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Response of *Listeria monocytogenes* to high hydrostatic pressure or freeze-thaw cycles following exposure to selected environmental stresses

by

Devin Kirk Dutilly

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
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Ames, Iowa

2011

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ABSTRACT

The purpose of this investigation was to examine the viability of *Listeria monocytogenes* Scott A NADC 2045 that endured selected environmental stresses and were then subsequently exposed to freeze-thaw cycles or high hydrostatic pressure. The environmental stresses investigated in relation to freeze-thaw cycle survival include acid shock (HCl, pH 4.0-6.0), alkali shock (NaOH, pH 8.0-11.0), ethanol shock (2.0% -0.5%), oxidative shock (H_2O_2, 50-500ppm), and acid adaptation. All shock stresses were applied to exponential phase cells whereas non-stressed exponential phase cells served as a control. Freeze-thaw cycles involved freezing at -18°C for 24 h and thawing at 30°C for 7 min. Injury evaluation for all freeze-thaw treatments were performed by comparing colony counts of the pathogen on tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) to counts on modified oxford agar (MOX). All samples were serially diluted (10-fold) in Buffered Peptone Water (BPW) and surface-plated on appropriate agar media. Inoculated agar plates were incubated at 35°C and bacterial colonies were counted at 72 h. Starvation of washed stationary phase cells in physiological saline (0.85% (w/v) NaCl) over 12 days was examined at 2-day intervals for viability and resistance to high hydrostatic pressure. Starvation preparation involved the static growth of *L. monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) and washing these cells twice in 0.85% NaCl. Cells were then suspended in fresh physiological saline and held at 25°C during the starvation period. Pressurization at 400 MPa from 1 to 75 s was achieved using the Food Lab High-Pressure Food Processor (Stansted Fluid Power Ltd, Essex, U.K.). Viability of pressurized *L. monocytogenes* was examined after serial dilution in 0.1% peptone and plating on TSAYE followed by incubation of 35°C for 48 h. Control cultures were non-starved stationary phase

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cells. Results for the freeze-thaw cycles and environmental stresses indicate no statistically significance difference in freeze resistance or injury of the stressed pathogen compared to the control. When examining controls, there was a decrease in viability after 1 cycle of 0.5 log CFU/ml and 0.74 log CFU/ml after 4 cycles. This decrease occurred irrespective of any prior environmental stress tested. No statistically significant freeze-thaw injury was found among cells that endured prior stresses or in freeze-thaw treated cells compared to control cells. For starved *L. monocytogenes*, approximately a 2 log CFU/ml decrease was seen in viability after 2 days of starvation. Viability remained stable for the remaining 10 days. Maximum D-values (at 400 MPa) of 19.88 s, 18.6 s and 18.5 s were observed after 8, 6, and 10 days of starvation, respectively. D-value (at 400 MPa) of the control was 11.85 s. Overall significance of freeze-thaw results for the food industry is that freeze-thaw resistance of *L. monocytogenes* does not seem to be affected by certain prior environmental stresses on this pathogen. Reductions after 4 freeze-thaw cycles in the controls were 0.74 Log CFU/ml, which represented a significant decrease in viability. Based on the results of the present study, the exposure of *L. monocytogenes* Scott A to certain environmental stresses does not increase the resistance of this organism to freeze-thaw cycles. Also, starved *L. monocytogenes* cells developed a higher resistance to high pressure processing compared to non-starved cells. The increased high pressure resistance of starved *L. monocytogenes* should be considered when aiming to design safe food processing protocols involving high hydrostatic pressure technology.

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INTRODUCTION

The food industry constantly searches for methods to reduce environmental and food contamination. Foodborne pathogens pose a serious risk to populations both in our domestic and international markets. Interest in discovering ways in which both pathogenic and spoilage microorganisms may survive current food processing methods is of growing interest.

Although many foodborne pathogens may generally cause illness, *Listeria monocytogenes* is an opportunistic organism that causes harm to individuals who are particularly vulnerable to disease. Such individuals include immunocompromised persons, pregnant women, elderly, the very young, and hospital patients enduring therapies that suppress the immune system. Recent 2011 estimates state that *L. monocytogenes* is responsible for approximately 1,500 illnesses and for 19% of all deaths related to foodborne pathogen infections in the United States. These estimates suggest a rate of death around 15.9%, where individual outbreak mortality rates may lend higher rates that cause serious public health concerns.

Stresses that *L. monocytogenes* are able to endure during infection of humans and animals, storage of foods, and survival in adverse environmental conditions may increase this pathogen's virulence and its resistance to subsequent food processing interventions. Historic and some novel processing interventions have been investigated and demonstrate increased resistance of *L. monocytogenes* to those interventions after exposure of this pathogen to prior environmental stresses. This increased resistance due to prior stress exposure is commonly referred to as cross-protection as is seen throughout the microbial world. Although not all

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stresses elicit cross-protection, there is a need to investigate the potential for such resistance to occur.

Additionally, stressed microbial populations may or may not possess the ability to recover from injury inflicted by food processing methods. This is of importance in determining the ability of the microorganism to proliferate and colonize in adverse food processing environments. Also, injury of bacterial populations may lead to decreased survival when microorganisms are in foods with adverse intrinsic properties such as low pH, low water activity and antimicrobial constituents. Measurement of injury provides important information on the physiological state of the microorganisms after stress exposures and has been demonstrated in relation to several food processing stresses.

Thorough investigation on cross-protection has not been investigated with the some physical interventions including freeze-thaw cycles and high hydrostatic processing. Reports of *L. monocytogenes* freeze-thaw injury during cross protection and other studies have reported both the presence and lack of freeze-thaw injury. Selected environmental stresses and their effects on the resistance of pathogens to conventional and emerging food processing methods still need investigation to ensure an elevated level of microbial safety within the food industry.

Given the precedence of cross-protection seen in published reports on stress-hardened *L. monocytogenes*, we hypothesize that *L. monocytogenes* exposed to selected environmental stress will elicit a resistance to subsequent freeze-thaw cycles. We also hypothesize that stressed *L. monocytogenes* will display increased injury in populations of this pathogen that survive freezing. The hypothesis related to high hydrostatic pressure follows the cross-

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protection theory: exposure of *L. monocytogenes* to selected environmental stresses will increase resistance to high hydrostatic pressure.

The objectives of this investigation are as follows:

1) Evaluate the viability of *L. monocytogenes* that were exposed to environmental stress followed by freezing and thawing.
2) Assess the freeze-thaw induced injury to *L. monocytogenes* that were exposed to selected environmental stresses.
3) Evaluate the resistance of *L. monocytogenes* to high hydrostatic pressure following several days of starvation in physiological saline.

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LITERATURE REVIEW

Listeria monocytogenes

History

Murray et al. (215) were the first to describe *Bacterium monocytogenes*, which infected monocytes and caused illness in laboratory rodents. Just one year later, scientist Pirie (245) found a bacterium in South Africa that infected rodents as well and termed it the "Tiger River bacillus" or *Listerella hepatolytica* and acknowledged the similarities that it held with the discoveries from Murray et al. of *Bacterium monocytogenes* (278). In 1940, Pirie declared that the name for the described organisms *Bacterium monocytogenes* and *Listerella hepatolytica* be combined into one and called *Listeria monocytogenes* (246). The disease caused by *Listeria monocytogenes* in sheep was called "circling disease" when circling movement of those infected animals were seen by Gill in 1926. That same year Nyfeldt had isolated the organism from three humans. As years followed *L. monocytogenes* was isolated from cattle, pigs and chicken as well (113). Currently, *L. monocytogenes* is acknowledged as a significant foodborne pathogen which causes a high fatality rate in humans annually. The focus of the food industry has been to control *L. monocytogenes* both in the food processing plant and in the food itself.

Morphology and Identification

L. monocytogenes is a rod-shaped Gram-positive non-sporeforming facultative anaerobic bacterium. This foodborne pathogen is marked by its small shape which measures 0.5 µm by 1-2 µm (diameter by length). *L. monocytogenes* has flagella at ambient

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temperatures (20-25°C) and is noted as having a minimal amount of flagella at or near body temperature (37°C) (95).

Listeria as a genus has 8 identified species including *L. grayi, L. seeligeri, L. welshimeri, L. ivanovii, L. innocua, L. monocytogenes,* and two recently identified species *L. marthii and L. rocourtiae* (111, 169). Identification of *L. monocytogenes* in food samples is commonly made using Pulse Field Gel Electrophoresis (PFGE) (34, 35, 38).

Traditional methods of identification can be used to differentiate *L. monocytogenes.* These methods examine several positive and negative results with the production of acid from various carbohydrates as well as testing for beta-hemolysis. *L. monocytogenes* produces acid in presence of L-rhamnose and alpha-methyl-D-mannoside. In the presence of D-xylose and D-mannitol no acid production is seen (25). A test for beta-hemolysis which is positive in *L. monocytogenes* is called the Christie-Atkins-Munch-Peterson (CAMP) test. This CAMP test is a presumptive test and can be used in combination with fermentable carbohydrate tests. The CAMP test uses *Rhodococcus equi* and *Staphylococcus aureus* which react and result in a display of synergistic hemolysis with *L. monocytogenes.* Proposed enhanced lysis of red blood cells is said to be due to the listeriolysin O (LLO) and phosphatidylinositol-specific phospholipase C (PI-PLC) and phosphatidylcholine-specific phospholipase C (PC-PLC) in *L. monocytogenes* and PLC in *S. aureus* and *R. equi* (196). The use of these biochemical, molecular and morphological identification tests allow for differentiation of *L. monocytogenes*. The differentiation of *L. monocytogenes* is of importance to the food industry based on the implication this pathogen has on human health.

Growth Characteristics

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L. monocytogenes has a wide range of growth characteristics which can be attributed to its commonly referenced thermotolerance as well as its classification as a psychrotrophic microorganism (154, 155, 172, 214, 227, 228, 287). Although these attributes differentiate *L. monocytogenes* from other non-spore forming bacteria, the following growth characteristics are generally seen in *L. monocytogenes*. Acknowledgment is made that continued research which involves strains with increase tolerances to temperature shifts, water activity, pH conditions and virulence expression exist (315).

Temperature Range

Listeria monocytogenes can grow at a wide range of temperatures and is characterized as being psychrotrophic, due to its ability to grow at refrigeration temperatures. One of the first exploitations of the psychrotrophic nature of *L. monocytogenes* was seen in the 1948 while attempting to detect this fastidious bovine brain pathogen (114). Compared to ovine infection, *L. monocytogenes* was noted as being less prevalent in bovine species. With the development and use of cold enrichment, researchers had noted that many negative test results were actually false-negative once samples were placed at $4^{o}C$ for some time (114). The use of cold enrichment to enhance the detection of *L. monocytogenes* can still be used today and was noted in the past literature as the “Gray’s cold-holding procedure” (331). This ability to grow at low temperatures was one early indication that *L. monocytogenes* might have the ability to present a public health risk.

The range of growth temperatures for *L. monocytogenes* is from $-1.5^{o}C$ to $50^{o}C$ (113, 136, 156, 239, 324). Junttila et al. (156) had performed research on *Listeria* spp. and found

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that *L. monocytogenes* strains were able to grow at a slightly lower temperature overall compared to non-pathogenic species. Temperature ranges that are optimum for growth of *L. monocytogenes* are between 25°C and 37°C (47). The range is associated with variations in optimum growth biomass and population numbers of the pathogen (100, 249, 268, 308).

Extreme temperatures, above 50°C and below -1.5°C, pose conditions which are not favorable for growth and survival of *L. monocytogenes*. Above 50°C, *L. monocyctogenes* begins to die (4). Research investigating heat susceptibility of this pathogen has been performed at 52°C and above to exhibit more rapid microbial death curves (289). Regarding survival at temperatures below -1.5°C, water activity seems to play an important role. As the water forms into ice crystals, an increase in salts occurs. Death of *L. monocytogenes* at freezing temperature is generally not seen as an effective intervention kill step. Freezing is most useful in the effect of preventing growth while causing cellular damage, which makes *L. monocytogenes* susceptible to higher concentrations of solutes in the suspending menstruum (14, 73, 230). Further discussion of the effects of freezing on *L. monocytogenes* are made in later sections.

pH Range

L. monocytogenes has a wide range of pH values over which it grows. Optimal growth conditions are between pH 6.0 and 8.0 with some researchers stating a pH of 7.0 as the optimum pH for growth (37, 239, 271). Shabala et al. (281) found that 25% of the strains tested were able to grow at a pH as low as 4.1 and 95% were able to growth at pH 4.2 in HCl adjusted BHI broth. Additional support for these low pH growth was seen by other researchers as well (241). The upper pH range for growth was reported by Petran and

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Zottola (239) to be pH 9.2. Lethality of *L. monocytogenes* can be seen at pH values below 3.5 (239). *L. monocytogenes* has been observed having slight decreases in populations above pH 10.0 (166, 198). Survival of *L. monocytogenes* is seen between lethality and growth pH values and this pathogen remains a very robust organism in terms of its ability to survive or grow in a wide pH range.

Water Activity Range

Petran and Zottola (239) reported that the optimal water activity (a_w) for growth of *L. monocytogenes* was ≥0.97. Water activity levels that permit growth of *L. monocytogenes* include those greater than 0.90 for glycerol and 0.92 for both sodium chloride and sucrose. Researchers reported that growth of *L. monocytogenes* was dependent not only on water activity, but upon which humectants were used (205, 219, 305). *L. monocytogenes* survived (up to 10 days) in fermented hard salami where the water activity was as low as 0.79 and up to 0.86. This water activity of 0.79 could be set as the lower limit for survival of *L. monocytogenes*. Water activity at <0.79 might elicit a greater decrease in survival overtime (152).

Virulence differs in different foods

L. monocytogenes has been found in foods which are both nutritionally and functionally diverse (83). It is evident that *L. monocytogenes* has the potential to contaminate many foods as well as survive in a variety of conditions. Midelet-Bourdin et al. (203) found that when comparing *L. monocytogenes* grown in raw salmon, cold-smoked salmon, milk and "potted minced pork" extracts, the minced pork extract produced *L.*

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monocytogenes that displayed decreased signs of virulence while viability of the microorganism did not decrease. Additionally, this study and others (31, 147) have reported an increase in virulence factors when *L. monocytogenes* was introduced into minimal medium from a nutrient rich medium. Other food compounds such as fats (69, 92) and lactoferrin, a glycoprotein present in milk, (11) have been shown to impact the ability for *L. monocytogenes* to colonize the gastrointestinal system. This suggests that the variation in growth conditions, either from minimal to highly nutritious media or different food systems, may warrant an increase need for risk assessment. Additional attention should be placed on the public health implications of the consumption of strains of *L. monocytogenes* (with decreased virulence) by immunocompromised populations (203).

Epidemiology

Natural environment and transmission of *L. monocytogenes*

L. monocytogenes is ubiquitous in the environment and is considered a saprophyte whose natural habitat is decaying vegetation (113). Samples from decaying plant material yielded isolation of *L. monocytogenes* from 7 of 12 samples. Additional sampling over two years yielded isolation of *L. monocytogenes* from soils and plants, game animal feces, decaying feed materials, and birds (329, 331). A survey of sewage, soil and fecal samples found a high prevalence of *L. monocytogenes* in sewage (60%) and low levels of the pathogen in soil (0.7%) and feces (0.6%) (188). Urban and populated environments have been reported to contain *L. monocytogenes* as well. This pathogen has been isolated from various urban environments including urban soils, leaves and debris, mulch, sidewalks, water

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runoffs, puddles, rivers and streams, lakes, storm drains and trash cans (273). Contaminated soil where survival of *L. monocytogenes* was seen upwards of 67 days may lend itself to contamination of water sources in contact with such soil (330).

Environmental water are likely sources of *L. monocytogenes*. Studies found that *L. monocytogenes* was present in approximately 12% (Switzerland) and 10% (Canada) of water samples examined. These studies indicate that contamination might not be completely random, but related to upstream contamination or other extraneous factors which may have contaminated the water (187, 274, 275). Examining water sources that were closer to direct contact with the food system found that *L. monocytogenes* could be isolated from saltwater fisheries 2% of the time and fresh water fisheries 10% of the time. An increase in positive samples to 16% and 68% were seen in fish slaughterhouses and smokehouses, respectively (118). *L. monocytogenes* and other *Listeria* spp. were isolated from both waters and sediments in the Humbolt-Arcata Bay, California area (57). This might reflect a general persistence within sediments that may last despite temporary interventions to decrease *Listeria* spp. in the waterways. There exists a high likelihood of *L. monocytogenes* isolation from the environment. Due to the wide-array of environments where *L. monocytogenes* has been isolated, it is easy to see how transmission onto animals and into food processing environments may occur.

To date, *L. monocytogenes* has been isolated from a large number of animals and food products. *L. monocytogenes* has been isolated from 37 different mammals and 17 bird species. In the human population, between 1 and 10% are carries of the organism (2, 256). *L. monocytogenes* has also been isolated from various food products including both raw and ready-to-eat (RTE) foods. The RTE products in which *L. monocytogenes* have been detected

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include cheeses (1, 109, 175, 181), vegetables and salads (15, 109, 173, 174, 177), fermented sausage (314), celery (68), deli meats (79, 93, 109, 176, 236), RTE seafood (109, 153, 204, 322), paté (79), and ice cream (18). Raw products in which *L. monocytogenes* have been detected include milk (265), beef (9, 15, 58), chicken (9, 15, 293), pork (9, 58), seafood (15, 208, 220, 307), and lamb (12). Both raw and RTE foods present a potential hazard for pathogen transmission through cross contamination and direct consumption of *L. monocytogenes* contaminated food. Almost all areas of the food processing industry are affected by *L. monocytogenes* as a potential food contaminant.

Maintenance of dry environmental conditions where feasible in food processing may be effective in decreasing foodborne contamination by *L. monocytogenes* from the processing plant environment. Wet processing environments are often present in food processing environments which use high amounts of water and constant application of sanitizers to control microbial contamination. Large amounts of water are also used during certain types of food processing including fresh and fresh-cut vegetables and meats. Wet environments offer a transmission vector for *L. monocytogenes* to cross production barriers (Fresh to RTE). It has also been noted that wet environments can hide evident sources of condensation drips and increase the likelihood of harborage of *L. monocytogenes* in cracked floor tiles or drains. In one processing plant, the rate of positive environmental *Listeria* samples was diminished to zero after implementation of dry processing interventions. The birth of this paradigm of reducing *Listeria* positive environmental samples by moving from wet processing conditions to dry was termed "dryer is better" (310, 312). Efforts such as dry processing environments might eliminate transmission of *Listeria* spp. within the processing plant, but might not eliminate the need for control of *L. monocytogenes* in foods. Also, such an approach may not

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be practical in some food processing plants due to the necessary use of water to facilitate processing and proper cleaning and sanitizing of equipment and surrounding areas.

Foodborne Listeriosis

Listeriosis, causative agent *L. monocytogenes*, has an increased infection rate with immunocompromised populations. This includes youth as well as the elderly and those individuals that are pregnant or have preexisting infections or health conditions (AIDS, cancer, diabetic, etc.) (256, 272). Where listeriosis is seen, 70% of patients can be said to have suppressed immune responses (182).

The infective dose of *L. monocytogenes* in humans varies depending on the person's immune status. The risk of contracting listeriosis from levels that are 100 CFUs or less is low for both at-risk populations and the general population (19, 54, 264, 291). This research has likely been applied in regulatory agencies in both Canada and some European countries where levels below 100 CFU/g or ml are accepted in some foods (81). Such regulations recognize the difficulty in eliminating *L. monocytogenes* from raw agricultural products and the relatively low risk in the consumption of small numbers of this organism.

Foodborne infection by *L. monocytogenes* occurs when an infectious dose of the pathogen is ingested via contaminated food. Such behaviors as the use of antacids can reduce the natural acid barriers of the stomach to increase risk of infection. Additional stomach related ulcer surgeries and drugs such as Nexium (Esomeprazole) which are designed to reduce the activity of proton pumps may also lead to increased risk of infection (133, 277). Once *L. monocytogenes* has entered our system it acts as an intracellular pathogen and it has the unique ability to cross the placental barrier. For these reasons many

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scientist use *L. monocytogenes* as a model organism to intracellular survival of pathogens (272).

Virulence factors which are expressed by *L. monocytogenes* allow successful invasion and infection within cells. Virulence factors such as internalin (Inl), listeriolysin 0 (LLO), and other hemolytic proteins, and ActA will be described in relation to enhancing *L. monocytogenes* survival within the host.

Initial interactions between internalins, surface proteins of *L. monocytogenes*, and E-cadherin, a host receptor, allow for the pathogen to induce endocytosis which allows the intercellular activity of *L. monocytogenes* (59, 83, 201). The entry into host phagosomes, host defense systems, is host induced and not necessarily pathogen induced. InlA protein is of particular importance within the group of internalin proteins allowing entry into mammalian endothelial cells. In mutants not containing other internalin proteins, but only InlA, invasion into cells was seen in InlA mutants and not in mutants without this protein (272). A similar examination of genomes had shown that *L. monocytogenes* strains contain 8 *inl* genes, (including *inlA*) which are not present in the genome of the nonpathogenic *L. innocua* genome (60).

Once entry of *L. monocytogenes* into the cells has occured, LLO is mainly responsible for release from the single layered member vesicle. LLO is an extracellular proteolytic pore-forming protein that permits hemolysis of the vesicle. This 60-kilodalton protein has been shown to be dependent upon the presence of cholesterol (99, 200, 256, 272). LLO is homologous protein to other cytotoxic proteins such as streptolysin O and pneumolysin (200). The LLO has a relatively high lethality and is associated with increased lethality when expressed in *L. monocytogenes* (70, 99, 272). The expression of LLO is

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increased at pH 5.5 and not present at pH 7.0. This indicates that when phagosome acidification occurs, increased expression of LLO will actually promote lysis and escape of *L. monocytogenes* into the cytoplasm (99).

Another set of hemolytic proteins are expressed in *L. monocytogenes* named phosphatidylinositol-specific phospholipase C (PI-PLC) and phospholipase C (PC-PLC). Unlike LLO, both PI-PLC and PC-PLC do not form pores. These two proteins cleave phospholipids facilitating escape from vesicles. PI-PLC mostly has a role in helping *L. monocytogenes* escape from the initial vesicle membrane (single layer membrane) because once *L. monocytogenes* begins cell to cell transmission the pathogen is located within a two membrane vesicle (272).

Once *L. monocytogenes* has escaped the first vesicle, the pathogen is able to replicate and use actin from the host cell cytoskeleton for motility within the cytoplasm. The ActA surface protein is able to polymerize actin filaments at its surface and propel using a nucleated actin tail. Depolymerization by the host cell occurs at an equal rate at *L. monocytogenes* polymerization leading the tail to appear at about an equal length during motility. This movement between and within cells and a replication rate of once per 50 minutes allows *L. monocytogenes* to effectively infect the host (272). These virulence factors and means of motility ultimately make *L. monocytogenes* able to intracellularly infect the whole host and lead to listeriosis.

Occurrences and Outbreaks

With the ubiquitous nature of *L. monocytogenes*, food processing plants have a difficult time ensuring that plant areas and foods are *L. monocytogenes* free. This has

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resulted in numerous outbreaks which have been examined by other authors (25, 32). The following information describes several current outbreaks of listeriosis.

Lessons can be learned from the examination of past outbreaks. The identification of processing, mechanical, or environmental errors which have led to an outbreak become a useful tool that can be used in avoiding future outbreaks. By avoiding future outbreaks, the food industry and agriculture as a whole avoids condemnation from the public perception and avoidable public health risks to the population. In the information that follows a few prominent outbreaks relating to 1) Mexican-style cheese, 2) deli meat and 3) cantaloupe will be examined. Of the three outbreaks, the second and third cases represent products that have a likelihood of being frozen for prolonged storage.

