prolonged illness can occur due to pathogens such as *L. monocytogenes* and usually requires antibiotic treatment. The prevalence of antimicrobial resistance among foodborne pathogens can be a potential problem in the treatment of humans with antibiotics. The spread and mechanisms of antimicrobial resistance among food-related bacteria have been widely studied. An area of much debate is the relationship between the use of antibiotics in animal husbandry and the development of antibiotic resistance in human pathogenic bacteria. Antibiotics have been used in animal husbandry to control bacterial diseases and for growth promotion (White et al., 2002). This usage can lead to the development of resistance to one or even multiple antibiotics in foodborne pathogens, which could ultimately be transmitted to human beings via the food chain. The widespread use of antimicrobial agents such as antibiotics, sanitizers or disinfectants in food processing or equipment cleaning and their effect on antimicrobial resistance is being investigated. Genetic factors such as the mobility of antibiotic resistance genes found on plasmids and transposons can increase the transfer of antibiotic resistance between bacteria. For example, resistance could be transferred from environmental bacteria to human foodborne pathogens (Sorum and L'Abee-Lund, 2002). The ability of bacteria to adapt to adverse environmental conditions is an important factor in the development of resistance. This is because an exposure of the organism to a sub-lethal level of an antimicrobial agent can lead to adaptation and development of resistance to higher levels of the antimicrobial or even cross-resistance to other agents.

4.1. Resistance to antibiotics

Antimicrobial resistance in the foodborne pathogen *Listeria* is emerging in recent years. Studies have shown that several species of *Listeria* isolated from humans or from food production or processing facilities are resistant to one or more antibiotics. Walsh et al. (2001) looked at 1001 isolates of *Listeria* from retail foods to determine their levels of resistance to eight antibiotics. About 10.9% of the isolates was resistant to one or more antibiotics. Resistance to penicillin or tetracycline was the most common and there was no resistance to the antibiotics commonly used for treatment of listeriosis. However, this does not eliminate the possibility that resistance to antibiotics used for listeriosis treatment such as ampicillin and gentamycin cannot be acquired, since penicillin and ampicillin belong to the same family of beta-lactam antibiotics (Walsh et al., 2001). On the other hand, Mayrhofer et al. (2004) determined the antimicrobial resistance of *L. monocytogenes* isolates from 304 meat samples. The study did not reveal any resistant isolate from the samples for the antibiotics tested. (Mayrhofer et al., 2004). Prazak et al. (2002) tested the sensitivity of twenty-one isolates of *L. monocytogenes* from cabbage, water and environmental samples to various antibiotics. The study showed that 20 isolates (about 95%) were resistant to two or more antibiotics. About 85% of the isolates was resistant to penicillin and one of the strains was also resistant to gentamycin. This study is important since it reveals the presence of multidrug resistant strains of *L. monocytogenes* in food and environmental samples (Prazak et al., 2002). The differences in results reported by the two studies could be due to the type of samples used and variability in the procedures used for antimicrobial sensitivity testing.