Between October 2008 and March 2009, an outbreak, with eight cases, of listeriosis was reported and epidemiological investigations trace the likely source back to Mexican-style cheese. The multiple state outbreak identified 3 cases in Illinois, 2 cases in Georgia, and 1 case in each North Carolina, Tennessee, and Wisconsin. *L. monocytogenes* isolates that matched the outbreak strain had been found in plant locations, cheese samples and human specimens (146). Although this cheese was pasteurized, post-pasteurization contamination from a production vat gasket contained a positive test for *L. monocytogenes.* Despite corrections to the process by removal of the causative vat, employee retraining, and modifications to employee traffic flow, *Listeria* contamination in the cheese persisted. The production plant was closed, processing equipment was removed and a rupture in the wall that separated raw and finished product areas was noticed (146). Although, no one source was named as the reservoir for *L. monocytogenes* in the plant, other studies have found that areas where moisture can easily build up can harbor *L. monocytogenes* in a processing plant

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environment (310, 312). This previously described scenario represents a perfect example how plant sanitation procedures may have failed in preventing the reoccurrence of contamination within a product line. It also emphasizes the importance of preventing the initial introduction of ubiquitous microorganisms such as *L. monocytogenes* into a plant environment as they are often difficult to eliminate.

Another outbreak had occurred with ready-to-eat deli meats that were processed and packaged at a Canadian based Maple Leaf Foods processing plant in 2008. The recalled products included roast beef, corned beef, turkey, and smoked products. As with many large food processors, the food processor did not sell those products directly to the consumer. Consequently, many of the users across Canada which had incorporated these meats in other products had issued recalls as well. Investigators eventually reported intricate meat slicing equipment (2 units) as probable cause of the outbreak (50). The outbreak resulted in a total of 23 deaths from 57 laboratory identified cases (67). This outbreak that claimed a 40% mortality rate, represents one of the reasons *L. monocytogenes* is seen with such importance in the realm of public health and safety. In the case of food processor contamination, there is often a greater spread of outbreaks as products are centrally produced and widely distributed. This outbreak was linked to a meat slicing machine that contained parts which are difficult to clean. This situation emphasizes the importance of engineering food processing equipment with special consideration of food safety and the routine use of proper disassembly for efficient cleaning and sanitizing of equipment.

As of September 21, 2011, an outbreak involving *L. monocytogenes* was identified and linked to cantaloupe which was grown in and distributed out of Colorado. The outbreak which involved "Rocky Ford" brand cantaloupes has cause 55 illnesses in 14 states which

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have been identified since August 4, 2011. One infection has been found in each of the following states: California, Illinois, Indiana, Maryland, Montana, Virginia, West Virginia, and Wyoming. Two cases have been reported in Wisconsin, four cases in Nebraska, eight in Oklahoma, nine in Texas, ten in New Mexico, and fourteen in Colorado. All interview and publicly released information from ill persons indicate that all cases involved hospitalization. In this case or listeriosis, nearly all infections were associated with immune compromised or persons over the age of 60 (251). Past researchers have examined cases of listeriosis infection and have occasionally seen melons as a potential risk factor in diets (320). Additional outbreaks involving in 10 listeriosis illnesses in 2010 were traced raw celery and an outbreak with sprouts in 2009 was also *Listeria*-associated (68, 87, 251) . Cantaloupe, sprouts and celery, products which are harvested from within or on top of soil, have plenty of opportunities to come in contact with surfaces potentially contaminated with *L. monocytogenes.* The consideration of exterior pasteurization or cleaning remains difficult based on the porous and imperfect exteriors which lend themselves to harboring soils. The fact that these foods are considered ready-to-eat products and, unlike fresh meats, no intervention kill step for pathogens is applied to the fresh-cut product, is one challenge that remains in the prevention of future fruit or vegetable outbreaks.

Given the variety of sources that are harborage sites for *L. monocytogenes* and the variety of foods that are linked to listeriosis infections in humans, processing and environmental conditions offer adequate opportunity for exposure of the pathogen to environmental stresses. In this regard, one must consider how stress-hardened *L. monocytogenes* may adapt physiologically and develop an increased resistance against other stresses.

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Stress Adaptation

Introduction

Foods and food processing environments may pose a set of challenges to microorganisms. As there is a growing diversity of foods on the market, foods and their associated processing areas may also provide a diverse environment for microbial survival. The variety of foods and food processing environments may impose stress on the microorganisms that coexist in these environments. In general, stress is encountered by microorganisms whenever an environment deviates from the ideal growth conditions. In addition, stress is said to exist whenever microorganisms express deviations in optimal growth patterns, sub-lethal injury, or any alteration to optimal functioning of metabolic reactions in the cell. Stress conditions occur constantly in microorganisms in the food processing environment and may include physical, chemical and nutritional stresses. Foods themselves may impose stress on microorganisms when one considers both intrinsic (pH, water activity, oxidation-reduction potential, available nutrients or inhibitory agents or barriers) and extrinsic factors (temperature, atmospheric conditions or competitive exclusion) that impact survival and growth of foodborne microorganisms. Food environments present unique opportunities for microorganisms to encounter fluctuations in nutrient availability which may include the exposure to periods of nutrient deficiency. Nutrient deprivation may occur in nature (outdoors) before the microorganism enters the food system or in production areas that may be difficult to clean. Chemical stresses that include pH or toxin stress may be encountered directly from intrinsic properties of the food or from processing interventions applied to the environment as cleaners or foods as processing aides. Food preservation and

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processing conditions expose microorganisms to some specific physical stresses such as irradiation, high pressure processing, ultraviolet-light, pulsed electric fields and heating, cooling, freezing or thawing. As one might imagine, these conditions provide many challenges for microbial survival (149, 298, 336).

Despite these numerous physical, chemical and nutritional challenges, microbial life is abundant and proliferating throughout the food supply chain. Given that some of these stresses do not fully inhibit microbial survival, microorganisms have adapted mechanisms to function in these diverse environments. The degree of stress and adaptive response may vary, but survival and proliferation of cells can depend upon multiple parameters of the exposure including both time and the extent of stress exposure (336). Bacterial systems are often studied in relation to model organisms which have traditional included *Escherichia coli* for understanding systems in Gram-negative bacteria and *Bacillus subtilis* in the case of Gram-positive bacteria (298). Understanding bacterial stress response systems in adverse environments can be readily studied in the model organisms such as *E. coli* (142). The stress response described in the sections to follow will focus on physiological responses to stress in relation to selected stress conditions that microorganisms may encounter in food processing environments. *B. subtilis* will largely be used as a model microorganism in instances where information on stress responses may be limited or lacking for *L. monocytogenes.* The sections will begin with a discussion of general stress regulation then proceed into discussion of responses to environmental stresses of pH (acid and alkali), oxidation, ethanol and starvation. Many stress responses overlap, are complicated in nature and are not understood completely. This overlapping of complicated stress responses can lead to cross protection of cells to multiple or subsequent stressed conditions. These sections include a discussion of

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topics relevant to the physiological responses in microbes and cross protection of bacteria against selected stresses from food processing environments.

Overlying Bacterial Stress Response

The overlying response of Gram-positive bacteria to environmental stress is regulated through alternative sigma subunits or sigma factors. These sigma factors, σ, and a core RNA polymerase are able to identify and bind to a DNA sequence that will enable the bacterium to make physiological alterations to endure an environmental stress (52). Each group of σ factors identifies and binds to specific promoter regions. Similar σ factors are grouped together as they bind to similar promoter sequences, initiate transcription and ultimately produce appropriate proteins. In this case, σ^B is said to be functionally responsible for general stress responses. The response and role of σ^B will be mentioned for it has been noted primarily in stress regulation (117). The presence of the alternate sigma factor, σ^B, has been identified in both *B. subtilis* and *L. monocytogenes*, but the characterization of σ^B has only been made in *B. subtilis* (161). For this reason much of the focus of research has used *B. subtilis* as a model Gram-positive bacterium, but studies on *L. monocytogenes* have examined the cross protection that σ^B might confer.

The regulation of σ^B is quite complex although the σ^B operon might only contain a few genes itself. The σ^B operon contains the four downstream genes of *rsbB, rsbW, sigB and rsbX* (140). Ten genes including these four and six other genes are said to add to the stress response in Gram-positive bacterium. These genes can be divided into two general signaling responses. These include *rsbQ* and *rsbP* which may be responsible for stress related to nutritional needs of the cell and *rsbR, rsbS, rsbT* and *rsbU* which responds to environmental

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stresses (88, 298). Some of these genes are known to contain regulation of σ^B through both negative and positive feedback regulation.

The complexity of this regulation has been seen as well. The σ^B is known to coregulate 176 genes which emphasizes the complexity of the transcription that may be seen throughout stress responses in *L. monocytogenes* (51). Additional, *L. monocytogenes* σ^B regulons have also been studied and associated with the regulation of over 150 genes (116, 253). The role of multiple sigma factors is seen through the co-regulatory analyses performed indicating that perhaps multiple yet under researched sigma factors may have relevance in stress responses. The sigma factor σ^{54} lacking in cells does not allow efficient uptake of carnitine, an osmoprotectant (226) and may play a role in cold response (179). The σ^H seems to be functionally important in the presence of pH changes (240, 258) and σ^C may respond during temperature stresses (342). Studies on characterization of subsequent sigma factors with respect to physiological implications and cross protection are at their infancy and warrant further investigation.

Despite the presence of these other sigma factors, σ^B has received early attention due to the encoding gene *sigB,* which was identified through the homologous *sigB* gene found in *B. subtilis* (225). Additionally, σ^B in *L. monocytogenes* may be an important sigma factor leading to virulence (337). The σ^B remains highly investigated over the years. Some studies that have examined the cross protective response in null σ^B mutants will be presented in subsequent descriptions of the physiological and cross protective response sections.

Adaptation to Acid Stress

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Acidic conditions are prevalent in our environment equally as much as other adverse conditions (145, 184). In the case of low pH there are particular situations in which microorganisms can encounter acidic conditions. The conditions exist in two particular cases: 1) acid adaptation and 2) acid shock. Adaptation conditions are a more gradual shift in pH over time, while shock occurs more rapidly and often for shorter periods of time. One example of adaptation exists during fermentation of food products. This can occur with vegetable products like olives where pH conditions are dropped to near pH 4.0 over extended days or even weeks (223). More commonly researched, acid adaptation is referred to in fermentation of meat products such as fermented sausages (138), or cheeses (45) where bioprocessing reduces pH according to regulatory measures and occurs over several hours (138). These reductions in pH are not always sufficient to eliminate *L. monocytogenes* from contaminated food ingredients (90). Additionally, some fermented meat processes incorporate the addition of molds to the exterior of the casings after fermentation to raise pH values between 6.0 to 6.3 (138).

In reference to the more acute acid exposure during acid shock, research has focused on acidic sanitizers, disinfectant washes on carcasses, and vegetable and meat dips. These environments related to sub-lethal conditions include areas are not properly cleaned, use of less than the recommended amount of sanitizer (134), biofilms being exposed to sanitizers (24, 44), and *Listeria* spp. that are surviving in dust or on floor areas that are in hard to reach/clean areas in a processing environment or food establishment (26). Each of these conditions can represent exposure and potential physiological adaptations to acidic conditions. Significant care must be taken to ensure that acid stress does not induce

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expression of protective genes or innate response in *L.monocytogenes* that procudes a stress-hardened state in the pathogen

L. monocytogenes employs multiple mechanisms to withstand acid conditions. These include general mechanisms to increase the pH within the cell, modification of cellular constituents, and regulation of genes. General mechanisms includes the glutamate decarboxylase (GAD) system which decarboxylates glutamate to form gamma-aminobutyrate (GABA) which consumes a proton from the cytoplasm ultimately increasing the pH (131). The significance of the GAD system in *L. monocytogenes* was illustrated by Cotter et al. (63); mutants that did not contain the GAD system were significantly disadvantaged during subsequent acid challenges (61, 64). Ryan et al. (270) identified a five-gene stress survival islet which encompassed the GAD system function indicating genetic regulation for growth in acidic (pH 5.2) and high salt concentrations (5% NaCl). Additional regulatory systems of pH in the cytoplasm include the arginine deiminase (ADI) system that increases the pH through the conversion of arginine to ornithine and a F_1F_0-ATPase that maintains pH intracellularly through the extrusion of protons (62, 269).

Gene regulation also takes place when acid conditions are present and have noted control by alternative sigma factors within the cell. Sigma B factor has an overriding role in homeostasis in a variety of environmental conditions (225). Multiple survival genes, 29 which facilitate DNA repair, are used during acid stress conditions (318). Some of these genes facilitate the SOS response, *recA*, and are part of the nucleotide excision repair mechanism, *uvrA*, in *L. monocytogenes* (162, 317). Additional mechanisms which find DNA binding proteins used to protect DNA during acid conditions are being uncovered (151). One unique acid triggered protein product by *L. monocytogenes* is Listeriolysin O (LLO) which is

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used to evade host responses. LLO, a pore-forming protein, is expressed by *L. monocytogenes* in the phagosome of the host during acidification (36, 160). This signaling mechanism emphasizes the diverse genes that *L. monocytogenes* contains to ensure survival.

Several membrane modifications have been described in acid stressed *Listeria* spp. *Listeria* cultures which were acid adapted over night to pH 4.7 displayed increased net cell hydrophobicity as well as decreased C_{15}:C_{17} and anteiso:iso fatty acid ratios (210). These modifications through acid adaptation lead to decreased ability for *L. monocytogenes* to adhere and have compounds infiltrate its membrane, and to decrease fluidity in the cytoplasmic membrane. The shorter chain fatty acids and increased protein concentration in the membrane are attributed to some of these cellular modifications (210). Ultimately, such modifications of the cytoplasmic membrane might lead to cellular resistances to subsequent food processing interventions.

Acid stress may induce cross protection to both chemical and physical food processing stresses. Acid shock at various pH conditions (4.0-6.0) were tested for stress hardening in further acid challenges (165). Researchers found that survival in an acid challenge at pH 3.5 was greatest when *L. monocytogenes* was habituated to pH 5.0, 5.5, and 6.0. Lou and Yousef (184) found similar results when cultures were adapted to pH 4.5 and 5.0 and challenged at pH 3.5. Additionally, pH 4.5 and 5.0 allowed *L.monocytogenes* to survive inimical conditions of ethanol (17.5%) and hydrogen peroxide (500 ppm) (184). Adaptation to pH 6.0 or 5.0 resulted in the decreased survival in NaCl concentration of upwards of 20% (309). Acid adaptation with citric acid stress hardened *L. monocytogenes* to trisodium phosphate and acidified sodium chlorite, but not to subsequent citric acid and peroxyacetic acid (7). Moorman et al. (209) used *L. innocua* to demonstrate that cells

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adapted to acid were more resistance to treatment with centrimide, a quaternary ammonium sanitizer.

One study examined challenging a mixture of *L. monocytogenes* strains at pH 3.5 repeatedly over 20 days (255). Findings exhibited that over the days, resistance to acid treatment was being formed in the cultures. The authors stated that this resistance was likely due to the formation of a subpopulation that was resistant rather than resistance mechanisms evolving, as one strain predominated after 20 days of treatment (255).

When examining thermal resistance, Farber et al. (82) and Lou and Yousef (183) both demonstrated that acid shock of *L. monocytogenes* drastically increased the pathogen's resistance to heating both in milk at 58°C and in laboratory menstrua at 56°C, respectively. Although findings were similar, Farber et al. (82) had noted that shocking the cells for 1 hour exhibited equal and superior thermal resistance compared to 2 or 4 h of acid exposure. The research team also demonstrated that heat resistance was neither enhanced nor reduced when gradual acidification was performed simulating conditions of food fermentation or adaptation to acid conditions. The type of acid used in the study had a great effect as *L. monocytogenes* had significant heat resistance when hydrochloric acid was used as an acidulant and not acetic acid (82). Lou and Yousef (183) screened multiple low pH conditions and reported that pH 4.5 yielded the greatest thermal resistance. With respect to resistance to heating in juice (watermelon, pH ~5.3), Sharma et al. (282), had shown acid adapted cells were less resistant to heating at 56°C; whereas no difference in heat resistance of *L. monocytogenes* was seen in cantaloupe juice (pH 6.3). Despite these findings, thermal resistance of acid adapted *L. monocytogenes* was reported in various other juices (orange, white grape, and apple) (194).

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Research in the area of the effect of acid stress on the resistance of *L. monocytogenes* to non-thermal technologies has been reported. After acid shock (3 h, pH 5.0), *L. monocytogenes* populations exhibited a resistance to UV light. In both fresh brine (9% NaCl) and sterile distilled water, acid shocked populations were reduced 5 log CFU/ml while unstressed cells were reduced over 7 log CFU/ml (197). Foley et al. (91) did not find irradiation resistance when using pH 5.5 for acid shocked *L. monocytogenes*. Foley et al. (91) found that in seafood salad, *L. monocytogenes* was actually sensitized to irradiation treatments over time. This can likely be attributed to the acid (pH 5.15) conditions of the seafood salad. Acid shock (pH 4.5) treatments prior to high pressure processing was found to enhance resistance of *L. monocytogenes* (333). These findings did not examine a variety of acid conditions and other pH values may need investigation to find pH of greatest resistance. Stress hardening can be seen with acid stress in *L. monocytogenes* that encounters diverse environmental conditions. The advent of new food and revisited technologies will require further investigation into acid stress responses.

Adaptation to Alkali

The presence of alkali in the food processing conditions may occur in various environments. Alkali cleaning solutions can be used to clean processing equipment. Alkali (NaOH and KOH) based detergents are currently in use for cleaning clean in place (CIP) equipment, containers, milk bulk and pasteurizing tanks, food contact surfaces and floors. The pH values for these cleaners have been reported up to pH 12.6 at solutions of 1% of the concentrate, but recommended application levels range from 0.19 to 10%. Research has

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suggested that survival in cleaning detergents is possible (304). Many of these alkaline detergents offer the advantage of being non-corrosive at lower concentrations and are commonly used in food processing environments where protein and fats may be abundant on equipment. Sodium hydroxide, sodium metasilicate and sodium carbonate have all been used in the formulation of alkali food processing detergents (189). The application of high pH chemical treatments in food processing has been seen as well (27, 29, 217, 280).

High pH has produced some morphological changes in *L. monocytogenes*. In the presence of pH 12, bulging of *L. monocytogenes* was seen; however, no apparent rupturing of the cytoplasmic membrane was noted after examination of the treatment media for cytoplasmic constituents (198). The effect of pH 7.4 to 9.7 on the morphology of *L. monocytogenes* was made. Alkali treatment resulted in cells that were longer, larger ended and had an increased volume. Alkali treatment in a buffered media led to increases in *L. monocytogenes* length with a longer exposure time. These studies also exhibited cells that were in chains and that some of the longer alkali pH exposures contained multiple nucleoids in cells (103). Filamentous growth of *L. monocytogenes* was also seen when cells were exposed to alkali condition greater than 9 (144). Membrane fatty acid composition increased to include more anteiso form of branched-chain fatty acids when *L. monocytogenes* was exposed to pH 9 (104).

The response of *L. monocytogenes* to alkali shock includes the expression of genes that enable the survival under these adverse conditions. An analysis of gene expression in *L. monocytogenes* yielded an immediate upregulation of genes involving ATP-binding transporters and hydrogen ion antiporters. Much of the upregulation involved transporter proteins and proteins related to metabolic function. The metabolic proteins include pyruvate

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dehydrogenase, *gad* and those involved in the degradation of amino acids. A general stress response including chaperone proteins and σ^B expression was also identified as being highly involved in the response (106). Along these lines, a specific calcium ATPase transporter was identified as having a likely role in alkali adaptation and survival in *L. monocytogenes* as well (84). These studies support that *L. monocytogenes* may be able to alter metabolic function to increase acid production, increase transporter proteins and alter the ability for the membrane to withstand exposure to alkali conditions (229). Mutants deficient in σ^B did not exhibit any outward morphological differences than the parent strain when exposed to alkali conditions. This might indicate that alkali phenotypic response is not directly linked to σ^B expression (103). σ^B control in alkali shock response should not be disregarded. It was shown that mRNA that was σ^B related was increased in alkali shocked *L. monocytogenes* (105). A genetic response to the formation of filament-like cells in *E. coli* related to the SulA protein expression which was seen in the suicidal response of cells. SulA may be implicated in a regulatory sequence that ultimately may cause the inhibition of cell division (311). *L. monocytogenes* alkali tolerance was investigated using multiple mutant constructions. Results indicated that alkali conditions caused greater sensitivity in 12 mutants. Although much is unknown about the exact function of the mutants, reduced transporter function or presence were thought to the reason for deceased tolerances to alkali conditions (97). Production of branched chain fatty acids was implicated as a alkali response that may cause a resistance to high pH conditions. This was shown through mutants that lacked the ability to increase branched chain fatty acids rapidly and thus were unable to survive in alkali conditions (288). Alkali response has some foundation in σ^B expression as well as specific stress responses seen phenotypically.

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Along with upregulation of gene expression, the cross protection of detergent or cleaner application and subsequent processing may occur. *L. monocytogenes* previously exposed to alkali treatment resulted in cells that were resistant to thermal treatment compared to the control (303). Thermal tolerance of alkali shocked *L. monocytogenes* was assessed using commercial alkali detergents as the alkali shock treatments. The thermal responses were mixed with both sensitivities and resistances to heat noted after commercial detergent shock treatment. When the alkali shock detergent treatment of *L. monocytogenes* was thought to only contain sodium hydroxide as the alkali agent, a thermal resistance was seen (304). Alkali shock of *L. monocytogenes* resulted in a slight increased sensitivity to subsequent benzalkonium chloride, but did not have an evident impact on the resistance or susceptibility to chlorine or cetylphyridinium chloride (304). Exposure of alkali stressed *L. monocytogenes* to pH 12.0 exhibited great survival compared to both an unstressed control and a mutant which lacked σ^B. Increases survival of alkali stressed *L. monocytogenes* was also seen when the pathogen was exposed to ethanol and osmotic stresses (102). The implications of cross protection may involve increased resistance of pathogens to other food processing technologies and further investigations into the stress-induced response in microorganisms and its impact of microbial resistance to emerging food processing technologies are important.

Adaptation to Oxidative Stress

Oxidative stress can be encountered by *L. monocytogenes* in multiple situations. During the aerobic respiration, bacteria produce toxic compounds which may act as oxidizing

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agents. These oxidizing byproducts of aerobic metabolism include hydrogen peroxide, hydroxyl radicals and superoxide (257, 298). Other sources of the oxidative stress may be encounter by *L. monocytogenes* and other microorganisms. *L. monocytogenes* can be exposed to oxidative stress upon invasion of a human host. The immune response of cells can produce oxidizing molecules in an attempt to eliminate pathogens such can be the case of neutrophilic attacks on *L. monocytogenes* (143). In some countries the additional of H_2O_2 in raw milk and liquid eggs is permitted. The activity of hydrogen peroxide in the raw milk lactoperoxidase system has also been acknowledged (257). The use of hydrogen peroxide and other oxidizing compounds (ozone, hypochlorite, etc.) in sanitizers and detergents have also been reported (189, 292, 298). The impact of oxidative stress may be seen in the destruction of enzymes, proteins, DNA and cellular membranes (292, 298).

Microbial defenses are in place in an attempt to eliminate the effects of reactive oxygen species (ROS) (298). A response to hydrogen peroxide in Gram-positive bacteria is the PerR repressor, which can detect concentration as low as 10 µM hydrogen peroxide (129). PerR, a protein that can bind metals, is responsible for regulation of catalase, a Dps homolog, Fur and alkyl hydroperoxide reductase, which all act in the defense of ROS. Fur has a relevant role is iron uptake in the cell. Another repressor is OhrR in *B. subtilis*. This regulation involves the enzyme organic hydroperoxide reductase (Ohr) and plays a role in eliminating ROOH in cells. OhrR is part of the family of MarR proteins which contains other proteins that are involved with pathogenesis and antibiotic resistance (298). Superoxide dismutase enzyme expression has an additional responsibility for ROS defense and is essential in the survival of *L. monocytogenes* upon the invasion of a host. This gene, *sod*, expression acts to protect against superoxide that is present inside a phagosome (13). In

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E.coli, exposure to bleach induced a heat shock chaperone protein which can protect the cell from further oxidative damage (338). The overlapping of several responses might suggest an interrelationship between oxidative and other cellular stresses (298).