4.2. Resistance to sanitizers and disinfectants

In addition to antibiotic resistance, the emergence and spread of resistance among foodborne organisms to sanitizers and disinfectants used by the food industry are also becoming a concern. Many studies are done to determine the susceptibility of *Listeria* to quaternary ammonium compounds (QACs), commonly used as disinfectants in food processing facilities. Mereghetti et al. (2000) studied ninety-seven epidemiologically unrelated *L. monocytogenes* strains for their sensitivity to QACs. The strains were isolated either from the environment, food products, animals or humans. Seven of the isolates that were of environmental or food origin had high MICs to the QACs tested such as benzalkonium chloride and cetrimide. The authors concluded that the resistance in these strains could explain the persistence of some organisms in food processing facilities. Further, all isolates contained the *mdrL* gene, which encodes an efflux pump responsible for conferring resistance to QACs. They suggested that rotation between two different sanitizers for cleaning of food facilities could prove useful to prevent the development of persistent and resistant strains (Mereghetti et al., 2000). To et al. (2002) investigated the adaptation and development of resistance in *L. monocytogenes* after exposure to sub-lethal concentrations of disinfectants used in the food industry. Two resistant and four sensitive strains of *L. monocytogenes* were grown in the presence of sub-lethal levels of benzalkonium chloride, a sanitizer widely used in food processing sites and the resistance acquired after adaptation was studied by evaluating the use of efflux pumps by the organism and comparing the cell surface properties of resistant and sensitive strains. The sensitive strains had an MIC that was five-fold higher and the MIC of resistant strains doubled after the period of adaptation. The efflux pumps were responsible for the adaptation of sensitive strains, whereas the originally resistant strains showed a change in their fatty acid profile after adaptation (To et al., 2002). Two persistent and two non-persistent strains of *L. monocytogenes* isolated from an ice cream and poultry plant were tested for their resistance and adaptive response to disinfectants (Lunden et al., 2003). The initial resistance of persistent and non-persistent strains to disinfectants was different and the strains adapted after a two-hour exposure to sub-lethal concentrations of disinfectants. The strains also showed adaptation to increasing levels of the disinfectant. However, the resistance to sodium hypochlorite disappeared in a week, whereas the resistance to QACs and tertiary alkylamine was maintained even 28 days after exposure. *L. monocytogenes* showed cross-adaptation to the same (related) or different family (unrelated) of disinfectants. The cross-adaptation of *L. monocytogenes* strains to related and unrelated disinfectants poses a question on whether the practice of rotation of sanitizers in the food industry suggested by Mereghetti et al. (2000) would be really effective in controlling the development of antimicrobial resistance (Lunden et al., 2003). Romanova et al. (2002) investigated the sensitivity of nineteen *L. monocytogenes* strains to sanitizers commonly used in the meat industry. Some of the isolates were from a listeriosis outbreak and some from the meat-processing facility. Five of the isolates showed a resistant phenotype to the sanitizers tested and they contained two plasmids. Similar to the findings of Mereghetti et al. (2000), all the isolates tested contained the *mdrL* gene, which encodes an efflux pump (Romanova et al., 2002).

4.3. Resistance to bacteriocins

Antimicrobial peptides produced by bacteria such as lactic acid bacteria are called bacteriocins. They are synthesized from ribosomes and are effective against closely related bacteria (Klaenhammer, 1993). Nisin is a 34-amino acid bacteriocin produced by *Lactococcus lactis* strains and is approved for use in food preservation in many countries; however, several other bacteriocins have shown the potential for future applications in food systems (Cleveland et al., 2001). Since its discovery and approval for use in foods, nisin has been used as a preservative in the dairy and meat industries to control pathogens such as *L. monocytogenes*. Nisin acts on target cells by permeabilizing the cytoplasmic membrane. The formation of pores leads to the leakage of cytoplasmic substances from the cell (Abee et al., 1994). However, similar to the use of antibiotics, the concern with the use of bacteriocins is the development of resistance in foodborne pathogens. Gravesen et al. (2002) investigated the frequency of resistance development in *L. monocytogenes* to two bacteriocins, pediocin PA-1 and nisin A, along with the effects of strain differences and environmental conditions. The resistance frequencies for pediocin investigated in about 20

strains were approximately 10^{-6}, irrespective of the environmental conditions, while the frequency of resistance to nisin was strain-specific and varied with environmental conditions from 10^{-7} to 10^{-2}. The paper sheds light on the development of resistance to bacteriocins in a food system and the influence of a number of environmental factors such as low temperature, acidic pH and presence of sodium chloride on the frequency of resistance development (Gravesen et al., 2002).