Oxidative stress response has been under the control of PerR, OhrR and σ^B. Some of the responses were mentioned above (127, 298). Hoper et al. (139) had shown that the expression of genes in ethanol expose may be linked to those seen in expose to ROS. This study also showed that that multiple proteins including *dps*, *sodA, spx, ycdF, yceD, yceE, ydaD* and *yqgZ* supported an oxidative response. These genes, when lacking, led to the inability to survive at 4°C (139). The presence of PerR and Fur regulons during oxidative stress indicated a response to damage and/or repair of proteins and DNA (301). *L. monocytogenes* lacking PerR exhibited a deceased survival during hydrogen peroxide exposure (259). The presence of plasmids in *L. monocytogenes* that control expression of superoxide dismutase and catalase production may have an influence on the specific resistance of *L. monocytogenes* to oxidative stress in the environment. Plasmids expressing enzymes that can detoxify oxidizing products may increase the ability for *L. monocytogenes* to survive in harsh conditions (143). The upregulation of 26 genes in *L. monocytogenes* grown in UHT milk did not impact hydrogen peroxide sensitivity compared to cells grown in laboratory media (180). The regulation of genes in oxidative stress response may lead to cross protection to subsequent stresses.

Exposure to oxidative stress may cause cross protection in microorganisms. *L. monocytogenes* exhibited a thermal resistance after exposure to hydrogen peroxide stress at levels up to 500 ppm (183). Oxidative stress (H_2O_2) resulted in stress hardening and increased resistance of the pathogen to lethal amounts of hydrogen peroxide (184). The

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exposure of *L. monocytogenes* to oxidative stress in the form of ozone did not alter the resistance to alkali treatments up to pH 12.0 compared to control cells (222). Thermal resistance of oxidative stressed cells was reported in both *E. coli* and *Salmonella typhimurium* (56, 319).

Adaptation to Ethanol Stress

The use of alcohol as a disinfectant can be found in food processing environments. Alcohol or ethanol based sanitizers are recommended in areas that must be kept dry. A survey examining 117 food industry sites noted that 26% of facilities used alcohol based products. This was the third most commonly used disinfectant reported in the survey (134). Ethyl alcohol is also found in food ingredient extracts as required by the federal regulations. An examples is vanilla extract, which requires that "ethyl alcohol is not less than 35 percent by volume" (85). The use of alcohol wipes to sanitize thermocouples prior to temperature readings has been mentioned in the Food and Drug Administration Food Code (86). The application of ethanol on pizza crusts is also permitted up to 2.0% by weight (66). These situations represent a likelihood that alcohol or ethanol exposure to bacteria may occur in a food processing or production environment. The ineffectiveness of 60 and 70% ethanol in food soil or organic matter to eliminate *L. monocytogenes* may represent an inactivation or lower exposure of alcohol to this microorganisms under real life food production situations (49) . Ethanol shock may occur when concentrations are present at sub-lethal levels resulting in physiological responses in the cell as well as the potential for cross protection.

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Morphological and physiological responses of the microbial cell can occur as a result of its exposure to ethanol. Microorganisms may undergo a shrinking in cell volume. Ethanol may act to aggregate and denature proteins. Modest levels of ethanol may provide production of heat shock proteins that can protect against this protein damage (296). Ethanol exposure in cells caused an appearance of membrane damage and instability as based on scanning electron microscopy (43). A wrinkling and pitting in ethanol shocked cells was also seen by others (55). Ethanol also can impact lipid ordering and increase stability of lipid bilayers (gel phase), effect enzyme function, increase permeability and decrease fluidity of the cells (316, 328). Cell leakage increased when cells were exposed to low levels of ethanol for longer periods of time (55). Morphological changes were accompanied by a cellular increase in catalase and osmoprotectants (296). Additionally, exposure to ethanol enhanced the ability of *L. monocytogenes* to attach to polystyrene, and this enhanced attachment was likely linked to regulation of σ^B and *cesRK* genes (112). Protein production certainly may play a part in ethanol exposure response in microorganisms.

In *Lactobacillus plantarum*, ethanol shock in a strain that was able to overproduce a heat shock protein (*hsp 18.55*), caused a decrease in membrane fluidity. An increase in membrane fluidity was seen with a deletion of this heat shock protein and ethanol shock (43). Production of heat shock proteins may be seen as a general stress response in overlapping alternate sigma factors (51, 89). Ethanol may increase the permeability of the cytoplasmic membrane in *L. monocytogenes* and alter the function of the membrane. Increased membrane permeability caused by ethanol resulted in an increase in sensitivity to stress treatments in combination with low levels of ethanol. Membrane permeability was seen when ethidium bromide was able to penetrate ethanol-treated cells, whereas cells that were

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not exposed the ethanol prevented penetration of this compound by the cell membrane (21). During a state of ethanol shock, the production of heat shock proteins may play a role in stability of the cytoplasmic membrane in *L. monocytogenes.* The stabilization of lipids in membrane and membrane permeability and fluidity has been linked to the heat shock proteins that are also expressed during the ethanol shock response (43, 216). These heat shock proteins have been reported to act intracellularly in protecting protein stability to prevent protein denaturation which is associated with the antimicrobial mechanism of action of ethanol (216). A protein that was indicated as a ribosome-associated chaperone protein protected *L. monocytogenes* during heating and ethanol exposure. This suggests that the antimicrobial mechanism of action of ethanol against bacteria may involve alteration of ribosomal function (30).

Cross protection may have a link to protein production resulting from ethanol shock. Ethanol induced the production of DnaK and GroE, which were also proteins produced in various other environmental stresses (heat, hydrogen peroxide, acid and nutrient depletion) (319, 325). Hoper et al. (139) was able to identify 37 genes that were expressed when *B. subtilis* was exposed to 10% ethanol. This assessment also linked many genes in ethanol shock to those in salt and cold exposure. As these genes were linked to σ^B regulon, the stress response of ethanol shock may lead to cross protection to other stresses. The σ^B alternate sigma factor is also responsible for Dps protein production in response to ethanol and heat shock. Dps proteins bind to DNA during cellular response to certain stresses and probably enhances the stability of DNA to protect this vital macromolecule from oxidative damage. Expression of ethanol and oxidative response genes were also seen through the expression or other σ factors (122). The expression of genes that may be regulated by HrcA include

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multiple chaperone proteins, membrane-bound enzymes (proteases) and heat shock proteins. Expression of these similar genes were seen after treatment of salt and heat as well as ethanol in other microorganisms (316). The relationship of multiple proteins whose production overlap in response to ethanol shock indicate that exposure of cells to ethanol may induce cross-protection of cells against other stresses.

Cross protection of ethanol shocked cells against certain stresses certainly results from the expression of the same genes for multiple stresses. Thermotolerance of ethanol shocked *L. monocytogenes* was shown to be greater than non-shocked cells with 4-8% ethanol increasing resistance the most (183). Ethanol shock resistance to subsequent ethanol treatment was also seen in other microorgansisms (55). Resistance to heating was also observed in ethanol shocked *L. plantarum* (43, 316). A similar heat resistance was seen in *Vibrio parahaemolyticus* with ethanol shocked cells, but freezing of the ethanol shocked cells actually decreased viability more than the control (55). Ethanol shock from exposure to 5% ethanol provided greater protection to *L. monocytogenes* against subsequent treatment of acidic conditions at pH 3.5, ethanol treatment of 17.5% and oxidative stress of 0.1% hydrogen peroxide (184). The impact of ethanol shock in *L. monocytogenes* has relevance to the food processing industry and has significance in cross protection as well.

Adaptation to Starvation

Natural environments of microorganisms present several hurdles to microbial survival and growth including the fluctuating availability of nutrients. As one could imagine, nutrient deprivation or starvation for microorganisms is likely to be more common than most other stresses. The length of starvation may extend sometimes as long as weeks in the natural

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environment (171, 244). Bacterial nutrient limitation may occur in washed food processing environments including any food contact surfaces or areas of water accumulation (128, 224). Bacteria suspended in water may be a good representation of complete starvation (244). Starvation stress can induce a general stress response system and is linked to biochemical pathways in the stringent response system. The stringent response system inhibits RNA synthesis for production of proteins associated with multiplication of the cell but allows the cell to divert resources to production of proteins to enhance cell survival. These responses may be similar to those seen during stationary phase or osmotic stress. The response to starvation remains quite intricate and involves multiple changes within the cell. These changes have been studied in both *B. subtilis* and *E. coli* (244, 298).

Morphological and physiological changes occur during starvation of cells. Bacterial cells become smaller and more rounded during starvation (326). Similarly, during long term survival *L. monocytogenes* cells appear small and rounded as well (335). This rounding and shrinking may increase the ability of the cell to absorb nutrients (171). Wen et al. (335) observed condensation of the cytoplasm in *L. monocytogenes* that may be linked to a decrease of water activity in cells that were in long term survival. Herbert and Foster (128) observed morphological changes to specific nutrient deprived *L. monocytogenes* cultures. Shortening and widening of the cells along with a decrease in partial septa present in the cells was observed after starvation. A decrease in viability between 1 and 3 Log CFU/ml was also observed during starvation. Cell wall synthesis was observed at the beginning of starvation, but not later in the starvation phase. This led to the concept that shortening of the cells may be due to reductive division (128). Studies involving *L. innocua* demonstrated that a short 24 h period of starvation was able decrease membrane fluidity. Net hydrophobicity was

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shown to be increased in starved *L. innocua* by means of *n*-hexadecane separation of the non-aqueous phase and absorbance measurements (210). Numerous physiological changes in the cell may be attributed to the increase in protein production.

The change to starvation state may induce a production of genes and proteins within the cell. In the study of *B. subtilis,* the protein production from vegetative growth to starvation conditions differed by 68 distinct proteins. It was noted that over 100 genes can be induced during starvation conditions and this limited number of proteins identified may due to the methods chosen or to the presence of overlapping genes expression (301). Specific nutrient regulation by σ^L, BkdR, TnrA and TRAP can be seen during nitrogen starvation (301). Glucose starvation was seen to be controlled by such factors as CcpA, CcpN and AroR (301) and phosphate starvation may illicit a response of PhoPR production (301). General nutrient deprivation regulons include CodY, σ^B and σ^H. CodY and σ^H expression may have more of an impact on transition into stationary phase including cell wall metabolism, enzyme production for intracellular and extracellular degradation and protein production for chemotaxis and nutrient transport (207). Although as illustrated above multiple specific stress responses can be seen during starvation, σ^B may encode for the general stress response protecting the cell from future harsh conditions that may arise (94, 122).

General stress alternate sigma factors are seen in both Gram-positive and Gram-negative microorganisms and represented as σ^B and σ^S, respectively. These sigma factors encode for *katE, dps* and *opuE* genes which are responsible for catalase, protective DNA binding proteins, and proline transport, respectively, and may aide in starvation survival (244). Starvation in *L. monocytogenes* protects the pathogen against subsequent acid

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exposure, where σ^B mutants had shown some relationship between this alternate sigma factor and acid resistance (128). In *E. coli*, σ^S associated genes may be responsible for physiological changes seen during starvation such as shortening of cells (5, 167). Other resistances in *L. monocytogenes* following starvation treatments have been reported. For example heat resistance of starved *L. monocytogenes* and *E.coli* has been demonstrated (150, 183). Regarding fermented products, resistance to nisin and diacetyl combinations increased in *L. monocytogenes* after 14 days of starvation (224). Mendonca et al. (199) demonstrated that starvation cross protected *L. monocytogenes* against electron beam irradiation in both saline and ground pork. During prolonged suspension in laboratory media, termed long term survival, *L. monocytogenes* exhibited greater resistance to both thermal processing and high pressure processing (335). As new and emerging technologies arise in the food industry, cross protection of starved cells should be tested to ensure safety of food products.

Freeze-Thaw in Foods

Introduction and Historical Use of Freezing

The effort to preserve foods by freezing dates back to 1000 BC in China. In France, the first flavored ice deserts were served in 1534 and later development of documents outlined the preparation of ice treats in 1700 and 1768. Artificial ice was first produced in 1755 and in the 1870s and 1880s fish was one of the first products that were commercially frozen. By 1881, the first international shipment of frozen meat took place between Australian and South American regions to Europe. Early frozen foods consisted of meats, fish and butter until 1928 when Clarence Birdseye developed a contact freezer that allowed

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for commercial production of his Birdseye frozen line of foods just one later. The use of previous observations and experimentation with quick freezing and blanching of products allowed for frozen food preparations to maintain high quality and become widely available by 1937 (14, 186, 238). Since frozen foods are often blanched or precooked before freezing, the use of high hygienic care must be taken to not contaminate these products prior to packaging.

Impact of Freeze-Thaw on Microorganisms

The impact of freezing on bacterial cells is multifold and has a general purpose in the food industry of suppressing bacterial growth. Although bacteria reside in a dormant state while being frozen, freezing and thawing of products has a lethal impact on bacteria. The first impact thought to occur is the formation of ice crystals that can disrupt the membrane of cells when formed both internally in the cytoplasm and externally in the surrounding environment. The lysis of cells from the rupturing and disruption of the membrane by physical formation of ice crystals is thought to occur during thawing (14, 96).

Ice crystal formation extracellular may not indicate immediate freezing intracellularly. First the frozen state of cell is not seen usually until below -5°C. Then a supercooling effect occurs internally which creates a pressure gradient with a higher pressure inside the cell compared to outside. The unfrozen water within the cell can either freeze in place or can move across the pressure gradient and freeze externally. When water freezes inside the cell, the pressure gradient is diffused when the temperature drops because a decrease in temperature leads to a decrease in pressure (73).

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When freezing rates are considered, slow rates allow great movement of water across the cellular membrane out of the cell. A slower freezing rate is defined when temperatures take longer than 30 minutes to transition from 0°C and -4°C. At this slower rate of freezing ice crystals are able to grow to a larger size in the medium surrounding microorganisms. As the water is bound in the form of large ice crystals, high concentrations of solute accumulate extracellularly, which then can lead to water exiting the cell to create an osmotic balance. Within a cell, water content has decreased which dehydrates the cell and an accumulation solute (73).

When examining fast freezing rate, intracellular ice crystals can be seen. The formation of intercellular ice crystals is dependent upon nucleation agents. Nucleation agents are water insoluble and are sites where the formation of ice crystals can occur with ease. The cellular membrane limits the nucleation of water that can occur between the inside and outside cell environments. At temperatures near -15°C, nucleation can occur readily. This formation of intracellular ice crystal can cause great damage to the cells (73). The overall nucleation of water limits the viability of thawed cells.

The injury and lethality of freezing occur primarily from the concentration of solutes and chemical compounds due to the removal of water during nucleation of diffusion as well as through physical damage to cellular membranes caused by ice crystal formation. Cellular damage from freeze-thaw exposure may also involve the formation of reactive oxidative species which are formed during freezing and thawing. The research in this area is still not well understood (14, 98, 130, 232, 297).

Thawing may also have an adverse effect on cellular viability. When thawing, ice crystals increase in size which may lead to greater damage in cells that were rapidly frozen

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and contain ice crystals internally. The growth of ice crystals during slower rates of thawing in general may lead to more lethal effects. The thawing of the mixture with a lower freezing point (concentrated solutes and environmental materials) may create an osmotic stress on microorganisms that are located in lower freezing point portions of the mixtures. This osmotic stress is short lived as thawing continues (73).

Freeze-thaw cycles may exhibit stresses of cold shock with decreases in temperatures and of osmotic shock with the concentration of solutes and solution observed when water becomes bound in the form of ice. There are several observed cellular modifications that are generally considered as a cold shock response. With temperature downshifts, the production of cold shock proteins (CSPs) becomes evident. Bayles et al. (22) and Phan-Thanh and Gormon (243) demonstrated that upwards of 38 CSPs are produced during cold shock. These proteins can vary in their role as RNA chaperones or protective proteins, transporters, signal promoters and translation enhancers (28, 137, 141). With decreases in temperatures *Escherichia coli* and yeast produced trehalose, which serves as both a cryoprotectant and osmoprotectant (231, 298). Modifications to increase membrane fluidity include reducing the higher melting point iso-branched fatty acids with anteiso fatty acids and decreasing fatty acid length (10). Many reports including those previously mentioned in this writing suggest that a cold shock responses might be linked to cryotolerance (23, 46, 178, 179, 306, 332). One author found that regulation might actually be a component of thermoregulation in *L. monocytogenes* due to a decrease in cryotolerance in cultures grown at both 4°C and 25°C, a non-cold-shocked temperature (17). As research continues in the area of cold shock response it is evident that mechanisms of survival are not clear. Microorganisms may undergo many adaptations during either repeated freeze-thaw cycles or cold shock.

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Osmotic Shock is termed an effect of the concentration of solutions when water is removed in the frozen state, which is then termed the "solution effect" (96). The solution effect is the impact on the suspending solution has on the cells when it is concentrated. Although the impact may include toxins or antimicrobials being concentrated in the system, here we will briefly discuss it in terms of solute concentration and osmotic shock to the cell. During the presence of high salt concentrations, *L. monocytogenes* exhibits an increased expression of *sigL* genes. This sigma factor regulates over 77 genes including those that increase tolerance to osmotic stress. The induction of these genes resulted in increased mRNA transcript as well (254). Cold shock proteins (CSP) are also produced by osmotic shock. These proteins have been stated as chaperone proteins that protect DNA and RNA during averse conditions. The expression of some CSP during osmotic conditions is seen to increase as much as 4.4-fold (80, 279). There is an apparent shrinking or decrease in cell volume during osmotic responses of cells as well (193). The exposure to osmotic shock has been seen to alter upward of 59 protein expression rates in *L. monocytogenes*. Some of these proteins have been implicated as transport proteins. These transport proteins such as BetL, Gbu and OpuC transport compounds into the cell to maintain some intracellular volume as well as lessen the effects of solute concentrations. These transporters can transport compounds such as glycine betaine and carnitine that may even help stabilize proteins intracellularly (71, 334). The impact of osmotic stress is widespread within the cell acting in some areas that could ultimately protect the cell from damage.

During cold shock and osmotic shock microorganisms endure general environmental stress which may make survival and replication difficult. The modifications mentioned previously allow microorganisms to endure stresses imposed by cold and osmotic shock.

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During the period of decreased temperature bacteria undergo a reduction in membrane fluidity (viscosity), DNA modifications that have been implicated at reducing transcription, RNA modifications and increase strain on enzyme reaction rate (47, 298). Cellular sites that are modified by cold and osmotic shock may be seen as targets for stresses that the cell undergoes.

Overall, general impacts on structural components of the cell have been noted in relation to survival of the cell. One impact is the potential denaturation of proteins Reasons for protein denaturation hypothesis include interacts of proteins upon dehydration of the cells that are altered once the cell is rehydrated damaging protein folding and functionality. The proximity of proteins in a shrunken cell may increase proteins interactions that were once unlikely to interact (73). Other scientists state that protein structure can be altered due to the concentration of solutes during the freezing process. This may ultimately lead to protein function decline and reduced water holding capacity seen in whole muscle foods (6, 73, 191, 266). Other reactions during freezing have been reported in animal muscles including cross linking of proteins and hydrolysis and oxidation of lipids (266). Other research suggests that weak covalent bond of lipoproteins may be impacted immensely by the concentration in cell constituents and changes while freezing concentrates constituents extracellularly. Damage to the membrane of *L. monocytogenes* has also been observed through transmission electron microscopy. Disruption of the cell wall in frozen and freeze-thaw treated cells has been shown to increase the sensitivity of cells to additional stresses as well as cause the release cell constituents to the environment. During the loss of water from cells, the cell wall, and plasma membrane, may physical interact and this interaction has been implicated in reducing the survival of cells as well (73).

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The impact of freezing and thawing on microbial cells due to the concentrated surrounding environments as well as the formation of ice crystals may be sub-lethal, or cause repairable injury, as well as lethality. The material in this review is not intended to be an extensive, detailed description of microbial response to cold shock or osmotic stress that may be associated with freeze-thaw applications. Instead the discussion that follows will merely examine the impact of freeze-thaw on *L. monocytogenes* as it relates to food processing.

Impact of Freeze-Thaw Conditions on *L. monocytogenes*

L. monocytogenes has been of great concern to food industries that routinely use freezing to preserve foods and thawing to prepare the foods for consumption. Freeze-thaw treatment has been shown to have lethal effects and in some instances, injury in *L. monocytogenes*. In this regard the effects of freezing on *L. monocytogenes* warrants discussion.

The impact of freezing at -18°C (slow freezing) and -198°C (fast freezing) and frozen storage on fate of *L. monocytogenes* has been investigated. Slow freezing and storage for 1 month at the same temperature in phosphate buffer and tryptose broth resulted in *L. monocytogenes* population death of 87% and 54%, respectively. These same treatments resulted in injury of 79% (phosphate buffer) and 45% (tryptose broth) in the surviving population. Fast freezing and storage for 1 month resulted in very little death or injury of *L. monocytogenes*. Fast freezing and storage at -18°C resulted in great lethality and injury compared to fast freezing and storage at -198°C. Cycles of freezing and thawing resulted in decrease survival and greater injury in *L. monocytogenes* (77). That same study demonstrated that the effect of ice crystal formation from rapid or slow freezing may have a

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great impact on the survival of *L. monocytogenes*. The importance of maintaining and minimizing temperature fluctuations in the cold supply chain can have an effect on *L. monocytogenes* survival as well.

A later study examined the impact of glycerol, lactose, fat, casein and salts on the survival and injury of *L. monocytogenes*. After 24 hours, approximately 50% of *L. monocytogenes* were dead in phosphate buffered solution. After 6 months approximately 90% of the initial population were considered dead. These results examined freezing slowly at -18°C. In the presence of glycerol over 6 months, *L. monocytogenes* were protected against freezing, but immediate protection (within 30 min) was not seen. Although protection (viability and injury) during 2 weeks and 6 months of frozen storage was observed with glycerol, casein, lactose and milk fat, glycerol provided the greatest protection over 6 months of storage and the least protection after 2 weeks of storage. Injury seen in *L. monocytogenes* frozen in phosphate buffer solution was approximately 65% after 24 hours of storage and 85% after 6 months of storage. Salts had little impact on protecting *L. monocytogenes* from death or injury (77). The protective effect of food components on *L. monocytogenes* has been demonstrated in both reducing lethality and injury in freeze storage. The reduction in impact on *L. monocytogenes* by individual components might suggest that a complex food matrix might greatly improve survival.

El-Kest (76) reported that strain and menstrua contributed to variation in survival and injury of *L. monocytogenes* following freeze-thaw cycles. In that study, samples were frozen at -18°C and thawed at 35°C. After 1 hour of freezing death in phosphate buffer resulted in lethality to 51-66% and injury to 6-51% of the population. After 48 hours, 58-93% of *L. monocytogenes* had died and 32-48% were injured. Another study by the same authors used

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L. monocytogenes Scott A and reported death after 1 hour of freezing of 27% and injury at 34% of the surviving population. After 48 hours the fate of this pathogen was 55% dead and 50% injury (77). In both of these studies, death and injury of *L. monocytogenes* in tryptose broth and milk were decreased as compared to phosphate buffer suspensions. Strain variation in lethality and injury of the pathogen in frozen and stored milk was evident and one strain exhibited great resistance in a laboratory medium compared to the food system (milk) (75, 76). Strain variation might exist in resistance to injury and death following freeze-thaw treatment. The study demonstrates that more complex matrices such as food might lessen the lethality and injury seen in *L. monocytogenes*.

The impact of growth phase on freeze-thaw susceptibility has been reported (17). Azizoglu et al. (17) reported that the viability of 2 strains of *L. monocytogenes* was higher in late logarithmic and stationary phase grown cells compared to mid-logarithmic cultures. This resulted in decreases in over 2 log CFU/ml over 18 freeze-thaw cycles and ca. 0.5 log CFU/ml reductions after 6 freeze-thaw cycles in mid-logarithmic phase cells. Stationary phase and late logarithmic cells experienced approximate population decreases of <0.5 log CFU/ml and between 0.5 and 1 log CFU/ml after 6 and 18 freeze-thaw cycles, respectively.

In that study (17), no evidence of increased injury in the frozen and thawed populations were observed. Comparison of plate counts on both TSAYE and modified Oxford selective medium was used in estimating injury in surviving populations of *L. monocytogenes.* The freezing treatment consisted of 1.5 ml (TSBYE) at -20°C at a rate of 0.039 ml/min. The thawing took place in a room temperature water bath for 10 min.