5. Advanced strategies for control of food safety

There has been an ongoing effort to control the foodborne pathogen *Listeria* in foods and in food processing facilities. Research performed by academia, government agencies and the food industry is aimed at developing new and improved methods to prevent the survival and growth of *Listeria*. The wide range of efforts to achieve food safety include better monitoring and reporting of foodborne diseases by government agencies, routine food sampling and testing, establishment of HACCP, inspection at food processing facilities, training of food workers and general awareness among consumers about food safety (Bryan, 2002). Several studies are being conducted that utilize various preservation techniques for the control of *Listeria* in foods and most of them aim at achieving food safety without compromising the sensory and nutritional qualities of foods.

5.1. General stress sigma factor (σ^B) as the target for food preservation

The advances in genomics have led to the identification of genes, the proteins they make and their functions. This information is proving very useful to develop new strategies to prevent the survival and growth of pathogens. van Schaik and Abee (2005) discuss the use of alternative sigma factor σ^B for inactivation and control of *Listeria* in the food industry. As discussed earlier, σ^B is an important regulator of the stress response in *Listeria*. Once the pathogen senses an environmental stress, the signal is relayed to σ^B and its activation initiates transcription of the σ^B regulon. The protein products being produced are a part of the stress response and confer protection to the cell. Since σ^B plays an important role in the stress response of the organism, an in-depth understanding on the mechanism of σ^B activation could be used to develop preservation technologies, which aim towards inactivating the sigma factor and thereby controlling the stress response of the pathogen (van Schaik and Abee, 2005).

5.2. Multiple hurdle technology

The growing demand for fresh, minimally processed foods by consumers has led to the need for natural food preservation methods such as the use of antimicrobial peptides to control the growth of foodborne pathogens, that have no adverse effects on the consumer or the food itself. The bacteriocin nisin has been widely used as a preservative to control the growth of pathogens in foods. Nisin has also proved very useful as a part of hurdle technology, where a combination of two or more treatments is used to obtain a more effective method of food preservation (Cleveland et al., 2001). The combined action of nisin and carbon dioxide on *L. monocytogenes* cells grown at 4 °C has been investigated (Nilsson et al., 2000). Nisin brought about a two-log reduction in wild type *L. monocytogenes* cells and acted synergistically with carbon dioxide to give a four-log reduction in cell count. Nisin had no effect on nisin-resistant cells grown in the presence of air or carbon dioxide. Carbon dioxide increased the lag phase of *L. monocytogenes* by six days and was more effective against nisin-resistant cells compared to the wild type strain. The presence of carbon dioxide increases the membrane permeability and the proportion of short-chain fatty acids in the cell membrane, which helps in the pore formation by nisin (Nilsson et al., 2000). Modi et al. (2000) studied the combined effect of heat and nisin on wild type and nisin-resistant *L. monocytogenes* cells. The heat sensitivity of wild type and nisin-resistant strains was the same in the absence of nisin. The synergistic effect of heat and nisin on nisin-resistant cells caused a 3.7 log reduction in the first 7 min of treatment. The sub-lethal heat treatment alters the membrane permeability along with nisin that causes poration of the cell membrane (Modi et al., 2000). Further, certain foods such as liquid whole egg and dairy products when processed by thermal treatments such as pasteurization result in undesirable changes in the sensory and nutritional qualities of the product. This has led to the development and use of non-thermal processing technologies such as Pulsed Electric Fields (PEF) in the food industry. Calderon-Miranda et al. (1999a,b) investigated the use of PEF and nisin in combination to inactivate at *Listeria innocua* in liquid whole egg and skim milk. The results showed an additive effect on the inactivation of *L. innocua* in both foods when the pathogen was exposed to PEF and the sensitized cells treated with nisin (Calderon-Miranda et al., 1999a,b). Multiple hurdle technology targets the bacterial cell in different ways resulting in better control of the pathogen. Arques et al. (2004) studied the effectiveness of reuterin, an antimicrobial compound produced by *Lactobacillus reuteri*, along with nisin against Gram-positive and Gram-negative organisms in milk. At the concentrations tested, reuterin exhibited bacteriostatic activity against *L. monocytogenes*. When reuterin was used in combination with nisin, it acted synergistically to inhibit *L. monocytogenes* (Arques et al., 2004). Bacteriocins such as nisin do not have a significant inhibitory effect on Gram-negative organisms; however, the use of nisin with other agents could probably serve as a method to enhance its effectiveness against a wider range of organisms. Branen and Davidson (2004) investigated the effect of ethylenediaminetetraacetic acid (EDTA) and lactoferrin on the antimicrobial activity of nisin. Low levels of EDTA used in the study synergistically enhanced the activity of nisin against *L. monocytogenes*. EDTA also increased the effectiveness of nisin against *E. coli*, a Gram-negative organism. EDTA functions as a chelator of divalent cations and permeabilizes the outer membrane of Gram-negative bacteria by releasing the lipopolysaccharide (LPS). This allows nisin to act easily on the cytoplasmic membrane. Lactoferrin alone did not show any bacteriostatic effect against the organisms tested, but in a combination treatment with 50% less nisin, lactoferrin totally inhibited *L. monocytogenes*. The use of bacteriocins with other treatment methods to achieve food preservation requires the use of lower concentrations of the bacteriocin, and