Freeze-thaw studies in other food systems produced variations in the viability of *L. monocytogenes*. One study examined the viability of 2 strains of *L. monocytogenes* held at -

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18°C for 7 weeks. Decreases in viability were not detected until the later 3 weeks of storage. After 7 weeks, approximately 2 log decrease in numbers of survivors occurred in both strains. The impact of freeze-thaw on viability of *L. monocytogenes* in ground beef prompted research on microbial protection offered by intrinsic food constituents (124). The reduction of *L. monocytogenes* on hot dogs stored at -18°C and -20°C was insignificant and was not dependent upon hot dog formulation (248, 302). Various thawing methods in hot dogs stored at -15°C over 50 days had no impact on survival of the pathogen. Although, it was noted that pretreatment of the inoculated hot dogs at 4°C for 30 days led to an appreciable decrease in viability (<1 log reduction) (285). Various other foods also caused the pathogen to exhibit increased resistance to freeze-thaw treatment (73). This concept of food constituent cryoprotection was demonstrated in studies using casein, glycerol, and lactose previously mentioned. The major ingredient composition, fat content, water content, etc., might supersede any minor formulation changes in regard to protective effects seen in freeze-thaw tolerance of microorganisms. This has not been directly addressed and may be of research importance in food manufacturing where minor formulation changes are made frequently.

Additional research examined the effect of freezing on *L. monocytogenes*. The results of one study in ground beef indicated that no reductions in viability occurred in cultures frozen at -7, -14, and -18°C (221). Ritz et al. (261) also tested citrate and phosphate buffers at pH of 4.5 and 7.0, respectively, and noticed that there was no difference in viability in *L. monocytogenes* following one freeze-thaw treatment across the same freezing temperatures (261). A close look at the growth phase of *L. monocytogenes* during these studies indicated that stationary phase cultures were used. Examination of the effects of freeze-thaw at these

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treatments was not performed in exponential phase which might have yielded different results in the extent of survival of the pathogen.

The results of freeze-thaw cycles in 10% TSBYE were also examined. One freeze-thaw cycle at -20°C was able to reduce *L. monocytogenes* populations by 1.42 log in that menstruum. Faster freezing rates of -196°C in liquid nitrogen resulted in no change in viability even after two cycles at this temperature. At -20°C freezing in the presence of polysorbate 80, three freeze-thaw cycles resulted in 0.69 log reductions and six freeze-thaw cycles resulted in 1.4 log reduction of *L. monocytogenes* (65).

Other studies have examined the ability to see freeze injury and survival of *L. monocytogenes.* The use a freeze-thaw treatment on *L. monocytogenes* resulted in less than a 0.6 log reduction on nonselective media (48). This study examined the use of various plating methods to enhance the recovery of *L. monocytogenes* that were injured by freeze-thaw cycles. The comparison of MOX and Tyrptic Soy Agar (TSA) displayed differences in enumeration indicating injury from freezing at -15°C. Recovery on a thin agar layer, which allowed gradual diffusion of selective agents, demonstrated increased recovery of injury colonies that were not statistically different from counts seen on TSA. Freezing performed on pork surfaces demonstrated an increased rate of survival of *L. monocytogenes* further supporting potential protective effects of food matrices (48).

A study by Golden et al. (108) found that two weeks of storage at -18°C resulted in little decrease in viability, but significant injury was observed. These results of viability, slight decreases, and injury between 72 and 82% were seen across various strains of *L. monocytogenes*. These measures were performed with selective media that contained 8% sodium chloride as the selective agent (108). Injury of freeze-thaw treated *L. monocytogenes*

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was also seen when using selective enrichment broth compared to nonselective broth after seven days of -20°C storage (39).

Other microorganisms have exhibited resistance to freeze-thaw and related injury. Fungi may become dehydrated and produce cryoprotective compounds in the cells to increase the organism's freeze-thaw resistance (170). The use of glycerol and trehalose has proven effective in cryoprotection of *L. monocytogenes* and trehalose may also protect the pathogen under osmotic, heating and desiccation stresses (75, 78). *L. monocytogenes* has been shown to produce trehalose, a carbohydrate which may enhance survival during adverse environmental conditions including freeze-thaw cycles (78). Although extracellular production of cryoprotective compounds may not be present in adequate amounts or at particular growth conditions to elicit cryoprotection, the addition of these compounds to the environment may result in sufficient protection (17, 75). Similar protection from trehalose and glycerol have been demonstrated in *Escherichia coli* and *Lactobacillus acidophilus* (72, 294).

The freeze treatment of *L. monocytogenes* can strongly vary the impact on survival and injury. The food matrix that is frozen can effect *L. monocytogenes* as well. When determining the public health impact, food matrix must be considered. The next section highlights studies where cross protection of *L. monocytogenes* to freeze-thaw cycles has been seen.

Cross Protection with Freezing-Thawing in *L. monocytogenes*

Cross protection of environmentally stressed *L. monocytogenes* against freeze-thaw cycles has not been thoroughly investigated. In fact, to my knowledge only few efforts have

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been made to examine the resistance of stressed *L. monocytogenes* to single and multiple freezing and thawing cycles. In one investigation the impact of growth temperatures on the resistance of *L. monocytogenes* to freeze-thaw cycles was explored. Freezing and thawing after 18 cycles resulted in 0.83 log CFU/ml reduction in viable *L. monocytogenes* cells grown at 37˚C, 4.40 log CFU/ml reductions in cells grown at 25˚C and 4.39 log CFU/ml reduction for cells grown at 4˚C. Although a similar trend was observed even in non-pathogenic *L. innocua* and *L. welshimeri*, some strain differences in freeze-thaw tolerances were noted as being significant (17). The resistance to freeze-thaw treatments seen in cultures grown at 37˚C could not be transferred to cultures grown at 4˚C when supernatant of the cultures grown at 37˚C was applied as the freezing menstrua of cells grown at 4˚C (17). This might suggest that resistance is not due to extracellular production of compounds. Another study (333) examined freeze-thaw tolerance of cold shocked (10°C for 4 h) *L. monocytogenes* under similar freeze-thaw treatments and reported increased resistance of the pathogen to 5 cycles of freezing and thawing. The study also indicated that tolerances may be partially attributed to sigma B regulation. These studies demonstrate that *L. monocytogenes* grown at lower than optimal temperatures exhibits a decreased cryotolerance while cold shock treatment may result in resistance to freezing. This difference may also be due to variations in strains used in the studies.

Azizoglu et al. (17) also examined *L. monocytogenes* grown on agar and in broth to determine if colony formation on surfaces impacted freeze-thaw tolerances. Cultures of *L. monocytogenes* grown on either agar surfaces or liquid broth had shown resistance to freeze-thaw cycles when grown at 4°C compared to the control grown at 37°C. When comparing *L. monocytogenes* grown on agar and in liquid broth at 25°C, there was a greater resistance to

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freeze-thaw cycles of the cultures grown on agar compared to cultures grown on liquid (17). These results may lead to studies on the formation of colonies on food surfaces placed in temperature abused settings. Further studies need to examine formation of colonies on surfaces relevant to environmental *L. monocytogenes* colonization.

Other studies demonstrated growth temperature dependence related to freeze-thaw tolerances. In the case of *Exiguobacterium* spp., a permafrost isolated bacterium, growth at 4°C induced a protective effect against subsequent freeze-thaw treatment of the organism. Also, agar growth at 24°C allowed for a resistance to freeze-thaw cycles that mirrored those seen in cultures grown at 4°C in broth (321). Studies in *Yersinia enterocolitica* reported similar results as observed in *Exiguobacterium* spp. Freeze-thaw tolerance was observed at both 4°C and 25°C when *Y. enterocolitica* was grown on agar compared to liquid at 37°C (16). A 4°C cold stress for 4 weeks was applied to *E. coli* which then displayed a resistance to 4 freeze-thaw cycle (-20°C for 24 h and 21°C for 30 min) (74). Resistance to freeze-thaw cycles were also observed in *Saccharomyces cerevisiae* following cold shock (231). The various responses to freeze-thaw cycle dependent upon prior temperature treatment do not lead to clear conclusions across microbial species.

The freeze-thaw response of acid shocked *L. monocytogenes* has been reported (333). This study examined the response of freeze-thaw cycle with prior acid shock at pH 4.5 only. The study did not indicate pH ranges tested or why pH 4.5 alone was determined for the shock application. Freeze-thaw cycles were considered -20°C for 24 h followed by 30°C for 2 min and repeated for 5 cycles. The acid shocked cells did demonstrate a substantial resistance to freeze-thaw cycles. The difference in survival was over 10 fold comparing acid shocked to untreated *L. monocytogenes* (333).

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An additional study mentioned the use of alkali adapted *L. monocytogenes* and determined the freeze-thaw tolerance over time. That study used -20°C for freezing along 12 days and 37°C for 5 min as the one freeze-thaw cycle tested. The study examined three alkali shocks and adaptations using pH 11.6, 10.0 and 10.4 and no significant lethality was observed after 12 days of frozen storage (302). The experimental treatments varied greatly from those presented in research described in the present thesis. The main question that the present research attempted to answer was as follows: Does prior exposure of *L. monocytogenes* to alkali shock alter the pathogens resistance to freeze-thaw cycles? In the study by Taormina and Beuchat (302) some of the alkali treatments were composed of multiple components (actual meat sanitizers) which may impose additional stress (due to the chemical nature of the sanitizer) in addition to alkali shock. That study also did not examine freeze-thaw cycles, but tolerance of *L. monocytogenes* to frozen storage instead. Additional differences represented in the study include their use of stationary phase cultures which were all exposed to cold (4°C) temperature in addition to alkali treatment. The research question being proposed in the follow work addresses the freeze-thaw response of environmentally stressed *L. monocytogenes*, which includes the impact of various levels of alkali shock.

The previously described investigations on the resistance of prior stressed *L. monocytogenes* to freeze-thaw treatments are a start to the research needed in the area of prior environmental stress and associated cryoprotection of *L. monocytogenes*. The acid shock investigation did not examine pH ranges that might induce varied response of the organism to freeze-thaw treatments. Considering the various levels of a particular stressor that a pathogen might experience in the food processing environment, it is important to investigate the organism's tolerance to freeze-thaw cycles after it was exposed to various

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levels of a particular stress. With the low number of studies investigating cryoprotection of environmentally stressed *L. monocytogenes* the present research aims to increase scientific knowledge in this area.

Use of Freeze-Thaw in the Food Industry and by the Consumer

Freezing of meats is commonly employed in the meat processing industry.. Initial freezing of whole or half meat carcass can be observed to increase the preservation during handling, loading and transportation between companies or overseas (267). Lawrie (168) acknowledged that in the production of lamb, carcasses may be broken down into small portions such as the shoulder and frozen. Given the use of smaller portions and consumer trends in individual portions, producers then thaw (45°C for up to 2.5 hours) these whole shoulders for subsequent processing. Once these larger portions of meat are cut into individual portions of meat, they are sometimes sold to consumers in a frozen state to increase shelf-life and salability to consumers (168). This is an example of how multiple freeze-thaw cycles may occur in the meat industry.

Once consumers purchase these portions, temperature abuse and improper defrosting/thawing methods have been reported in the literature. *L. monocytogenes* has the ability to replicate if present in thawed products that are held at refrigeration temperatures and above. Consumer transportation of food products from the supermarket may represent significant area for temperature abuse and thawing and refreezing in the home. Data have shown that transportation with ice cream from the grocery to the home can take 0.5 hours and have a mean air temperature of 21.0°C (107). Thawing practices in the home have also been reported. Of those surveyed on thawing practices of hamburger patties, 16% stated that they

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thawed raw hamburger patties on the counter and 56% thawed them in the refrigerator (242). In Jamaica, a study found that 70.9% of those surveyed consumers admitted using room temperatures to thaw frozen foods (163). Further evidence was compiled when a meta-analysis of studies found that 42.3% used improper thawing methods (234).

Additional freeze-thaw situations can be seen due to the purchasing habits of hams in the food processing industry. It is a common understanding in the meat industry that both hams and other meat protein (fish) have an increased seasonal demand and production need. In the case of hams, this seasonality drives producers to purchase large amounts during low demand and low prices. Hams are then stored in large freezer warehouses to ensure meat availability at a stable and sustainable price when the consumer demand increases (20, 110, 252). When seasonal needs peak and production needs increase, large scale thawing of these freezers occurs to enable the distribution or use of these hams in processing. During the freezing process, temperature variation may occur within the freezers as well. Examining traditional laboratory freezers, manufacturers state that auto-defrosting freezers allow a temperature change as low at 3˚C and as high as 15˚C (164). A frozen ham may experience a variety of temperature that may encompass freeze-thaw conditions depending on the location of the ham within the warehouse, type of cooling system employed, and the size of the warehouse freezer. When explaining the quality attributes scientist discussed that slight thawing can be seen with minimal temperature fluctuations. This thawing leads to recrystallization of ice or larger ice crystals that are implicated in loss of quality. This similar phenomenon is seen with freezer facilities that use air-cooled devices compared to pipe-cooled devices. Additionally, in the distribution of frozen hams Kaale et al. (157) reported that temperature management during transportation and storage of chilled food

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products can be difficult. All of these factors can lead to multiple freeze-thaw cycles within a ham product.

Another way in which *L. monocytogenes* may encounter freeze and thaw conditions is in the environment. When microorganisms are washed or unknowingly recylced within the environment they may be exposed to stresses (elevated growth temperature from infected animals, run-off from sanitized animal facilities, etc.) (17). Organisms present in cooler climates that experience early freezes or continual freezes during the seasons may be impacted by freeze tolerance in pathogen populations present in the soils. Since *L. monocytogenes* is ubiquitous in the environment it inevitably will be exposed to a variety of environmental stresses and may contaminate foods as a stress-hardened pathogen. This in turn can lead to increased resistance of the pathogen to freeze-thaw cycles applied to food products.

The citrus processing industry also is compelled to acknowledge freeze-thaw impact on microbial survival when harvesting freeze damaged crops. Economic devastation due to frosts has been noted in both 1963 and the 1980s and fueled research into uses of frozen and thawed raw citrus fruit pulp. This fruit is still used in juice production to retain some value from a freeze damaged crop (185). Although, in most instances juice products are pasteurized, the introduction of *L. monocytogenes* to a plant can occur when harvesting trucks drop off freeze damage crops for processing. In instances where unpasteurized juices are sold, niche market production, introduction of *L. monocytogenes* resistance to the lethality of acid may be a serious concern (239). Environmental freeze-thaw cycles may directly impact the food system. Microbial soil populations can change with freeze-thaw cycles associated with environments (299). A prolonged survival due to increases freeze-

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thaw resistance or susceptibility and presence in soils surrounding agricultural processing settings may lead to alter the life-cycle of *L. monocytogenes* as an environmental contaminant. This in turn may be linked to an increased risk of *L. monocytogenes* in produce or livestock harvest from area with cold climates that experience periodic or prolonged freezing.

High Hydrostatic Pressure

Introduction and History

The food industry is highly focused on the needs and demands of the consumer market. As of recent, consumer desires have moved towards decreased use of preservatives and lower levels of processing in foods. Traditional foods, fermented meats and dairy products, salted fish, cooked vegetables and meats, etc., have had a place in history. These traditional foods are perceived to be purer by consumers and often contain fewer food additives. The category for some of the foods which involve reduced processing is minimally processed foods. These minimally processed foods must ensure food safety without the use of additional additives or alterations to quality. In this area the use of high hydrostatic pressure has emerged as a novel processing technique. High hydrostatic pressure (HHP) is also termed high pressure processing (HPP) and ultrahigh hydrostatic pressure processing (UHP) in some case (257) and will be referenced interchangeably through the paper as HPP.

The use of HPP is novel in its application, but has been in use since the 19th century. Hite (132) had applied HPP principles in an attempt to increase the stability of milk, meat and juices. Initial studies, focused on milk preservation, used self developed pressure units

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and achieved an extended microbial shelf-life for this product for up to 5 days at room temperature. These studies noted that spoilage was not consistent with unpressurized sample, suggesting different microorganisms might be at fault for spoilage of pressurized samples. Early studies also examined the effect of heat and pressure which elongated the shelf-life of milk (132). Although some research progress was made up until the 1980s, the Japanese made great efforts to use HPP in an attempt to find alternatives to radiation preservation, which was unfavorable to consumers. In 1993, the use of HPP in Japan allowed the marketing of the first commercial products. High acid jams and juices and low protein foods occupied much of the early investigations in the application of HPP, which was taken from other disciplines of research (Physics, Geology and Chemistry) and adapted to the field of food science (257, 263). As high pressure in the food industry is still in its infancy there is much research that remains to be pursued. The review below will discuss some of the research in the effects of HPP on biological systems and *L. monocytogenes* as it pertains to the food industry.

Industrial Principles and Application of High Pressure Processing

The use of high pressure processing offers multiple advantages in processing over other technologies. The use of high hydrostatic pressure offers a great amount of control of processing parameters, temperature, pressure, and time, over other commonly used technologies such heating where product quality and lethality are affected only by temperature and time of exposure. The cost of HPP is said to be comparable to irradiation and less expensive than drying, smoking, salting and fermentation. Although heating may have a lesser cost in processing, the processing impact and intensity of processing is much

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less in HPP (125). Along those same lines, there is noted decreased impact on nutrient and quality content of products. The stability of vitamins and minerals is an extremely appealing attribute of HPP (53, 125). The stability of nutrients in HHP foods may be due to the impact on covalent bonds. Pressures less than 2,000 MPa have little impact on covalent bonds seen in the primary structure of molecules such as vitamins, minerals, and peptides. In the case of starch, alterations and gelatinization can be seen under pressure (125, 257). The major impact of HPP is on ionic bonds, lipid bilayer structure, and hydrophobic interactions seen in secondary and tertiary structures of compounds and biological components. This might also be a result of the physical compression seen during pressurization. Compression can be seen and result in different and decreased function of compounds upon removal of pressure. Proteins, enzymes and membrane inactivation can be a result of disruption in these weaker bonds (257). DNA has seen a stabilization under pressure as HPP may stabilize hydrogen bonding (123, 263). The utility of HPP has been observed in the food industry and continued interest has arisen.

High pressure units have made an increasing impact in the area of food processing. As the need for fewer additives and cleaner labels are desired by consumers, numbers of HPP equipment have risen exponentially. In 1990, only 1 industrially used HPP unit was in place. As of 2009, 128 HPP units are installed (41, 125). The use of HPP equipment will only continue to climb as more research investigates potential uses for this technology. Vegetable (33-35%), meat (28-29%), liquid beverage (15-17%), and seafood (15%) products continue to have the predominant uses for HPP in the food processing industry. The application of of HPP continues to be aimed at inactivation of enzymes, preservation of appearance, increased shelf-life and most importantly increase post-processing product safety (41, 125, 190).

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Current product usage includes processing of fruit juices, jams, jellies, sliced fruits, tofu, ham, shellfish, guacamole, salsa, fermented meats, RTE deli meats, salmon, surimi, poultry, pork, fish, spaghetti and rice (41, 192, 257, 263).

One of the unique processing advantages in HPP is that the rate of lethality or impact on the product is not dependent upon the dimensions of the product itself as seen in thermal processing. HPP applies pressure isostatically transferring and ensuring equal distribution of pressure to the entire product (125, 190). The instantaneous transmission of pressure to product which can either be liquid product (juices) directly placed into the vessels or packaged product (guacamole, RTE meat slices, etc.) is seen combined with a 10-15% shrinkage of the product under pressures seen in commercial usage. Additional impact of pressure usage results in temperature increases in the vessel due to adiabatic heating of the product, which is seen at an approximate rate of 3°C/100 MPa. The impact of compression and heating are reverses once depressurization occurs (257). These characteristics of HPP differentiate this processing technique from other non-thermal and thermal processing techniques on the market. There is also a great promise of HPP in leading the way as an effective method for food safety. Below is a general discussion of the impact and lethality of HPP on bacteria as it relates to food safety followed by the impact seen in *L. monocytogenes.*

High Pressure Processing Impact on Microorganisms

The impact of HPP on microorganisms is great and can lead to multiple sites of damage within a microorganism. One way that HPP acts on the cell is the disruption of protein folding leading to denaturation. As high pressure has an impact on weak bonds within a cell, protein structure which is often held together by hydrogen bonds can be

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unfolded irreversibly when HPP is applied. The HPP effect on weak bonds can also impact protein-protein interactions within the cell. The presence of water in a system can also impact the denaturation process by interacting with regions of unfolded proteins preventing refolding. This can result in the loss of function of enzymes associated with cell function and membrane transport as well (263, 343). Additional research with pressures between 100 and 220 MPa has shown dissociation of proteins and denaturation which impacted the integrity of the membrane. In some cases protein modifications can lead to transmembrane pores, which can replace altered proteins. Higher pressures irreversibly affected protein within the membrane and those below 100 MPa resulted in reversible impact on protein conformation and destruction (159). Primary structures (peptide bonds) are not directly affected by pressure up to 1000 MPa (339). In *Salmonella*, HPP altered proteins present in membranes, which suggests that HPP may target proteins during microbial inactivation (260).

Lipid structures are said to be one of the most pressure-sensitive cellular areas and the target of HPP inactivation in microorganisms (263, 339). When exposed to pressure the lipid bilayer shrinks laterally and has a straightening of acyl-chains resulting in an increased thickness of the bilayer. Additionally, the bilayer changes from a liquid-crystalline state to a gel-like state. This gel state is displayed through an orderly structure of extended chain fatty acids. An increased ordering of the membrane may cause proteins to be disassociated from the member as well (339). Additional changes might occur in relation to the membrane which include the separation of the cell wall from the membrane which was observed in *Lactobacillus* (233).

Ritz et al. (262) had used scanning electron microscopy (SEM) and observed bud scars in pressurized *L. monocytogenes*. The images also noted that disruptions in cell

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membranes might inactivate cells, but leave cell morphologically similar to unaffected cells. In this study, membrane integrity was not uniform within the cell population suggesting a resistance to pressure in some of the cell population. The membrane potential decreased in cells that were pressurize suggesting some sort of damage to the cell membrane or the mechanisms that ensure homeostasis in the cells (262).

Various studies have examined the role of HHP on protein synthesis, ribosomes and nucleic acids as well. Protein synthesis is reversibly inhibited above 68 MPa (218). Studies have shown that ribosome dissociation under pressure can lead to cell death. During examinations of protein production, pressures 40-60 MPa were sufficient in dissociating ribosomes (115, 218). Research of pressurized *E. coli* suggests that ribosome damage might be related to cell death (218). Increasing pressure to levels used in the food industry may ultimately result in death of cells (53, 135). Research has also shown that DNA stabilization occurs at lower pressures up to ~270 MPa. Beyond this up to 1,000 MPa no denaturation was seen in DNA. This was explained by potential volume changes seen in compression under hydrostatic pressure (123). The compression is also suggested at stabilizing hydrogen bonds found in DNA, mentioned earlier (263). Direct observation of yeast under pressure noted a 25% decrease in volume (compression) of the cells at 250 MPa and a 10% decrease in cell volume remained even after pressure was remove (237). The Le Chatelier's principle on pressure suggests that reactions are favored under pressure when they involve a decrease in volume, while reactions involving an increase in volume are disfavored (53, 341). This might suggest that DNA transcription and protein production under pressure might relate to volume they need to function. In these instances, cell death might be better explained by damage to ribosomes caused by HPP.

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Although the determination of the primary impact of HPP on microbial life has not been elucidated, it is safe to say that HPP decreases cell viability by multiple mechanisms (286). The role of HPP in the food system is ever growing and the application of this technology has already had a great impact on food product stability. Application of this technology to *L. monocytogenes* in foods follows.

Impact of High Pressure Processing related to *L. monocytogenes* in foods

The impact of food constituents and high pressure processing on *L. monocytogenes* has been examined and may have a significant impact on the extent of pressure that foods must undergo to eliminate this pathogen. The food matrix can have a protective effect on *L. monocytogenes*. The following will examine some factors which impact the barotolerance of *L. monocytogenes* in both foods and laboratory media.