this helps to prevent the risk of development of bacteriocin-resistant population of cells (Branen and Davidson, 2004). A study by Ettayebi et al. (2000) investigated the synergistic action of nisin and thymol against the pathogens *L. monocytogenes* and *B. subtilis*. Thymol is an essential oil component in thyme and has previously been shown to have antimicrobial activity (Ettayebi et al., 2000). The results showed that nisin Z and thymol when used alone resulted in only a partial inhibition of both pathogens. But the two agents acted synergistically in combination treatment and sub-inhibitory concentrations of both nisin Z and thymol were sufficient to reduce the growth of both pathogens. Thymol alters the bacterial membrane structure resulting in greater permeability for nisin. This results in a higher concentration of nisin within the bacterial cells, thus permitting the use of lower nisin concentrations when used synergistically to obtain the same level of antibacterial activity (Ettayebi et al., 2000).

5.3. Encapsulation technology

Commercial preparations of nisin such as Nisalpin (2.5% pure nisin A) are commonly added directly to foods for preservation. However, the loss of nisin activity over time in various food systems has been reported (Benech et al., 2002b). The problem of nisin degradation and loss of its activity during storage of foods drew the attention of researchers to the technique of microencapsulation. Encapsulation technology is a method of enclosing materials into capsules before delivery into a system. Encapsulated materials are protected from adverse effects of heat, moisture, pH changes and their activity is maintained for prolonged periods of time (Gibbs et al., 1999). This method has been widely used for encapsulation of drugs in the field of medicine (Maswadeh et al., 2000) and in other areas of food science such as delivery of flavor compounds (Zasypkin and Porzio, 2004) and vitamins (Lee et al., 2002) into foods and microencapsulation of probiotic bacteria (Kailasapathy, 2002). Substances such as fats, starches, proteins and lipids are commonly used to encapsulate materials using techniques such as spray drying, extrusion coating and entrapment in liposomes. The release of encapsulated materials can be initiated by various conditions such as temperature, pH or moisture (Gibbs et al., 1999). Several recent studies have investigated the use of encapsulation to deliver antimicrobial agents such as nisin into food systems (Benech et al., 2002a,b; Were et al., 2004). The use of free nisin in cheeses to inhibit the growth of spoilage and pathogenic microorganisms results in the inhibition of starter cultures, ultimately affecting the acidification and flavor of the final product. Further, the use of bacteriocin-producing strains during cheese manufacture alters the quality of the final fermented product. Studies by Benech et al. (2002b) have investigated the use of nisin encapsulated in liposomes for delivery into cheddar cheese and its antimicrobial activity against *L. innocua*. Nisin A and nisin Z are the two natural variants of nisin, with nisin Z being more soluble than nisin A. This is due to the presence of asparagine at position 27 in nisin Z and histidine in nisin A (Benech et al., 2002b). Cheddar cheese prepared with either nisin Z encapsulated in liposomes or cheese with in situ production of nisin Z using a nisinogenic starter was compared over six months of ripening for the inhibitory activity on *L. innocua*. A greater reduction in *L. innocua* was observed in cheese made with nisin Z-containing liposomes compared to the cheese made with nisin Z-producing starter. After the six-month ripening phase, cheese made with nisin Z-liposomes had less than 10 CFU/g of *L. innocua* and nearly 90% of the nisin activity was retained. On the other hand, cheddar cheese made with the nisin-producing starter contained 10^4 CFU/g of *L. innocua* and only 12% of the nisin activity. The authors suggested that the encapsulation of nisin in phospholipid vesicles resulted in its higher concentration maintained over a longer period of time and greater activity (Benech et al., 2002b). Benech et al. (2002a) also used anti-nisin Z antibodies and Transmission Electron Microscopy (TEM) to study the localization of nisin Z molecules in the cheddar cheese matrix and understand the mechanism of its inhibitory action against pathogens. The images from TEM showed nisin to be encapsulated within liposomes and also associated with the hydrophobic liposomal membrane. They predicted that the quick release of encapsulated nisin into the food system would provide short-term antimicrobial activity, whereas the immobilized nisin would provide an inhibitory effect over a longer period of time due to its slower desorption from the membrane (Benech et al., 2002b). Were et al. (2004) evaluated the antimicrobial activity of nisin Z encapsulated in phospholipid liposomes against *L. monocytogenes*. The results showed the increased ability of nisin in liposomes to inhibit bacterial growth compared to free nisin (Were et al., 2004). The technique of delivery of bacteriocins in an encapsulated form into food systems for improving its stability and antimicrobial action is very promising, but it requires further research for optimizing the technology for use in various food products.