Comparisons of the impact to *L. monocytogenes* regarding the amount of time, temperature and pressure have been made. Pressurization of *L. monocytogenes* at 207 MPa, 276 MPa and 345 MPa for 10 min at 25°C in tryptic soy broth with 0.6% yeast extract (TSBYE) resulted in reductions of 0.79, 0.85 and 3.05 Log CFU/ml, respectively. Treatment of *L. monocytogenes* with the similar parameters of 276 MPa at 25, 35, 45 and 50 °C resulted in reductions of 0.85, 1.90, 3.60 and 8.08 Log CFU/ml, respectively. Log reductions with increasing time at 345 MPa were 2.64 and 3.05 CFU/ml after 5 and 10 minutes of treatment, respectively. Only moderate reductions were seen through both increases in pressures and times at a particular pressure. This study demonstrated that moderate increases in pressure and time at low pressures may not have as much of an impact on *L. monocytogenes* as much as temperature increases above 35°C (8). Low pressures for longer hold times have been

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used to reduce any negative change in food quality; however, they produced relatively small decreases in cell viability (8, 202, 235, 250, 300). That same study also examined the impact of decreased pH on the survival of *L. monocytogenes*. It showed that with decreases in pH from 6.5 to 4.5 under pressure decreases in survival were also seen. Lactic acid seems to have the greatest impact at reducing the viability of *L. monocytogenes* under pressure irrespective of strains and pH conditions tested (pH 6.5, 5.5 and 4.5) (8). Various parameters (time, temperature and pressure) can be used to effectively control the safety of food products. Increasing temperature had a greater impact on *L. monocytogenes* survival during pressurization, but the addition of acids may also add to the safety of foods or ensure the use of low temperatures when processing acidic foods.

The use of model food systems to identify the impact of fats, proteins and glucose on *L. monocytogenes* under pressure has been investigated. Bovine serum albumin (BSA) as a protein source at various concentrations in phosphate buffered saline (PBS) was used to examine the resistance of *L. monocytogenes* to high pressure treatments. Multiple (3) strains were examined for resistance. Overall at 375 MPa, higher BSA content served as a greater protective agent in all strains tested suggesting a protective effect when greater protein is present in a food system (286). Examining the barotolerance of *L. monocytogenes* in various glucose concentrations lead to similar results. The barotolerance of the three strains varied along with variation due to some of the concentrations of the glucose. Ultimately, there was a greater resistance seen in all three strains that were suspending in 10% glucose PBS solution compared to PBS suspensions with 1, 2 and 5% glucose suspensions. Protein (BSA) seemed to have a greater protective effect compared to glucose overall (286). To see if fats served as a protective agent during pressurization, olive oil was chosen. A 30% mixture of

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olive oil with lecithin (0.75%) in PBS resulted in a greater resistance to pressure treatments in three strains of *L. monocytogenes* compared to pressurization in PBS alone (286). Protection from fat content in ovine milk was also observed in *L. innocua* (101). This suggests that *L. monocytogenes* may have a greater chance of survival in complex food matrices that contain higher levels of glucose, lipids and protein.

The protective effect of salt or water activity on in *L. monocytogenes* foods has been demonstrated as well. A study examined the impact of water activity on the survival of *L. monocytogenes* pressurized in two different types of cheese. *L. monocytogenes* exhibited an increased resistance to pressure in the cheese with the higher salt content and lower water activity. Considering that the two cheeses might have contained various other differences due to variations in the dry material content, a controlled study with one cheese and various salt additions (0-5%) was performed and supported the baroprotective nature of salt content. For 0% added salt (a_w=0.984) and 5% added salt (a_w=0.904) viable numbers of the pathogen after pressurization (400 MPa; 12°C; 10 min) were 3.61 and 6.69 Log CFU/g respectively. The viable numbers of the pathogen before application of high pressure was 7.12 log CFU/g in both cases. That same study examined lactose and galactose for a protective effect during pressurization of *L. monocytogenes*. The study did not produce a difference in viability from samples with or without lactose, but did reveal a protective effect against pressurization when 5 mg/g galactose was added to the cheese suspension prior to pressure treatment (212). Similar protection to high pressure treatment has been attributed to higher salt content in hams that contained different amounts of salt (211). Research with *L. innocua* in TSBYE resulted in a reduction of approximately 4 Log CFU/ml in 0.5% NaCl media, where only about a 1 Log reduction was seen in media at 3.5% NaCl after pressurization at 600 MPa for

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5 min (20°C). That study also demonstrated the protective effect of salt in oysters (290). The use of salt to in food processing is abundant. Consideration of introducing HPP into processed foods with high salt content must be taken seriously as lower HPP parameters might not provide an effective kill step for *L. monocytogenes* when salt content is increased.

Cross Protection with High Pressure Processing in *L. monocytogenes*

The effect of prior adverse growth conditions or exposure of *L. monocytogenes* to other stress may impact the antibacterial effectiveness of HPP against this pathogen. There have been several studies that have delved into this topic of cross protection. Some of these studies will be reviewed below.

The impact of prior growth temperature and its effect on barotolerance in *L. monocytogenes* has been researched. One study examined various growth temperatures from 4°C to 43°C and exposed each of these growth temperature treatments at stationary phase to 400 MPa for 2 min at 21°C in UHT whole milk (283). Stationary cells were used as they had been shown to provide the greatest pressure resistance in studies with *L. monocytogenes* (119). A greater pressure resistance was observed in *L. monocytogenes* grown at 43°C compared to cells grown between 10 and 25°C. There was a 6 Log greater reduction in viability of the pathogen grown between 10 and 25°C compared to 43°C. Also, a slight increase in growth temperature from optimal (35°C) to 40°C greatly increase resistance of *L. monocytogenes* to HPP in whole UHT milk. Pressure resistance of stationary-phase *L. monocytogenes* grown at cold temperatures (4 to 15°C) was not statistically different compared to its resistance when the pathogen was grown at 30 or 35°C (283). Exponentially grown *L. monocytogenes* displayed a slight resistance to high pressure when grown at 8°C

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compared to 30°C (195). Those results indicate a greater susceptibility of *L. monocytogenes* to pressure in exponential phase of growth. Elevated growth of *L. monocytogenes* at 43°C protected the pathogen against subsequent pressure treatment (119). The illness of an animal or lack of refrigeration leading to elevated temperatures in milk may render *L. monocytogenes* more resistant to HPP. This is important when examining procedures to ensure the microbial safety of milk treated with this emerging technology.

Heat shock has been shown to elicit a barotolerant response in *L. monocytogenes.* This pathogen was heat shocked at 48°C in whole ultrahigh-temperature (UHT) milk for various amounts of time and exposed to pressurization at 400 MPa for 90 seconds at ambient temperature. Heat shocking *L. monocytogenes* in UHT milk between 2 and 60 minutes resulted in an increased resistance to high pressure compared to the non-heat shocked cells. The greatest resistance to pressure was seen in cells that were heat shocked for 5, 10, 15 or 30 minutes. Another experiment examined the resistance in D-values at 400 MPa in *L. monocytogenes* heat shocked for 10 minutes in UHT milk. Those experiments tested whether protein inhibition achieved through the use of chloramphenicol prior to heat shocking cells had an impact on barotolerance in UHT milk. Heat shocked cells resulted in a D-value of 126.8 seconds whereas, the control had a D-value of 35.3 seconds. The D-value of the samples that were heat shocked with chloramphenicol did not differ from the control. This demonstrated that barotolerance of heat shocked *L. monocytogenes* might be strongly associated with protein production (121). *E. coli* exhibited a similar barotolerance associated with the production of heat shock proteins as well (3). Heating is commonly used in the food processing environments. When combining low heating prior to pressure we see a great

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resistance to HPP which is of concern in food safety. Special care must be taken when developing processes that might involve low heating of product prior to pressurization.

Examining the cold shock response of *L. monocytogenes* prior to HPP has also been investigated. Exponentially grown *L. monocytogenes* were cold shocked for 4 hours at 10°C in brain heart infusion broth and then re-suspended in fresh media and tested for pressure resistance at various pressures for 20 minutes. Pressure treatments at 200, 250, 300 and 350 MPa all proved great resistance in cold shocked *L. monocytogenes*. Lethality for unstressed cells was 100-fold greater than cold shocked cells at 300 MPa for 20 minutes. Cold shock proteins (CSPs) were shown to be produced in both pressure treated and cold treated cells. Cold shock for longer periods of time, 20 hours compared to 4 hours, resulted in an increase of 4-fold and 1.5-fold in the production of CSPs, respectively. CSPs were implicated as a possible explanation for the resistance seen to pressurization (332). This is of concern if products were to be held at cold temperatures directly prior to pressurization. Cold holding conditions might pose an increases food safety threat and processing conditions might need to be adjusted to increase processing lethality.

The effect of prior acid shock at pH 4.5 for 1 hour in exponentially grown cells on the barotolerance of *L. monocytogenes* has been examined. Various pressures between 150 and 400 MPa for 20 minutes were used. The results indicated that increased pressure resistance was seen in acid shocked cells compared to non-shocked cells. At 350 MPa, the survival was 90% of the acid shocked cells compared to controls cells where below 0.0001% of the cells survived. When *sigB* mutants were acid shocked and subsequently exposed to 350 MPa for 20 min, population survival was more similar to the non-acid shocked control. This indicated that *sigB* might play a role in the acid shock response leading to barotolerance in *L.*

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monocytogenes (333). The response of microorganisms to acid in relation to food processing is important because organic acids are widely used in food processing and cleaning and sanitation systems can use low pH cleaners or sanitizers to destroy pathogens.

Few studies have examined the influence of othe environmental stresses on the resistance of *L. monocytogenes* to high pressure processing. The effect of growth at restricted water activity was investigated. The results indicated that *L. monocytogenes* was sensitive to pressure when grown at $a_w<0.96$ in laboratory media (212). Hayman et al. (120) found similar results supporting the pressure protection of *L. monocytogenes* that previously exposed to lower water activity levels. The literature has also shown that *L. monocytogenes* in "long-term-survival-phase" exhibited a resistance to HPP. The "long-term-survival-phase" simulated growth cessation and survival in TSBYE up to 30 days (335). To my knowledge research relating directly to the impact of starvation on barotolerance of *L. monocytogenes* has not been reported.

As the scientific literature lacks research on the impact of many other prior stresses on the barotolerance of *L. monocytogenes*, there is a need to complete our understanding of how environmental stresses that are common in food processing environments affect the barotolerance of *L. monocytogenes*. Accordingly, the impact of starvation on barotolerance of *L. monocytogenes* Scott A was investigated.

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MATERIALS AND METHODS

Bacterial culture and culture conditions

Stock cultures of *Listeria monocytogenes* Scott A NADC 2045 stored at -80°C were thawed and streak-plated onto tryptic soy agar with 0.6% yeast extract (TSAYE). Individual colonies from streaked plates were selected and grown at 35°C overnight in brain heart infusion (BHI) broth for freeze-thaw research and in tryptic soy broth with 0.6% yeast extract (TSBYE) for high hydrostatic pressure research. These overnight cultures were used to prepare working cultures via preparation of two consecutive 24-h transfers into the appropriate broth medium (35°C).

Inoculation procedure and exposure of cells to selected stresses

One ml of working culture was aseptically added to pre-warmed (35°C) 100 ml of BHI broth in 250 ml screw cap Erlenmeyer flasks. All flasks were exposed to shaking (150 rpm) inside a gyrorotary water bath (New Brunswich Scientific Co. Inc., Model G76) and held until cultures reached mid-exponential phase based on an optical density (OD) of approximately 0.50 at 600nm. Stresses were then applied to the exponentially grown cells for 1 h at 35°C (shaking, 150 rpm) or for approximately 2 doublings of the control, (non-treated cells). Description of application of each individual stress is listed below. This treatment of cells for 1 h with each respective stress was considered a short duration stress and thus indicated as a "shock" treatment and not a stress adaptation treatment. Stresses that did not require exponential growth (acid adaptation and starvation) are described later in this section.

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All cells were harvested and washed the same way unless indicated otherwise. After treatment of cells, they were harvested by centrifugation (Sorvall Super T21, 25°C, 10 min, 10,000 x g) and washed twice in buffered peptone water (BPW, Difco). Washed cells were placed (0.5 ml) into appropriately labeled sterile 15 ml conical tubes (Fisher Scientific) containing 4.5 ml BHI broth. Tubes were then placed in freeze-thaw treatment or high hydrostatic pressure as described later.

Acid Shock

Hydrochloric acid (HCl, Fisher Scientific, 12 N) was added directly to exponentially growing *L. monocytogenes*. Each respective flask had a final concentration of 36, 30, 24, 18, and 12 and 0 (control) mM HCl, which represented acid shock treatment labels of pH 4.0, 4.5, 5.0, 5.5, 6.0, and 7.0 (control), respectively. An equal volume of each HCL solution or sterile distilled water (for control) was added to each flask to avoid variations in volumes of the culture.

Oxidative and Ethanol Shock

Ethanol (100%) was added to exponential cultures to a final concentration of 2%, 1.5%, 1.0%, 0.5%, and 0% (control). Oxidative shocked cultures were achieved by the addition of hydrogen peroxide (Fisher Scientific, 3% v/v) to a final concentration of 500, 250, 150, 50, and 0 ppm in respective flasks.

Alkali Shock

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Sodium Hydroxide (NaOH, Fisher Scientific, 10 M) was added to separate flasks to reach a final concentration of 70, 65, 55, 45, 35, and 0 (control) mM labeled respectively as pH 11, 10.5, 10.0, 9.0, 8.0, 7.0 (control). The flasks had a range of pH values for pH 11 (pH 9.48 to 9.99), pH 10.5 (pH 9.20 to 9.65), pH 10.0 (pH 8.61 to 8.71), pH 9.0 (pH 7.88 to 8.10), pH 8.0 (pH 7.28 to 7.45), and pH 7.0 (pH 5.61 to 6.29).

Acid Adaptation

Acid -adapted *L. monocytogenes* cultures were growth in tryptic soy broth supplemented with 0.6% yeast extract with 1% (w/v) added dextrose and non-adapted or control cells were grown in the same medium without dextrose. Acid adapted and non-adapted cells, were grown non-shaking for 20 hours in 250 ml flasks each containing 100 ml of appropriate broth media. The final pH values for cultures of acid-adapted cells varied from pH 4.20 to 4.32 and for non-adapted cells from pH 6.19 to 6.30. The cultures were then harvested by centrifugation and washed as previously described.

Starvation

L. monocytogenes was grown statically to stationary phase in 800 ml TSBYE (35°C). The cells were harvested by centrifugation (10,000 x g, 10 min, 4°C) and washed twice with 0.85% (w/v) NaCl (physiological saline). The pelleted cells were re-suspended in 0.85% NaCl and the cell suspension was held at 25°C in a sterile 2 L Erlenmeyer flask and sampled over 12 days in 2 day intervals. These cells were considered starved. Control cultures were prepared from overnight cultures (stationary phase cells).. Samples were taken for viability as well as for high hydrostatic pressure treatment.

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Freeze-Thaw Cycles

Freeze thaw method was used as indicated in the literature and described by Wemekamp-Kamphuis (333). All freeze cycles were performed in 5ml BHI at -18°C in sterile 15ml conical tubes (Fischer Scientific). Tubes were placed at this temperature for approximately 24 h in a Kenmore freezer model 253.9280213. BHI broth (5 ml) at this temperature reached -18°C within 5 h within the freezer. Thawing took place by exposing the frozen culture for 7 minutes in a 40 liter water bath (Isotemp 228 Fisher Scientific) set at 30°C. Samples were then mixed by vortexing and sampled immediately for viable counts. After sampling, tubes were placed directly into freeze conditions.

Application of High Hydrostatic Pressure

Samples (5-ml) of starved cells in 0.85% w/v NaCl were processed using High hydrostatic pressure (HHP). The samples were heat-sealed in polyester pouches (400, KAPAK Corporatin, Minneapolis, MN, USA). Pouches were pressurized at 0 MPa (control) and 400 MPa for 1, 15, 30, 45, 60 and 75 s at 25°C. The pressurization unit used was a Food Lab High-Pressure Food Processor (Stansted Fluid Power Ltd, Essex, U.K.) that was operated with a 50% distilled water and 50% propylene glycol pressurization fluid (GWT Global Water Technology, Inc. Oakbrook Terrace, IL CAS Number 57556). Temperatures within the pressurization vessel and pouches were measured with the Stansted fluid power FPG55000 RAP system and Scan 1000 Supervisory Control and Data Acquisition system (Hexatec, U.K.). Temperature recordings indicated that a maximum temperature reached was 39.3°C with an adiabatic heating rate of 2.8°C/100 MPa. The pressurization rate was

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350 MPa/min and rapid decompression occurred. The pressurization occurred within 90 s and decompression occurred within 6 s.

D-values are defined as the time (at a given pressure) required for 1 log (90%) decrease in the initial viable population of *L. monocytogenes*. D-values at 400 MPa were calculated from the slopes of the survival graphs (log CFU, y-axis versus pressurization time, x-axis). The negative reciprocal of slopes of the survivor curves were taken and expressed as the D-values.

Microbiological Analysis and pH

Freeze-thaw samples were serially diluted (1:10) in BPW and plated in duplicate on both tryptic soy agar with 0.6% yeast extract (TSAYE, Difco) and Modified Oxford Agar (MOX) for all treatments. All inoculated agar plates were incubated at 35°C and bacterial colonies were counted after 72 h.

The percentage of injured *L. monocytogenes* survivors was calculated for all freeze-thaw treated samples. Injury calculations were performed using the following equation:

$$\% \text{ injured} = \frac{\text{count (CFU/ml) on TSAYE} - \text{count (CFU/ml) on MOX}}{\text{count (CFU/ml) on TSAYE}} \times 100$$

For all pH measurements taken, the supernatants were filter sterilized (0.22 µm, PTFE) and measurements were taken using an Accumet Basic model AB15 unit (Fisher Scientific). Pressure-treated samples of cell suspensions containing starved *L. monocytogenes* were serially diluted in 0.1% (w/v) peptone (Difco) and plated in duplicate on TSAYE. Inoculated agar plates were incubated at 35°C and bacterial colonies were counted at 72 hours.

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Statistical Analysis

All experiments were replicated three times. Analysis of data was performed using a modified mixed model PROCEDURE GLIMMIX in Statistical Analysis System software program version 9.2 (SAS Institute Inc., Cary, NC). Normal distribution was used for all response variables. Both linear and categorical comparisons were made over freeze-thaw cycles. F-tests were used to test significance of treatment and day. Tukey-Kramer pair-wise procedures were used when comparisons were made between two data points. Significance was determined using adjusted p-values (significance 0.05).

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RESULTS

Impact of treatments on *L. monocytogenes*

All of the treatments, shock treatments (acid, ethanol, alkali and oxidative) and acid adaptation, did not statistically reduce the initial population of *L. monocytogenes* compared to the controls (P>0.05). These comparisons are seen in Tables 1-5.

Acid Shock

The actual pH values of the broth media containing acid-shocked exponential-phase *L. monocytogenes* ranged from pH 3.98-4.16 (for pH 4.0), 4.15-4.42 (for pH 4.5), 4.35-4.78 (for pH 5.0), 4.67-4.98 (for pH 5.5), 5.03-5.32 (for pH 6.0), and 5.70-6.09 (for pH 7.0). Viability of acid- shocked *L. monocytogenes* was assessed after 4 freeze-thaw cycles (Figure 1). The impact of one freeze-thaw cycle on initial populations of acid-shocked *L. monocytogenes* was a decrease of 0.8315, 0.9440, 0.7762, 0.3811, 0.7766, and 0.8536 Log CFU/ml for cells exposed to pH 4.0, 4.5, 5.0, 5.5, 6.0, and 7.0, respectively. The reduction in *L. monocytogenes* due to one freeze-thaw cycle was statistically significant for treatments pH 4.0 (P=0.0176), 4.5 (P=0.0029), 5.0 (P=0.0396), 6.0 (P=0.0395), and 7.0 (P=0.0125) within each treatment. Interestingly, there was not a statistically significant difference in decrease in initial viable counts of the acid-shocked (pH 5.5) pathogen following one freeze-thaw cycle (P=0.9661).

Treatment decreases after 4 freeze-thaw cycles represented an overall decrease in treatment populations compared to initial populations. These overall deceases were 0.9121 Log CFU/ml for pH 4.0 (P=0.0050), 1.251 Log CFU/ml for pH 4.5 (P<0.0001), 1.0514 Log CFU/ml for pH 5.0 (P=0.0005), 0.3626 Log CFU/ml for pH 5.5 (P=0.9808), 0.8221 Log

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CFU/ml for pH 6 (P=0.0203), and 0.9357 Log CFU/ml for pH 7 (P=0.0034). Statistically significant decreases were seen in all treatments due to freeze-thaw cycles except with treatment pH 5.5, which did not produce a statistically significant decrease from the initial *L. monocytogenes* populations (P>0.05). When viability of acid-shocked *L. monocytogenes* after 4 freeze-thaw cycles were compared to the control (pH 7), no statistical significant difference was found (P=0.05) (Table 1).

Oxidative Shock

Oxidative shock treatments of *L. monocytogenes* resulted in viability changes which were monitored after the application of multiple freeze-thaw cycles (Figure 2). Decreases after 1 freeze-thaw cycle from the initial populations in Log CFU/ml were 0.3361 for 0 ppm (control) (P=1.000), 0.1646 for 150 ppm (P=1.000), 0.5063 for 250 ppm (P=0.9924) and 0.2387 for 500 ppm (P=1.000) treatments. An increase of 0.02587 Log CFU/ml (P=1.000) was seen in cells shocked with 50 ppm prior to the first freeze-thaw cycle. Decreases in viability in the initial viable populations of the pathogen were observed in all samples after 4 freeze-thaw cycles. Log reductions were 1.2159 for the control (P=0.2626), 0.5425 for 50 ppm (P=0.9832), 1.3355 for 150 ppm (P=0.0217), 1.7741 for 250 ppm (P=0.0004) and 0.8013 for 500 ppm (P=0.6399) samples. Significant reductions were only seen within the treatments of 150 ppm and 250 ppm. No significant reductions (Table 2) were seen when viable numbers of the treated cells were compared to those of control after 4 freeze-thaw cycles (P>0.05).

Alkali Shock

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Application of multiple levels of alkali shock to *L. monocytogenes* was evaluated for its' impact on freeze-thaw resistance (Figure 3). Alkali shock of *L. monocytogenes* at pH 7 (control), pH 8, pH 9, pH 10, pH 10.5 and pH 11 resulted in a difference in viability of -0.04267 (P=1.000), -0.09967 (P=1.000), -0.76339 (P=0.7589), +0.1478 (P=1.000), -0.07576 (P=1.000) and +0.08084 (P=1.000) Log CFU/ml after 1 freeze-thaw cycle, respectively. After 4 freeze-thaw cycles populations were changed by -0.1264 (P=1.000), -0.189 (P=1.000), -0.9579 (P=0.3306), +0.1247 (P=1.000), -0.1988 (P=1.000), +0.00656 (P=1.000) Log CFU/ml in control, pH 8, pH 9, pH 10, pH 10.5 and pH 11 treated samples, respectively. Treatment viabilities compared to the control (Table 3) after 4 freeze-thaw cycles resulted in statistical similarity (P>0.05).

Ethanol shock

L. monocytogenes shocked with various concentrations of ethanol were exposed to 4 freeze-thaw cycles and viabilities were assessed (Figure 4). Decreases in Log CFU/ml after 1 freeze-thaw cycle for cells exposed to ethanol at 0%, 0.50%, 1%, 1.5% and 2% were 0.8067, 0.8221, 0.7231, 0.7639 and 0.7359, respectively (P<0.05). Log decreases of these treatments after exposure to 4 freeze-thaw cycles were 0.8949 for the control (0%) (P=0.0010), 0.7068 for 0.50% (P=0.0014), 0.5116 for 1% (P=0.0670), 0.445 for 1.5% (P=0.3756) and 0.6413 for 2% (P=0.0055) (Table 4). Some statistical difference was seen within treatments, but viability of treatments after 4 freeze-thaw cycles were not significantly different compared to the control (P>0.05).

Acid adaptation

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Viability of acid adapted and non-adapted *L. monocytogenes* was compared following freeze-thaw cycles (Figure 5). Non-adapted cells exhibited Log CFU/ml decrease of 0.1934 and 0.4807 after 1 and 4 freeze-thaw cycles, respectively (P>0.05). Acid adapted cells decreased 0.07473 and 0.1768 Log CFU/ml after 1 and 4 freeze-thaw cycles, respectively (P>0.05). Statistical decreases were not observed in initial cell counts and any freeze-thaw cycle with both adapted and non-adapted samples (P>0.05). Comparison (Table 5) was made between *L. monocytogenes* of the non-adapted and acid adapted cells after 4 freeze-cycles and no statistical difference was seen (P=0.9155).

Freeze-thaw cycles alone

Viability of *L. monocytogenes* after freeze-thaw treatments was assessed for all control treatments (Figure 6). Log reduction after 1 freeze-thaw cycle was 0.4974 Log CFU/ml (P=0.0144). Log reduction of 0.74 (P=0.0002) was observed after 4 freeze-thaw cycles and represented the lowest viability of all freeze-thaw cycles compared to the control (non-freeze-thawed cells) (Table 6). All freeze-thaw cycles produced a statistical significant difference in viability of the pathogen compared to viability of the control (P<0.05), but were not statistically different among treatments after each application of a freeze-thaw cycle (P>0.05).

Freeze-thaw injury

Percent injury was assessed for each shock (acid, ethanol, alkali and oxidative) and acid adaptation treatment and control (Tables 7-11). Percent injury values varied greatly, but

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no statistically significant differences were found either due to freeze-thaw cycles or treatments (P>0.05).

Survival after starvation

Figure 7 shows the viability of *L. monocytogenes* Scott A NADC 2045 over 12 days of starvation in 0.85% (w/v) NaCl. There was an initial decrease in viable counts from 9.42 to 7.74 Log CFU/ml, (1.68 Log CFU/ml reduction) from Day 0 (control) to Day 2 of starvation (P=0.0023). Statistically significant differences (P< 0.01) were observed on comparing the viability of non-starved *L. monocyogenes* (control) with that of starved cells of the pathogen in 0.85% (w/v) NaCl for 2 to 12 days. No significant differences in viability of *L. monocytogenes* were observed after 2 days of starvation (P>0.100).

Log reductions after 1 second of high hydrostatic pressurization (400 MPa)

Log_{10} reductions in initial viable counts of both *L. monocytogenes* control and starved cells that were exposed to HHP (400 MPa) at 25°C for 1 second are shown in Figure 8. Reductions in initial viable counts of starved cells following pressure treatment were substantially lower during days 4 to 12. Log reductions for *L. monocytogenes* Scott A were greatest on Day 2 of starvation. In this regard a 3.34 Log CFU/ml reduction in initial viable count was observed. Pressure treatment of control (non-starved) samples under these pressurization conditions resulted in a 2.92 Log reduction. The lowest reductions after 1 second of pressurization were seen after starvation for 4, 10 and 12 days, with reductions of 1.36, 1.10 and 1.29 Log CFU/ml, respectively. Statistical similarity was seen between most samples except Day 2 of starvation.

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A trend of decreasing Log reductions was observed as *L. monocytogenes* Scott A was starved for more than 2 days. This trend proved statistically significant with a decreasing slope over days of -0.1529 (P=0.0025).

HHP D-value analysis of starved *L. monocytogenes*

D-value (time at 400 MPa for achieving 90% reduction in numbers of viable cells) for *L. monocytogenes* at each day of starvation in 0.85% NaCl is shown in Figure 9. The D-value for control (non-starved cells; Day 0) samples was 11.85 seconds. At 2 days of starvation the D-value increased by 2.47 seconds. The highest resistance compared to the control was seen at 8 days of starvation; D-value=19.88 seconds (P=0.0321). After 10 and 12 days of starvation the D-values were 18.55 and 17.28 seconds, respectively. There were no statistically significant differences in other D-values (at days 2, 4, and 6) compared to the control; however D-values consistently increased with increased starvation time between during 2 to 8 days.

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DISCUSSION

Food processing environments offer many opportunities for the induction of stress response in microorganisms. The food industry may use minimal processing technologies which can present stresses in food processing systems. Stresses may enhance and cross protect against other stresses. With regard to freeze-thaw (19, 340) and high hydrostatic pressure applications (123-125, 339, 340, 342) some stresses have been investigated on the impact of cross protection or stress hardening in *L. monocytogenes*.

The assessment of freeze-thaw tolerance in foodborne pathogens that have endured prior environmental stress has received little attention over the years. There are federal regulations regarding freezing of foods and freezing is even considered an "antimicrobial agent" by the United States Department of Agriculture (313). Nevertheless, Archer (14) suggests that freezing has been overlooked for consideration as a treatment that may impact microorganisms greatly. The presence of *L. monocytogenes* remains a public safety issue in foods and care must be taken when handling products to ensure the safety and prevention of outgrowth of this psychrotrophic bacterium. Exposure of foodborne microorganisms to freeze-thaw cycles in the food industry occurs in the processing of meats (especially hams), smoked salmon (247) and during transportation and storage of foods (107, 157, 168, 267).

The research presented in this thesis aimed to address if prior stress on *L. monocytogenes* Scott A affected the survival of the pathogen after its exposure to subsequent freeze-thaw cycles. Examination of the effect of freeze-thaw cycles on viability of *L. monocytogenes* after the pathogen was exposed to each of the stress condition was performed (Figure 1-5). None of the prior stress treatments elicited a resistance to freeze-thaw cycles tested. Acid pre-treatment of *L. monocytogenes* (Table 1), pH 5.5, produced a slight

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resistance of the pathogen to freeze-thaw cycles. This resistance within treatment to freeze-thaw cycles was not seen when comparisons were made to the control. There was no statistical significant difference in freeze-thaw resistance of *L. monocytogenes* (exposed to pH 5.5) and that of the control. Other researchers (333) have found that acid shock (pH 4.5 for 1 h) of *L. monocytogenes* resulted in an increased resistance of the pathogen over 5 freeze-thaw cycles. Experimental freezing and thawing conditions were similar to the current study as were the acid shock pH conditions. In the current study, pH 5.5 treatment ranged from pH 4.67-4.98 due to the addition of acid to a growing culture. We used a different strain in the present study and therefore, strain variation might have contributed to differences in freeze-thaw resistances (75, 76) observed in results of the present study compared to those previously mentioned (333). A *L. monocytogenes* mutant lacking σ^B had only partial resistance to freeze-thaw cycles compared to the parent strain (333). Wemekamp-Kamphuis et al. (333) suggested that the acid adaptation response which caused resistance may not fully be linked to σ^B. Okada (226) described the presence of multiple alternative sigma factors, which may play a role in stress response of *L. monocytogenes*. Any resistance may not be attributed to just one physiological response as multiple stress responses may overlap (122). The results of the present research do not support previous findings by Wemekamp-Kamphuis et al. (333) that acid shock leads to freeze-thaw resistance in *L. monocytogenes*.

Overall analysis of the data leads to the conclusion that decreases in viability of *L. monocytogenes* exposed to cycles of freezing and thawing were independent of any of the shock (acid, ethanol, oxidative and alkali) or adaptation (acid) treatments examined (Figure 6 and Table 6). A trend suggests a decreasing slope of 0.1578 over freeze-thaw cycles applied

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(P<0.0001). Other investigations have been made into freeze-thaw tolerance of *L. monocytogenes*. In this respect cross protection was observed in cultures grown on agar at 4°C compared to 37°C. When cultures were grown in broth, the resistance of *Listeria* spp. to freeze-thaw occurred when cells were grown at temperatures of 37°C and not in 4°C cultures (17). Cold shock (10°C for 1 h) increased resistance to freeze-thaw cycles. Cold shock proteins were hypothesized as leading to freeze-thaw resistance in *L. monocytogenes*, while a moderate contribution of σ^B may have led to that type of resistance (333). Some stresses such as starvation produce "spore-like" cells that might contribute to the freeze-thaw resistance as seen in *Exiguobacterium*, a psychrotrophic permafrost isolate (321). Results of the present study indicated that decreases in viability were pronounced after the first freeze-thaw cycle. Based on results of research involving *Saccharomyces cerevisiae,* it was uncertain whether the observed resistance of that yeast to the freeze-thaw cycles was due to a subset of freeze-thaw resistant cells that survived freezing. Additionally, exposure to hydrogen peroxide induced resistance to freezing in that yeast (231). Despite reports of cross protection in other microorganisms, results of the present study indicate that certain stresses do not alter the resistance of *L. monocytogenes* Scott A to cycles of freezing and thawing.

Freeze-thaw injury in *L. monocytogenes* has been previously reported (77). Freeze injury may cause cellular leakage, membrane damage and alternations to proteins and DNA in cells (14, 73, 340). In the present study, *L. monocytogenes* did not show significant injury as measured by subtracting bacterial colony counts on selective media (MOX) from those on nonselective media (TSAYE). The similar measurement of injury was made by Jasson et al. (148) and a similar phenomenon was reported. The authors stated that the negative injury

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value was a "mathematical concept" in the calculations and was seen when injury was assessed for heat, cold, and freezing stresses in *L. monocytogenes*.

Freeze injury was not reported as being significant when bacterial colony counts on non-selective media were compared to those on two selective media with chemical agents (Agar Listeria Ottavani & Agosti, ALOA) or agar medium with added salt (4% (w/v)) (148). In the current research, statistical significant injury in *L. monocytogenes* was not found following exposure of the pathogen to freeze-thaw treatments. This research does not support past studies which have used TSAYE and TSAYE + NaCl as the nonselective and selective media, respectively (75, 77, 108, 206) and also MOX as the selective medium (48). The use of a nutrient rich base (columbia agar base) in the selective medium (MOX) may account for the unexpected differences in resuscitation of sub-lethally injured *L. monocytogenes* on MOX agar medium compared to the TSAYE medium. Differences in the base media might result in different counts on media after a stress has been applied. In freezing of *E. coli* cell damage can release cell constituents. These cell constituents such as polypeptides may have acted to protect and enhance the recovery of other cells (213). This may account for lower injury percentages and reduced impact of freezing on cells, but has not been tested in the present study. Further research should be conducted to test these hypotheses in *L. monocytogenes* Scott A. Additional research could be conducted on analyzing the impact of each particular selective agent on resuscitation of sub-lethally injured *L. monocytogenes.*

Various stresses associated with food processing methods may cause injury to one or more vital structures and functions in bacteria (42). Considering that damaged bacterial structures or functions may or may not be sensitive to certain selective agents, careful

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selection or development of selective agar medium for a particular stress is needed to ensure accurate evaluation of sub-lethally injured *L. monocytogenes* in foods and food processing environments. In addition, development of non-selective agar media for optimal resuscitation of sub-lethally injured *L. monocytogenes* is also warranted because sub-lethally injured microorganisms can have fastidious nutritional requirements until they repair their injury.

The importance of high hydrostatic pressure in the food industry has escalated over the past years as an increasing number of food processors install commercial units (41, 125). The use of stressed *L. monocytogenes* in high pressure research has received recent attention. Studies have been performed investigating the impact of growth phase (119, 335), water activity (120), heat shock (121), cold shock (332) and acid shock (333) on survival of *L. monocytogenes* exposed to subsequent high hydrostatic pressure treatment. Pressure resistance has been reported to vary between strains of *L. monocytogenes* (286). The aim of this research was to assess the impact of complete nutrient starvation on the barotolerance of *L. monocytogenes*.

Reductions of starved *L. monocytogenes* were seen in the order of 1.68 Log CFU/ml after 2 days of starvation in 0.85% NaCl after which viable populations of the pathogen remained relatively constant during the remainder of the study. Reductions in viable counts from 1-3 Log_{10} CFU/ml have been reported in the literature as being a common response to starvation. Mendonca et al. (199) noted that a decrease in the starved population of *L. monocytogenes* based on plate counts were 1.5 Log CFU/ml. Similar decreases in starved populations of *S. aureus, E. coli* and *Salmonella typhimurium* have been seen (284, 295, 327). The apparent stability in viable numbers of starved cells following an initial decrease

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in viability has been hypothesized to be related to cryptic growth and nutrient support for the maintenance of the remaining population (128). Viable-but-not-culturable (VBNC) cells may exist in these starved populations during a "dormant" type cell phase (199, 257). Increased adhesion during starvation may contribute to clumping of cells and thus a seemingly lower viability when examining cells using plate count methods (40, 126). This proposed explanation for the decrease seen in viable counts has not been addressed here. Further research should be conducted to examine the impact of VBNC state and adhesion proteins on the initial decrease in viability of *L. monocytogenes* during starvation.

The initial decrease in viability of *L. monocytogenes* Scott A resulted in variation of reductions from 1.10 to 3.34 Log CFU/ml (Figure 8). A lower reduction represents greater resistance of the pathogen to the pressure treatment. The initial reductions were large in some instances of starvation treatment and less as *L. monocytogenes* was starved for a greater number of days. While not statistically significant, these results represent biologically significant differences. The lack of statistical significance may be attributed to larger variations seen in the data. This initial large decrease in viability due to pressurization was also reported for other pressurization experiments involving *L. monocytogenes* (286). The initial reductions might be due to populations of a *L. monocytogenes* sample that do not have uniform pressure resistance. The pressure death kinetics relating to initial pressure treatment may differ greatly depending upon strains as well (8, 158, 262, 286). Decreases consistent with the ones seen in this study (1-3 Log CFU/ml) were seen after viability was measured during come-up pressurization at both 700 MPa and 500 MPa. Multiple strains of *L. monocytogenes* including Scott A exhibited these dramatic decreases when exposed to 700 MPa for 0.1 seconds and *L. monocytogenes* Scott A demonstrated this phenomenon after

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treatment with 500 MPa for 0.1 seconds. This pattern of decreases due to initial pressurization was not observed in treatment with 300 MPa in that study (323). Although pressure is exerted isostatically in high pressure processing, cell populations of *L. monocytogenes* did not all exhibit the same outward signs of cellular damage after pressurization (262). In the present study, variations in pressure resistance observed in *L. monocytogenes* especially at days 6 and 8 of starvation might be attributed to a transitional state in the physiology of the starved cells in which not all of the cells attained the same extent of stress hardening and resistance to high pressure.

The pressure resistance of *L. monocytogenes* observed once the samples were starved might relate to the presence of free water within the cell (120, 335). Starvation may induce shortening and widening of cells (128, 171) or condensing of cytoplasm (335) which may lower the amount of free water in the cytoplasm. The impact of protein denaturation in high pressure treatments relates to water through hydration and refolding of proteins. Lowered intracellular water activity might result in proteins that are less irreversibility denatured and thus facilitate a greater extent of reestablishment of native protein structure and function (286, 343). Wen et al. (335) hypothesized that in long term survival of cells there may be a decrease in water activity within the cells. That study also exhibited an increased barotolerance in *L. monocytogenes* upon long term survival. This similar phenomenon may be present in complete nutritionally starved cells as similar alterations to cell morphologies may be present. Other increased pressure resistances due to starvation stress have been reported for *Lactobacillus* spp. (276).

For the food industry, starvation in foodborne pathogens is an important phenomenon because water used in the cleaning and rinsing of food contact surfaces general offers a low

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nutrient environment for microorganisms. In this respect, stress from extended exposure to a nutrient-depleted environment is known to produce microbial resistance to subsequent chemical and physical stresses (153).

Based on the results of the present study, starved *L. monocytogenes* may survive high pressure treatments that may otherwise inactivate non-starved cells. The development of high pressure processes for destroying *L. monocytogenes* while maintaining desirable quality characteristics of food depends on accurate evaluation of the organism's barotolerance (D-value). Even small differences in high pressure D-values can have a significant effect on the antimicrobial efficacy of high pressure processing because of the exponential nature of microbial inactivation by this technology. Further research is needed on the resistance of other stressed pathogens to high pressure processing. This would offer food processors with more realistic, conservative information on microbial resistance to high pressure and permit the design of adequate pressure treatments that ensure the safety of high pressure treated foods.

Resistance to other processing technologies such as thermal processing has been shown. Lou and Yousef (183) found that hydrogen peroxide, acid and ethanol stress could induce resistance to heating in *L. monocytogenes*. Resistance to heating was shown in *L. monocytogenes* exposed to alkali treatment, but prior exposure to chlorine lead to a susceptibility to heating (303). UV resistance was seen in *L. monocytogenes* populations that were acid shocked (197). Starved *L. monocytogenes* had seen a resistance to irradiation treatments (199). These studies and the current research suggest that resistance of stressed cells to different food processing technologies may exist. There is an urgent need to further investigate potential of environmental stresses to cross protect pathogens against new and

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emerging food processing and preservation technologies and antimicrobial food preservatives.

The present research is of great significance to the food processing industry. The fact that prior stresses tested do not elicit a resistance of *L. monocytogenes* to freezing may allow processors to continue to use sequential minimal processing techniques on foods destined to be frozen without increasing the food safety risks regarding enhanced survival of *L. monocytogenes* in foods that are subjected to freeze-thaw cycles.

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CONCLUSIONS

The following conclusions are made based on the results of the present study:

1. Starvation of *L. monocytogenes* Scott A in physiological saline at ambient temperature increases the resistance of this organism to high hydrostatic pressure
2. Starvation of *L. monocytogenes* Scott A in physiological saline at ambient temperature for 8 to 12 days produces the highest pressure resistance in the pathogen
3. Prior environmental stresses tested in the present study do not alter the sensitivity of *L. monocytogenes* Scott A to freeze-thaw cycles.
4. The extent of sub-lethal injury in surviving populations of *L. monocytogenes* Scott A that have been exposed to stress prior to freezing, is not significantly different from that of non-stressed populations.

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FIGURES AND TABLES

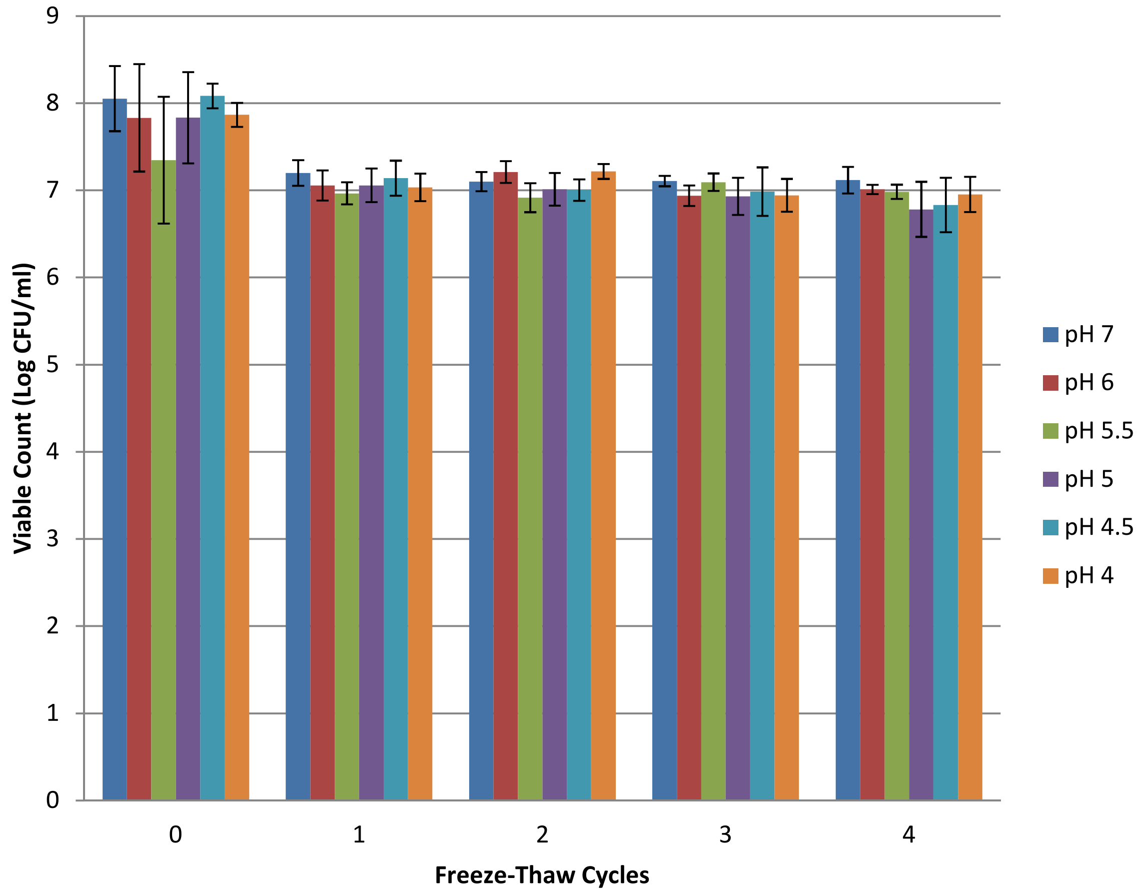

Figure 1. Viability of acid shocked (pH 4.0, 4.5, 5.0, 5.5 or 6.0) *Listeria monocytogenes* Scott A after multiple freeze-thaw cycles. Viability data represents mean Log CFU/ml ± standard deviation. "Blue" color represents control treatment. "Red" color represents pH 6.0. "Green" color represents pH 5.5. "Purple" color represents pH 5.0. "Light blue" color represents pH 4.5. "Orange" color represents pH 4.0.

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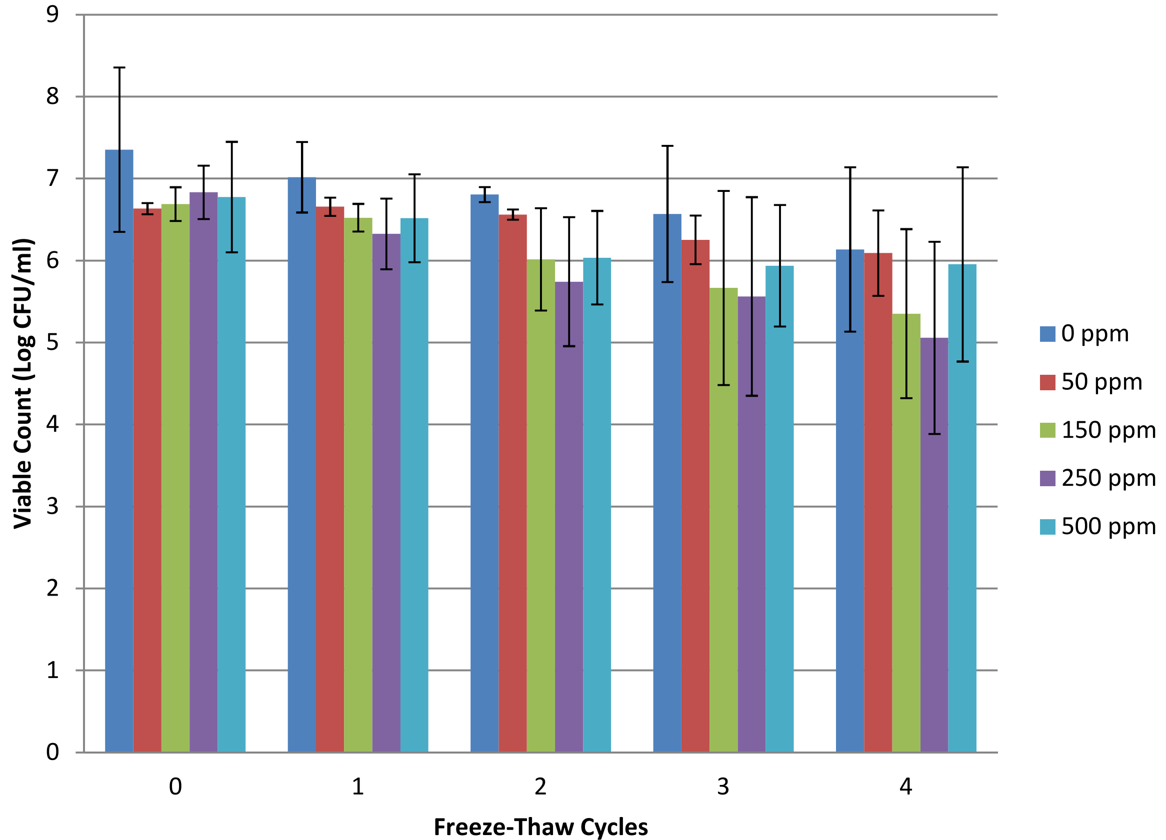

Figure 2. Viability of oxidative shocked (H2O2; 500, 250, 150 or 50 ppm *Listeria monocytogenes* Scott A after multiple freeze-thaw cycles. Viability data represents mean Log CFU/ml ± standard deviation. "Blue" color represents control treatment. "Red" color represents 50 ppm. "Green" color represents 150 ppm. "Purple" color represents 250 ppm. "Light blue" color represents 500 ppm.