5.4. Active packaging technology

Contamination of foods can occur during any stage of the manufacturing or processing phase. Despite the difficulty and uncertainty in identifying the source of contamination in food-borne disease outbreaks, several surveillance reports have shown that post-process contamination of foods has been a major cause in many of the outbreaks. The sources of recontamination identified are unprocessed raw materials added to finished processed foods, food contact surfaces and environments, defective packaging and food handling personnel (Reij and Den Aantrekker, 2004). The review by Reij and Den Aantrekker (2004) provides a comprehensive list of outbreaks that have been caused due to post-process contamination of foods by various pathogens. Outbreaks of *L. monocytogenes* have occurred due to contamination of butter and hot dogs in the processing environment, contamination of cooked meat from the dicing machine and rillettes being contaminated from a filling and packaging machine (Reij and Den Aantrekker, 2004). To deal with the problem of post-process contamination, research has led to the development of active packaging, wherein materials are incorporated into the packaging to either control the atmosphere within the package (such as moisture content, pH, oxygen level) or inhibit the growth of spoilage and pathogenic organisms on the food product (Ozdemir and Floros, 2004). The growing consumer demand for minimally processed, conveniently packaged and extended shelf-stable foods has drawn much attention to the field of antimicrobial

packaging, in which edible films and coatings are used to deliver antimicrobial agents such as lysozyme, triclosan and nisin into food systems (Cagri et al., 2004; Cha and Chinnan, 2004). Sebti et al. (2002) developed a biodegradable packaging by incorporating nisin and stearic acid in it, which serve as an antimicrobial agent and moisture barrier respectively. The pH of the hydroxyl propyl methyl cellulose (HPMC) film was adjusted to 3 to prevent the nisin and stearic acid from interacting. The packaging showed high inhibitory activity against *L. monocytogenes* and *S. aureus* (Sebti et al., 2002). Mauriello et al. (2004) developed a polyethylene film activated using a bacteriocin produced by *Lactobacillus curvatus* 32Y. The antimicrobial packaging was developed by the coating method and its efficacy tested against *L. monocytogenes*-contaminated pork steak and ground beef. The results showed about a one log reduction in cell numbers, with the highest antimicrobial activity after 24 h at 4 °C (Mauriello et al., 2004). In recent years, many studies have investigated the use of a variety of antimicrobial edible films and coatings for food products. For example, Nisaplin-containing cellophane-based coating for chopped meat (Guerra et al., 2005), antimicrobial efficacy of corn zein films containing nisin against pathogens such as *L. monocytogenes* (Hoffman et al., 2001), nisin and lauric acid impregnated soy-based films for turkey bologna and their inhibitory activity on *L. monocytogenes* (Dawson et al., 2002), inhibition of *L. monocytogenes* on turkey frankfurters coated with zein films containing nisin, sodium diacetate and sodium lactate (Lungu and Johnson, 2005).