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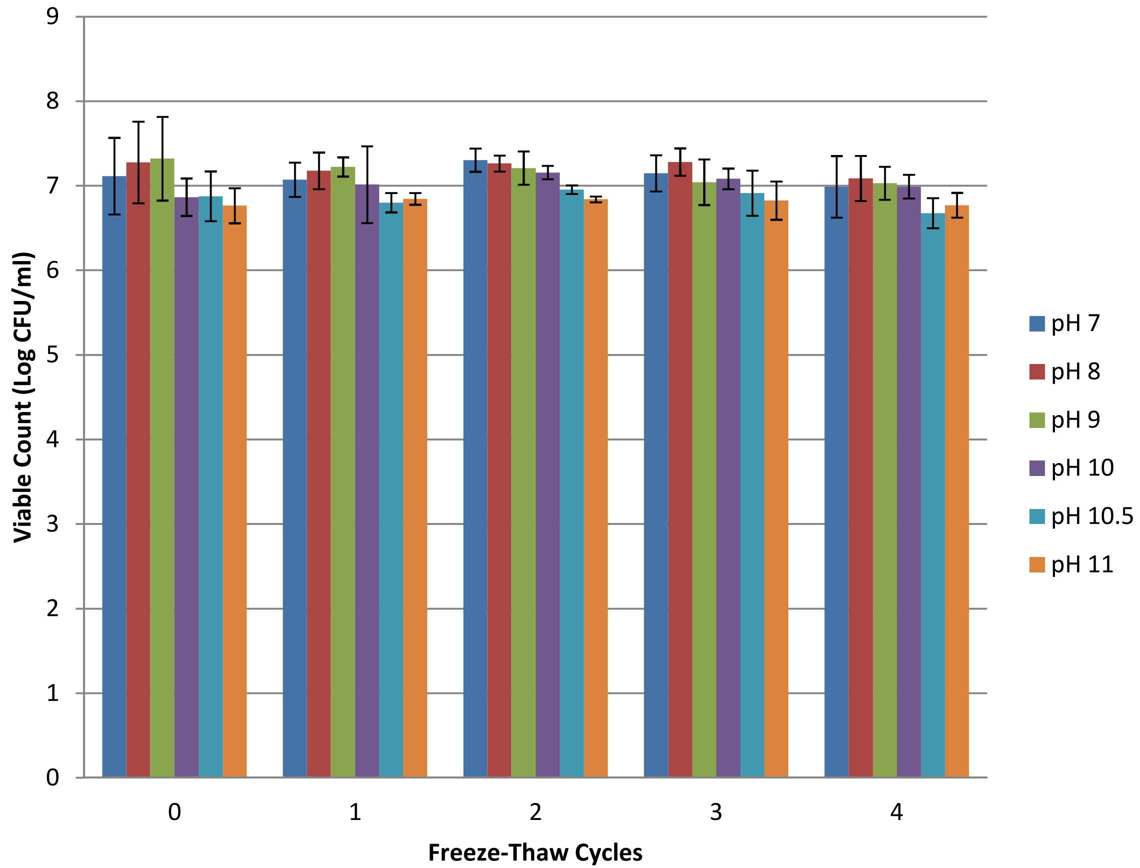

Figure 3. Viability of alkali shocked (pH 8.0, 9.0, 10.0, 10.5 or 11.0) *Listeria monocytogenes* Scott A after multiple freeze-thaw cycles. Viability data represents mean Log CFU/ml ± standard deviation. "Blue" color represents control treatment. "Red" color represents pH 8.0. "Green" color represents pH 9.0. "Purple" color represents pH 10.0. "Light blue" color represents pH 10.5. "Orange" color represents pH 11.0.

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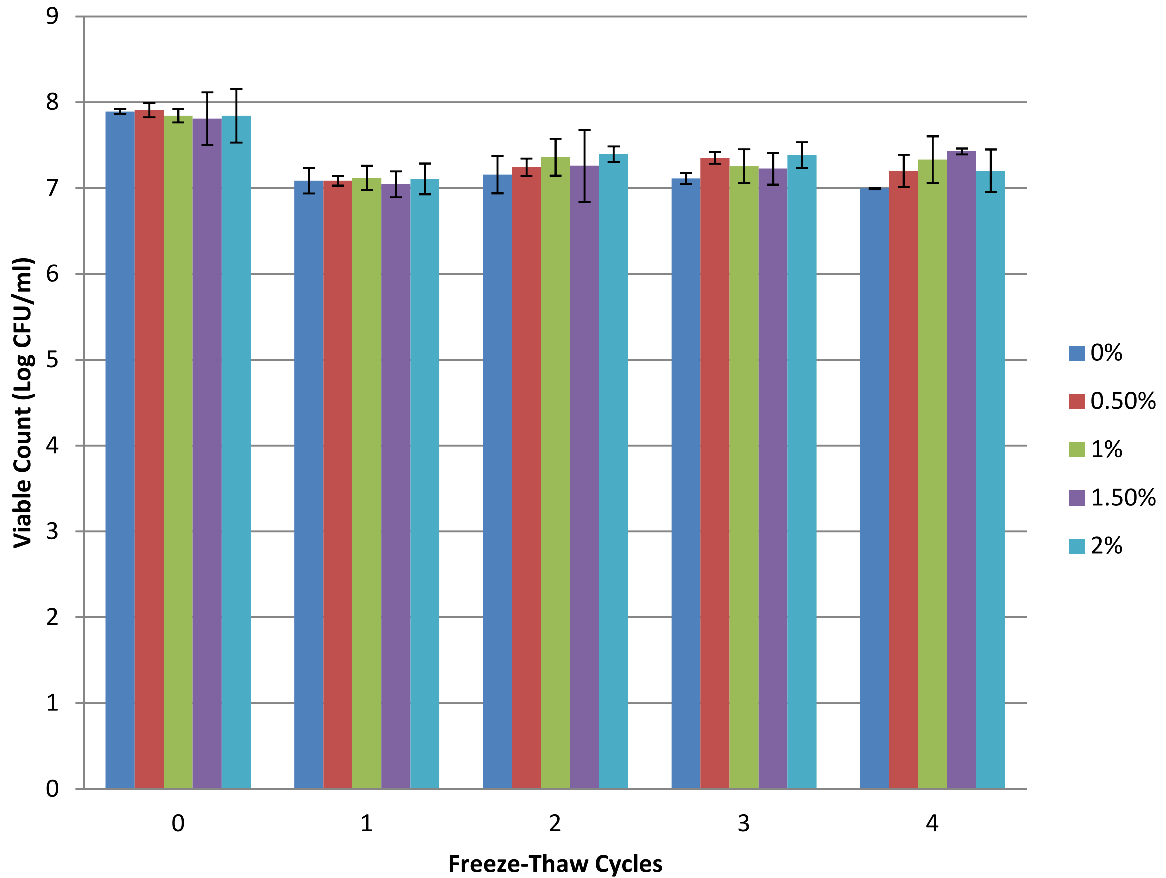

Figure 4. Viability of ethanol shocked (0.5, 1.0, 1.5 or 2.0%) *Listeria monocytogenes* Scott A after multiple freeze-thaw cycles. Viability data represents mean Log CFU/ml ± standard deviation. "Blue" color represents control treatment. "Red" color represents 0.5%. "Green" color represents 1.0%. "Purple" color represents 1.5%. "Light blue" color represents 2.0%.

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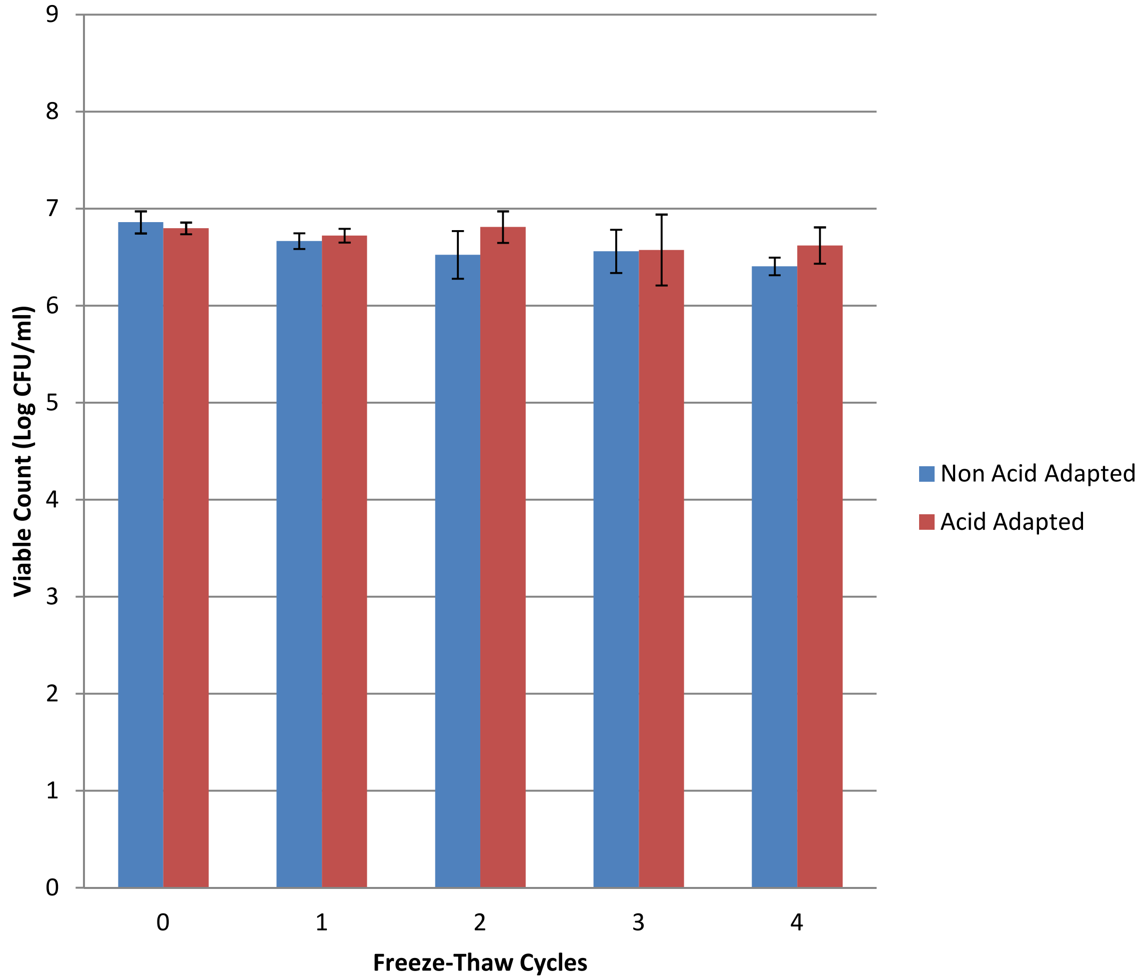

Figure 5. Viability of acid adapted *Listeria monocytogenes* Scott A after multiple freeze-thaw cycles. Viability data represents mean Log CFU/ml ± standard deviation. "Blue" color represents Non Acid Adapted cells. "Red" color represents Acid Adapted cells.

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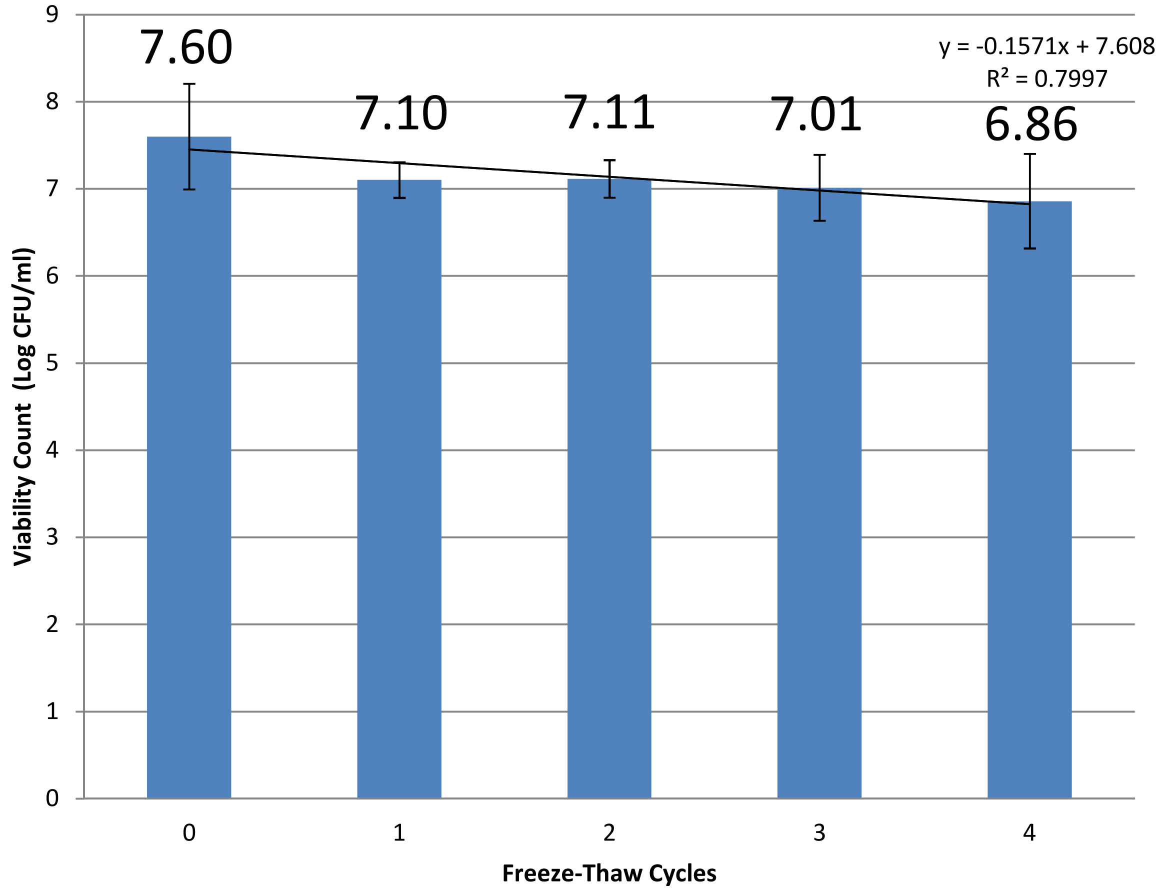

Figure 6. Viability of exponentially grown *Listeria monocytogenes* Scott A after multiple freeze-thaw cycles.

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Table 1. Comparison of the viability of acid shocked (HCl; pH 4.0, 4.5, 5.0, 5.5 or 6.0) *Listeria monocytogenes* Scott A based on the survival of the pathogen following either 1 or 4 freeze-thaw cycles. Viability (Log CFU/ml) decreases are also provided and compared across each acid treatment. Statistical comparisons also made between viability of control and treated cells just after acid treatments (bottom row below "Cycle 0" column). Similar comparisons were made between viability of control and treated cells after 4 freeze-thaw cycles of each treatment (bottom row below "Cycle 4" column). "+" indicates statistical significance ($p < 0.05$). "Not" indicates no statistical significance. "-" indicates that values are not present.

Decrease (Log CFU/ml)	***Significance***	***Cycles***		***Treatment***	***Cycles***		***Significance***	***Decrease (Log CFU/ml)***
0.8536	+	Cycle 1	← Cycle 0	Control	Cycle 0 →	Cycle 4	+	0.9357
0.8315	+	Cycle 1	← Cycle 0	pH 4.0	Cycle 0 →	Cycle 4	+	0.9121
0.994	+	Cycle 1	← Cycle 0	pH 4.5	Cycle 0 →	Cycle 4	+	1.251
0.7762	+	Cycle 1	← Cycle 0	pH 5.0	Cycle 0 →	Cycle 4	+	1.0514
0.3811	Not	Cycle 1	← Cycle 0	pH 5.5	Cycle 0 →	Cycle 4	Not	0.3626
0.7766	+	Cycle 1	← Cycle 0	pH 6.0	Cycle 0 →	Cycle 4	+	0.8221
		-	-		Not	Not		

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Table 2. Comparison of the viability of oxidative shocked (H_2O_2; 500, 250, 150, or 50 ppm) *Listeria monocytogenes* Scott A based on the survival of the pathogen following either 1 or 4 freeze-thaw cycles. Viability (Log CFU/ml) decreases are also provided and compared across each oxidative treatment. Statistical comparisons also made between viability of control and treated cells just after oxidative treatments (bottom row below "Cycle 0" column). Similar comparisons were made between viability of control and treated cells after 4 freeze-thaw cycles of each treatment (bottom row below "Cycle 4" column). "+" indicates statistical significance ($p < 0.05$). "Not" indicates no statistical significance. "-" indicates that values are not present.

Decrease (Log CFU/ml)	***Significance***	***Cycles***		***Treatment***	***Cycles***		***Significance***	***Decrease (Log CFU/ml)***
0.3361	Not	Cycle 1	← Cycle 0	Control	Cycle 0 →	Cycle 4	Not	1.2159
+0.02587	Not	Cycle 1	← Cycle 0	50 ppm	Cycle 0 →	Cycle 4	Not	0.5425
0.1646	Not	Cycle 1	← Cycle 0	150 ppm	Cycle 0 →	Cycle 4	+	1.3355
0.5063	Not	Cycle 1	← Cycle 0	250 ppm	Cycle 0 →	Cycle 4	+	1.7741
0.2387	Not	Cycle 1	← Cycle 0	500 ppm	Cycle 0 →	Cycle 4	Not	0.8013
		-	-		Not	Not		

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Table 3. Comparison of the viability of alkali shocked (NaOH; pH 8.0, 9.0, 10.0, 10.5 or 11.0) *Listeria monocytogenes* Scott A based on the survival of the pathogen following either 1 or 4 freeze-thaw cycles. Viability (Log CFU/ml) decreases are also provided and compared across each alkali treatment. Statistical comparisons also made between viability of control and treated cells just after alkali treatments (bottom row below "Cycle 0" column). Similar comparisons were made between viability of control and treated cells after 4 freeze-thaw cycles of each treatment (bottom row below "Cycle 4" column). "+" indicates statistical significance ($p < 0.05$). "Not" indicates no statistical significance. "-" indicates that values are not present.

Decrease (Log CFU/ml)	*Significance*	*Cycles*		*Treatment*	*Cycles*		*Significance*	*Decrease (Log CFU/ml)*
0.04267	Not	Cycle 1	← Cycle 0	Control	Cycle 0 →	Cycle 4	Not	0.1264
0.09967	Not	Cycle 1	← Cycle 0	pH 8	Cycle 0 →	Cycle 4	Not	0.189
0.76339	Not	Cycle 1	← Cycle 0	pH 9	Cycle 0 →	Cycle 4	Not	0.9579
+0.1478	Not	Cycle 1	← Cycle 0	pH 10	Cycle 0 →	Cycle 4	Not	+0.1247
0.07576	Not	Cycle 1	← Cycle 0	pH 10.5	Cycle 0 →	Cycle 4	Not	0.1988
+0.08084	Not	Cycle 1	← Cycle 0	pH 11.0	Cycle 0 →	Cycle 4	Not	+0.00656
		-	-		Not	Not		

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Table 4. Comparison of the viability of ethanol shocked (0.5, 1.0, 1.5, or 2.0%) *Listeria monocytogenes* Scott A based on the survival of the pathogen following either 1 or 4 freeze-thaw cycles. Viability (Log CFU/ml) decreases are also provided and compared across each ethanol treatment. Statistical comparisons also made between viability of control and treated cells just after ethanol treatments (bottom row below "Cycle 0" column). Similar comparisons were made between viability of control and treated cells after 4 freeze-thaw cycles of each treatment (bottom row below "Cycle 4" column). "+" indicates statistical significance ($p < 0.05$). "Not" indicates no statistical significance. "-" indicates that values are not present.

Decrease (Log CFU/ml)	*Significance*	*Cycles*		*Treatment*	*Cycles*		*Significance*	*Decrease (Log CFU/ml)*
0.8067	+	Cycle 1	← Cycle 0	Control	Cycle 0 →	Cycle 4	+	0.8949
0.8221	+	Cycle 1	← Cycle 0	0.50%	Cycle 0 →	Cycle 4	+	0.7068
0.7231	+	Cycle 1	← Cycle 0	1%	Cycle 0 →	Cycle 4	Not	0.5116
0.7639	+	Cycle 1	← Cycle 0	1.50%	Cycle 0 →	Cycle 4	Not	0.445
0.7359	+	Cycle 1	← Cycle 0	2%	Cycle 0 →	Cycle 4	+	0.6413
		-	-		Not	Not		

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Table 5. Comparison of the viability of acid adapted *Listeria monocytogenes* Scott A based on the survival of the pathogen following either 1 or 4 freeze-thaw cycles. Viability (Log CFU/ml) decreases are also provided and compared across each treatment. Statistical comparisons also made between viability of control and treated cells just after each treatment (bottom row below "Cycle 0" column). Similar comparisons were made between viability of control and treated cells after 4 freeze-thaw cycles of each treatment (bottom row below "Cycle 4" column). "+" indicates statistical significance ($p < 0.05$). "Not" indicates no statistical significance. "-" indicates that values are not present.

Decrease (Log CFU/ml)	*Significance*	*Cycles*		*Treatment*	*Cycles*		*Significance*	*Decrease (Log CFU/ml)*
0.1934	Not	Cycle 1	← Cycle 0	Non Adapted	Cycle 0 →	Cycle 4	Not	0.4807
0.07473	Not	Cycle 1	← Cycle 0	Adapted	Cycle 0 →	Cycle 4	Not	0.1768
		-	-		Not	Not		

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Table 6. Comparison of the viability of untreated (control) *Listeria monocytogenes* Scott A based on the survival of the pathogen following either 1 or 4 freeze-thaw cycles. Viability (Log CFU/ml) decreases are also provided and compared across untreated samples. "+" indicates statistical significance ($p < 0.05$). "Not" indicates no statistical significance. "-" indicates that values are not present.

Decrease (Log CFU/ml)	***Significance***	***Cycles***		***Treatment***	***Cycles***		***Significance***	***Decrease (Log CFU/ml)***
0.5	+	Cycle 1	← Cycle 0	Control	Cycle 0 →	Cycle 4	+	0.74
		-	-		-	-		

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Table 7. Percent injury (mean percent ± standard deviation) of acid adapted and non-adapted *Listeria* monocytogenes Scott A after exposure to freeze-thaw cycles.

	Acid Adaptation	
Freeze-Thaw Cycles	Non-Adapted	Adapted
0	-13.1 ± 17.2	3.89 ± 19.6
1	15.2 ± 6.55	16.8 ± 7.38
2	4.37 ± 5.47	11.1 ± 6.06
3	36.1 ± 39.12	-52.6 ± 142.9
4	-5.75 ± 14.37	6.01 ± 28.8

Table 8. Percent injury (mean percent ± standard deviation) of oxidative shocked (H_2O_2, 50, 150, 250 and 500 ppm) *Listeria* monocytogenes Scott A after exposure to freeze-thaw cycles.

	Oxidative Shock				
Freeze-Thaw Cycles	Control	50 ppm	150 ppm	250 ppm	500 ppm
0	17.6 ± 13.7	12.8 ± 20.5	20.2 ± 14.8	13.7 ± 18.9	11.3 ± 6.56
1	-5.47 ± 14.6	19.4 ± 28.4	8.54 ± 9.10	8.56 ± 19.5	14.1 ± 8.85
2	5.65 ± 1.56	29.2 ± 49.9	17.9 ±72.6	9.43 ± 71.8	47.8 ± 22.4
3	-29.4 ± 42.1	8.63 ± 5.49	-24.6 ± 74.3	13.7 ± 23.7	17.0 ± 13.2
4	-14.0 ± 19.8	5.52 ± 24.8	9.79 ± 28.93	12.3 ± 12.8	37.9 ± 18.1

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Table 9. Percent injury (mean percent ± standard deviation) of acid shocked (HCl, pH 4, 4.5, 5.0, 5.5 and 6) *Listeria* monocytogenes Scott A after exposure to freeze-thaw cycles.

	Acid Shock					
Freeze-Thaw Cycles	Control	pH 6	pH 5.5	pH 5	pH 4.5	pH 4
0	30.8 ± 20.5	-4.66 ± 36.7	-339.8 ± 621.2	17.0 ± 7.48	15.1 ± 15.9	8.95 ± 32.8
1	25.3 ± 6.41	16.1 ± 9.38	17.3 ± 4.80	28.3 ± 5.31	15.6 ± 8.94	17.8 ± 11.6
2	6.58 ± 26.1	12.1 ± 6.81	26.8 ± 6.55	21.6 ± 24.8	20.4 ± 4.90	27.0 ± 2.11
3	15.3 ± 15.6	23.4 ± 17.7	2.53 ± 14.4	8.3 ± 15.2	31.6 ± 18.7	7.80 ± 23.2
4	18.5 ± 16.8	23.1 ± 5.77	16.9 ± 14.2	1.31 ± 9.38	7.40 ± 23.1	22.5 ± 4.63

Table 10. Percent injury (mean percent ± standard deviation) of ethanol shocked (2.0%, 1.5%, 1.0%, and 0.5%) *Listeria* monocytogenes Scott A after exposure to freeze-thaw cycles.