An in-depth understanding of the material being used to develop the film or coating and the antimicrobial agent added to make it active is essential because the release kinetics of antimicrobial agents differ depending on the kind of packaging material being used and the agent itself. The ideal bioactive packaging would have the release of the antimicrobial agent at a rate that achieves the highest inhibitory action against microorganisms in the food system. Chung et al. (2001) investigated the effect of slow release of propyl paraben from a polymer coating versus direct addition of propyl paraben against *Saccharomyces cerevisiae*, which causes spoilage of foods. The slow release of the agent from the carboset coating showed a slow and continuous inhibition of *S. cerevisiae*. On the other hand, direct addition resulted in cell outgrowth after a period of incubation, and the cells were more tolerant to propyl paraben when compared to cells from the slow release culture (Chung et al., 2001). Buonocore et al. (2003) developed a mathematical model to describe the release kinetics of antimicrobial agents from cross-linked polyvinyl alcohol into water. The antimicrobial agents lysozyme, nisin and sodium benzoate were used in the study. They determined the diffusion of water molecules into the polymeric matrix and the reverse diffusion of the antimicrobial agent from the film into the water to develop the model (Buonocore et al., 2003). Since the antimicrobial action of bacteriocins is highly dependent on the mode of delivery into the food system, Chi-Zhang et al. (2004) evaluated the efficacy of instant addition of nisin and slow release of nisin into a broth system. The instantaneous addition is similar to nisin formulated into foods and the slow release of nisin mimics the release of the bacteriocin from a packaging film to the food. Both types of delivery methods resulted in the inhibition of *L. monocytogenes*; however, bacterial cells developed resistance to nisin over time, with the resistance being higher when the cells were exposed to instant addition of nisin. Further, excess of nisin in the system did not inhibit the pathogen due to development of nisin resistance. The study suggests that the combination of instantaneous and slow release of nisin into a food system is the most efficient way to obtain the greatest antimicrobial effectiveness (Chi-Zhang et al., 2004).

6. Conclusion

The foodborne pathogen, *Listeria* emerged in the late 20th century and has been a cause of many outbreaks of listeriosis with high case fatality rates. The economic impact due to big product recalls, severity of the disease, hospitalization and treatment costs has drawn the attention of researchers towards the development of preventive measures to control the spread of *L. monocytogenes*. Ongoing research is focused towards deciphering the mechanisms behind the ability of the pathogen to survive and grow under suboptimal conditions such as low temperature, acidic pH and osmotic stress. An in-depth understanding of the physiology of the organism, establishment of HACCP in the food industry, regulated product sampling and testing, along with better detection and surveillance systems to report foodborne disease outbreaks is valuable in controlling the pathogen. As mentioned earlier, the increasing demand of consumers for minimally processed, ready-to-eat foods, and globalization of the food industry where a greater proportion of foods of ethnic origin are being imported may contribute to an increase in the incidence of foodborne diseases such as listeriosis. However, concerted efforts by academia, government and the food industry, as well as consumer awareness can prove beneficial to develop innovative strategies for the control of *Listeria* in foods and food processing environments and meet the consumer demand for minimally processed, ready-to-eat foods, without any loss in sensory and nutritional attributes.

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