	Ethanol Shock				
Freeze-Thaw Cycles	Control	0.50%	1%	1.50%	2%
0	19.0 ± 4.45	6.12 ± 4.50	15.8 ± 18.5	17.4 ± 6.81	16.3 ± 13.0
1	18.8 ± 16.3	2.63 ± 13.1	-4.70 ± 12.9	4.06 ± 12.4	-2.79 ± 17.4
2	56.5 ± 52.6	-2.80 ± 29.0	27.9 ± 4.47	23.5 ± 22.2	16.5 ± 7.92
3	-14.4 ± 5.13	8.87 ± 7.4	12.0 ± 18.2	4.08 ± 21.9	15.7 ± 25.9
4	-3.81 ± 4.0	9.82 ± 5.47	21.9 ± 15.9	24.6 ± 3.85	24.3 ± 29.3

Table 11. Percent injury (mean percent ± standard deviation) of alkali shocked (NaOH, pH 8.0, 9.0, 10.0, 10.5, and 11.0) *Listeria* monocytogenes Scott A after exposure to freeze-thaw cycles.

	Alkali Shock					
Freeze-Thaw Cycles	Control	pH 8	pH 9	pH 10	pH 10.5	pH 11
0	14.9 ± 33.7	5.36 ± 25.0	45.9 ± 46.1	8.97 ± 8.35	14.9 ± 11.4	18.0 ± 22.5
1	21.9 ± 6.09	27.1 ± 11.6	34.0 ± 33.2	24.9 ± 16.3	26.8 ± 12.4	4.24 ± 22.4
2	14.9 ± 7.71	15.8 ± 9.96	19.2 ± 13.2	12.4 ± 7.88	2.55 ± 14.7	10.8 ± 28.5
3	16.0 ± 15.1	11.7 ± 21.6	6.73 ± 14.1	26.1 ± 9.34	-7.91 ± 32.6	19.6 ± 16.9
4	29.3 ± 27.8	33.8 ± 6.58	12.6 ± 9.13	23.3 ± 24.6	8.52 ± 10.5	22.9 ± 15.0

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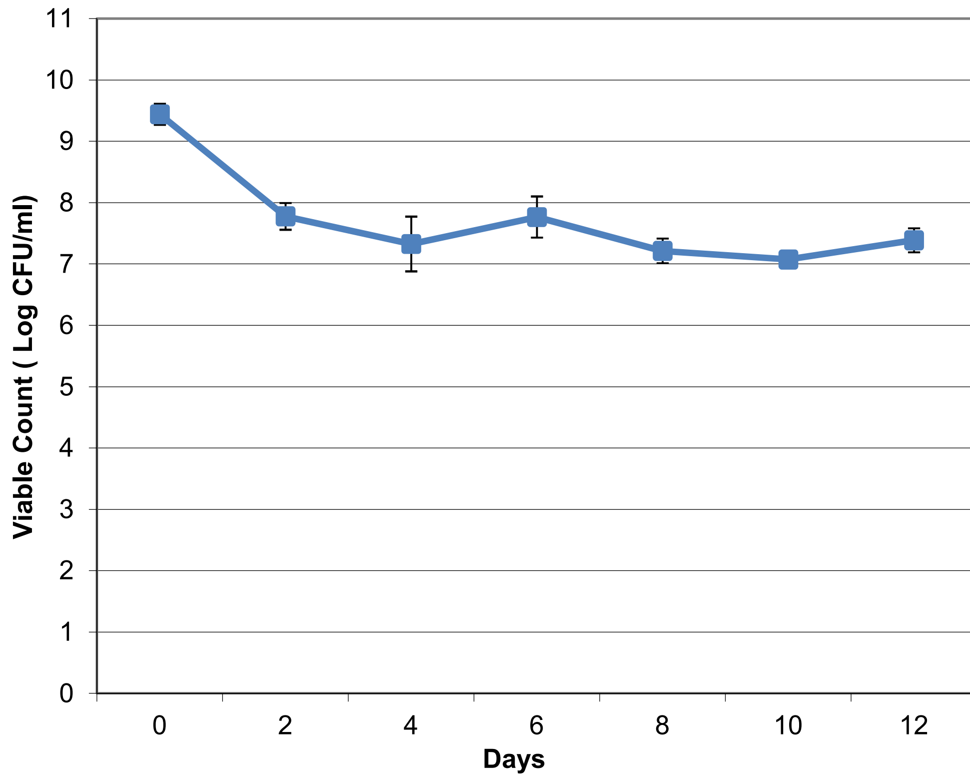

Figure 7. Viability of *Listeria monocytogenes* Scott A during 12 days starvation in 0.85% NaCl (w/v) at 25°C.

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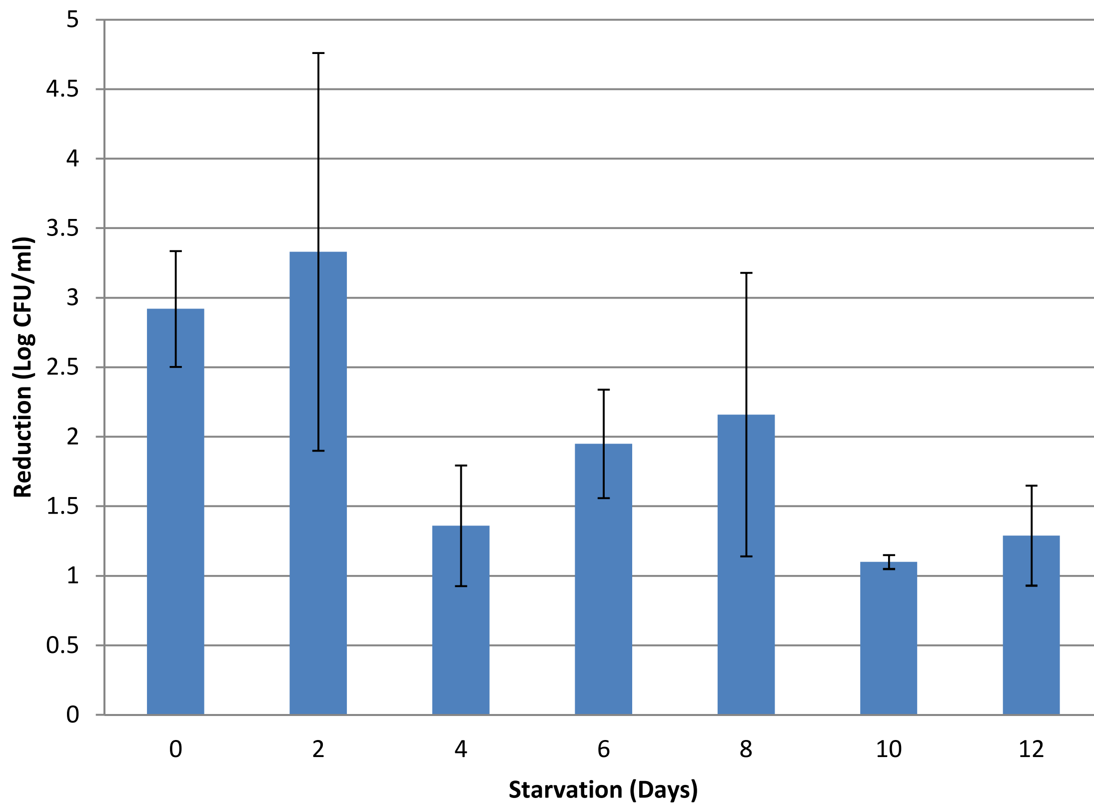

Figure 8. Influence of starvation on the extent of reduction in initial viable counts of *L. monocytogenes* Scott A following 1 second of exposure to high hydrostatic pressure (400 MPa).

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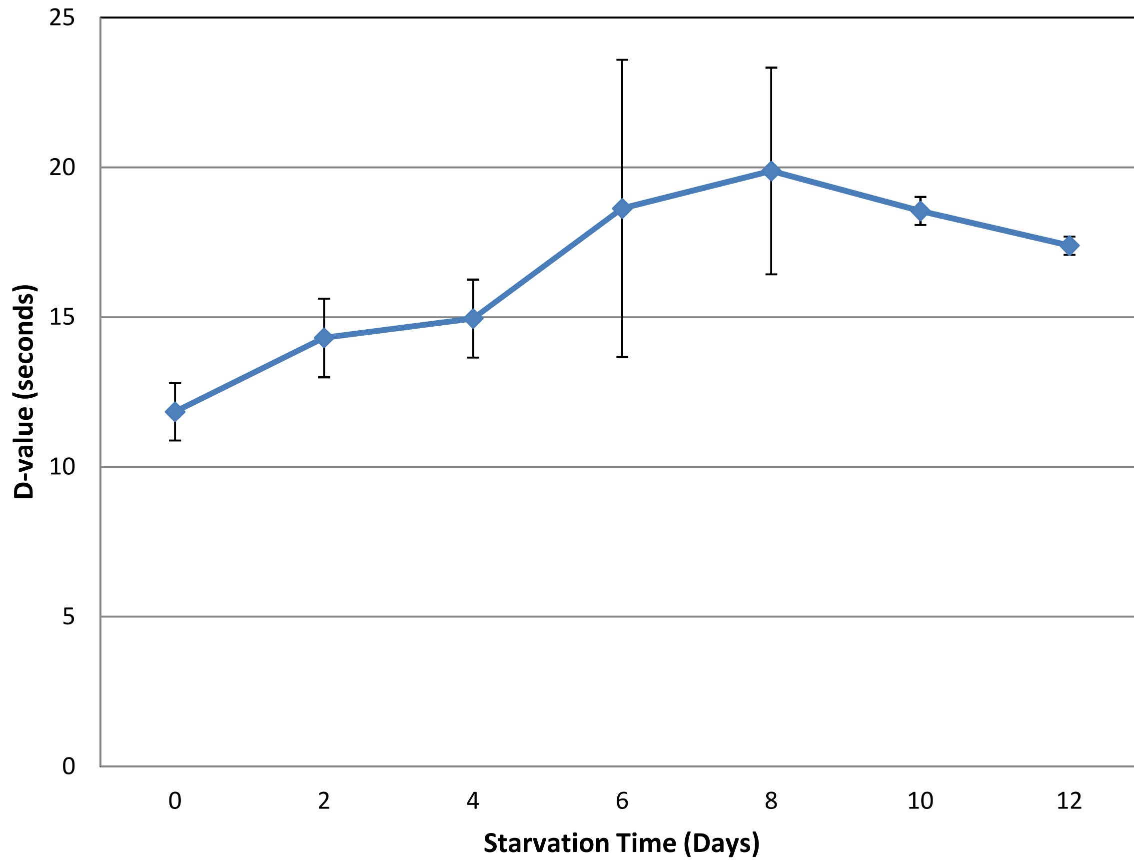

Figure 9. Changes in pressure resistance (D-Value) of *Listeria monocytogenes* Scott A during starvation in 0.85% NaCl (25°C). Cells were exposed to 400 MPa for various times. D-Values were calculated based upon the negative reciprocal of the slope of these treatment survival lines.

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REFERENCES

1. **Acciari, V., M. Torresi, G. Migliorati, E. Di Giannatale, P. Semprini, and V. Prencipe.** 2011. Characterisation of *Listeria monocytogenes* strains isolated from soft and semi-soft cheeses sampled in a region of Italy. Veterinaria Italiana **47:**15-23.
2. **Acheson, D. W. K.** 2004. Food-borne illnesses, p. 480-498. *In* M. Schaechter (ed.), The Desk Encyclopedia of Microbiology. Elsevier Academic Press, San Diego, California.
3. **Aertsen, A., K. Vanoirbeek, P. De Spiegeleer, J. Sermon, K. Hauben, A. Farewell, T. Nyström, and C. W. Michiels.** 2004. Heat shock protein-mediated resistance to high hydrostatic pressure in *Escherichia coli*. Applied and Environmental Microbiology **70:**2660-2666.
4. **Ahmed, Y., and L. Beatrice.** 2007. Characteristics of *Listeria monocytogenes* Important to Food Processors, p. 157-213, Listeria, Listeriosis, and Food Safety, Third Edition. CRC Press.
5. **Aldea, M., T. Garrido, and A. Tormo.** 1993. Gearbox gene-expression and growth-rate. World Journal of Microbiology & Biotechnology **9:**414-420.
6. **Alfonso, T.** 2004. Frozen Meat, Handbook of Frozen Foods. CRC Press.
7. **Alonso-Hernando, A., R. Capita, M. Prieto, and C. Alonso-Calleja.** 2009. Adaptation and cross-adaptation of *Listeria monocytogenes* and *Salmonella enterica* to poultry decontaminants. Journal of Microbiology **47:**142-146.
8. **Alpas, H., N. Kalchayanand, F. Bozoglu, and B. Ray.** 2000. Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. International Journal of Food Microbiology **60:**33-42.
9. **Amoril, J., and A. Bhunia.** 1999. Immunological and cytopathogenic properties of *Listeria monocytogenes* isolated from naturally contaminated meats. Journal of Food Safety **19:**195-207.
10. **Annous, B. A., L. A. Becker, D. O. Bayles, D. P. Labeda, and B. J. Wilkinson.** 1997. Critical role of anteiso-C15:0 fatty acid in the growth of *Listeria monocytogenes* at low temperatures. Appl Environ Microbiol **63:**3887-3894.
11. **Antonini, G., M. Catania, R. Greco, C. Longhi, M. Pisciotta, L. Seganti, and P. Valenti.** 1997. Anti-invasive activity of bovine lactoferrin against *Listeria monocytogenes*. Journal of Food Protection **60:**267-271.
12. **Antoniollo, P., F. Bandeira, M. Jantzen, E. Duval, and W. Da Silva.** 2003. Prevalence of *Listeria* spp. in feces and carcasses at a lamb packing plant in Brazil. Journal of Food Protection **66:**328-330.
13. **Archambaud, C., M. A. Nahori, J. Pizarro-Cerda, P. Cossart, and O. Dussurget.** 2006. Control of *Listeria* superoxide dismutase by phosphorylation. Journal of Biological Chemistry **281:**31812-31822.
14. **Archer, D.** 2004. Freezing: an underutilized food safety technology? International Journal of Food Microbiology **90:**127-138.

المنارة للاستشارات
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15. **Arumugaswamy, R., G. Ali, and S. Hamid.** 1994. Prevelance of L*isteria monocytogenes* in foods in Malaysia. International Journal of Food Microbiology **23:**117-121.
16. **Azizoglu, R., and S. Kathariou.** 2010. Impact of Growth Temperature and Agar Versus Liquid Media on Freeze-Thaw Tolerance of *Yersinia enterocolitica*. Foodborne Pathogens and Disease **7:**1125-1128.
17. **Azizoglu, R., J. Osborne, S. Wilson, and S. Kathariou.** 2009. Role of Growth Temperature in Freeze-Thaw Tolerance of *Listeria* spp. Applied and Environmental Microbiology **75:**5315-5320.
18. **Baek, S., S. Lim, D. Lee, K. Min, and C. Kim.** 2000. Incidence and characterization of *Listeria monocytogenes* from domestic and imported foods in Korea. Journal of Food Protection **63:**186-189.
19. **Bakardjiev, A., B. Stacy, S. Fisher, and D. Portnoy.** 2004. Listeriosis in the pregnant guinea pig: a model of vertical transmission. Infection and Immunity **72:**489-497.
20. **Banon, S., J. Cayuela, M. Granados, and M. Garrido.** 1999. Pre-cure freezing affects proteolysis in dry-cured hams. Meat Science **51:**11-16.
21. **Barker, C., and S. F. Park.** 2001. Sensitization of *Listeria monocytogenes* to low pH, organic acids, and osmotic stress by ethanol. Applied and Environmental Microbiology **67:**1594-1600.
22. **Bayles, D., B. Annous, and B. Wilkinson.** 1996. Cold stress proteins induced in *Listeria monocytogenes* in response to temperature downshock and growth at low temperatures. Applied and Environmental Microbiology **62:**1116-1119.
23. **Bayles, D., M. Tunick, T. Foglia, and A. Miller.** 2000. Cold shock and its effect on ribosomes and thermal tolerance in *Listeria monocytogenes*. Applied and Environmental Microbiology **66:**4351-4355.
24. **Belessi, C., A. Gounadaki, A. Psomas, and P. Skandamis.** 2011. Efficiency of different sanitation methods on *Listeria monocytogenes* biofilms formed under various environmental conditions. International Journal of Food Microbiology **145:**S46-S52.
25. **Bell, C., and A. Kyriakidaes.** 2005. *Listeria*: A practical approach to the organism and its control in foods, 2nd ed. Blackwell Publishing Ltd, Ames, Iowa.
26. **Berrang, M., R. Meinersmann, J. Frank, and S. Ladely.** 2010. Colonization of a Newly Constructed Commercial Chicken Further Processing Plant with *Listeria monocytogenes*. Journal of Food Protection **73:**286-291.
27. **Beuchat, L., C. Pettigrew, M. Tremblay, B. Roselle, and A. Scouten.** 2004. Lethality of chlorine, chlorine dioxide, and a commercial fruit and vegetable sanitizer to vegetative cells and spores of *Bacillus cereus* and spores of *Bacillus thuringiensis*. Journal of Food Protection **67:**1702-1708.
28. **Beumer, R., M. Giffel, L. Cox, F. Rombouts, and T. Abee.** 1994. Effect of exogenous proline, betaine, and carnitine on growth of *Listeria monocytogenes* in a minimal medium. Applied and Environmental Microbiology **60:**1359-1363.
29. **Bialka, K., A. Demirci, S. Knabel, P. Patterson, and V. Puri.** 2004. Efficacy of electrolyzed oxidizing water for the microbial safety and quality of eggs. Poultry Science **83:**2071-2078.

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30. **Bigot, A., E. Botton, I. Dubail, and A. Charbit.** 2006. A homolog of *Bacillus subtilis* trigger factor in *Listeria monocytogenes* is involved in stress tolerance and bacterial virulence. Applied and Environmental Microbiology **72:**6623-6631.
31. **Bohne, J., Z. Sokolovic, and W. Goebel.** 1994. Transcriptional regulation of prfA and PrfA-regulated virulence genes in *Listeria monocytogenes*. Molecular Microbiology **11:**1141-1150.
32. **Braden, C., and D. Norton.** 2007. Foodborne Listeriosis, p. 305-356. *In* E. T. Ryser and E. H. Marth (ed.), Listeria, Listeriosis, and Food Safety, 3rd ed. CRC Press, Boca Raton, FL.
33. **Bremer, P., and S. Ridley.** 2004. Safety of Frozen Foods. *In* K. Murrell, Y. Hui, M. Lim, I. G. Legarreta, and P. Cornillon (ed.), Handbook of Frozen Foods. CRC Press, New York, NY.
34. **Brosch, R., C. Buchrieser, and J. Rocourt.** 1991. Subtyping of *Listeria monocytogenes* serovar 4b by use of low-frequency-cleavage restriction endonucleases and pulsed-field gel electrophoresis. Research in Microbiology **142:**667-675.
35. **Brosch, R., C. Buchrieser, B. Sixl-Voigt, and J. Rocourt.** 1991. Use of pulsed field electrophoresis of DNA restriction fragments for comparing *Listeria monocytogenes* strains isolated from human infections and food in Austria. Zentralbl Bakteriol **275:**557-560.
36. **Bubert, A., Z. Sokolovic, S. Chun, L. Papatheodorou, A. Simm, and W. Goebel.** 1999. Differential expression of Listeria monocytogenes virulence genes in mammalian host cells. Molecular and General Genetics **261:**323-336.
37. **Buchanan, R., M. Golden, R. Whiting, J. Phillips, and J. Smith.** 1994. Nonthermal inactivation models for *Listeria monocytogenes*. Journal of Food Science **59:**179-188.
38. **Buchrieser, C., R. Brosch, and J. Rocourt.** 1991. Use of pulsed field gel electrophoresis to compare large DNA-restriction fragments of *Listeria monocytogenes* strains belonging to serogroups 1/2 and 3. International Journal of Food Microbiology **14:**297-304.
39. **Buduamoako, E., S. Toora, R. Ablett, and J. Smith.** 1992. Evaluation of the ability of primary selective enrichment to resuscitate heat-injured and freeze-injured L*isteria monocytogenes* cells. Applied and Environmental Microbiology **58:**3177-3179.
40. **Bunning, V. K., R. G. Crawford, J. T. Tierney, and J. T. Peeler.** 1992. Thermotolerance of heat-shocked *Listeria monocytogenes* in milk exposed to high-temperature, short-time pasteurization. Applied and Environmental Microbiology **58:**2096-2098.
41. **Campus, M.** 2010. High Pressure Processing of Meat, Meat Products and Seafood. Food Engineering Reviews **2:**256-273.
42. **Capozzi, V., D. Fiocco, M. Amodio, A. Gallone, and G. Spano.** 2009. Bacterial Stressors in Minimally Processed Food. International Journal of Molecular Sciences **10:**3076-3105.
43. **Capozzi, V., S. Weidmann, D. Fiocco, A. Rieu, P. Hols, J. Guzzo, and G. Spano.** 2011. Inactivation of a small heat shock protein affects cell morphology and

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membrane fluidity in *Lactobacillus plantarum* WCFS1. Research in Microbiology **162:**419-425.

44. **Carpentier, B., and O. Cerf.** 2011. Review - Persistence of *Listeria monocytogenes* in food industry equipment and premises. International Journal of Food Microbiology **145:**1-8.
45. **Cataldo, G., M. Conte, F. Chiarini, L. Seganti, M. Ammendolia, F. Superti, and C. Longhi.** 2007. Acid adaptation and survival of *Listeria monocytogenes* in Italian-style soft cheeses. Journal of Applied Microbiology **103:**185-193.
46. **Chan, Y. C., S. Raengpradub, K. J. Boor, and M. Wiedmann.** 2007. Microarray-based characterization of the *Listeria monocytogenes* cold regulon in log- and stationary-phase cells. Applied and Environmental Microbiology **73:**6484-6498.
47. **Chan, Y. C., and M. Wiedmann.** 2009. Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. Critical Reviews in Food Science and Nutrition **49:**237-253.
48. **Chang, V., E. Mills, and C. Cutter.** 2003. Comparison of recovery methods for freeze-injured *Listeria monocytogenes*, *Salmonella typhimurium*, and *Campylobacter coli* in cell suspensions and associated with pork surfaces. Journal of Food Protection **66:**798-803.
49. **Charbonneau, D., J. Ponte, and B. Kochanowski.** 2000. A method of assessing the efficacy of hand sanitizers: Use of real soil encountered in the food service industry. Journal of Food Protection **63:**495-501.
50. **Charlebois, S., and H. Horan.** 2010. Institutional and relational determinants in high- and medium-extent food product crises: the inner perspective of a public health crisis. International Journal of Environmental Health research **20:**299-312.
51. **Chaturongakul, S., S. Raengpradub, M. Palmer, T. Bergholz, R. Orsi, Y. Hu, J. Ollinger, M. Wiedmann, and K. Boor.** 2011. Transcriptomic and Phenotypic Analyses Identify Coregulated, Overlapping Regulons among PrfA, CtsR, HrcA, and the Alternative Sigma Factors sigma(B), sigma(C), sigma(H), and sigma(L) in *Listeria monocytogenes*. Applied and Environmental Microbiology **77:**187-200.
52. **Chaturongakul, S., S. Raengpradub, M. Wiedmann, and K. Boor.** 2008. Modulation of stress and virulence in *Listeria monocytogenes*. Trends in Microbiology **16:**388-396.
53. **Cheftel, J.** 1995. Review: High-pressure, microbial inactivation and food preservation. Food Science and Technology International **1:**75-90.
54. **Chen, Y., E. Ross, V. Scott, and D. Gombas.** 2003. *Listeria monocytogenes*: Low levels equal low risk. Journal of Food Protection **66:**570-577.
55. **Chiang, M. L., W. L. Ho, and C. C. Chou.** 2006. Response of Vibrio parahaemolyticus to ethanol shock. Food Microbiology **23:**461-467.
56. **Christman, M., G. Storz, and B. Ames.** 1989. oxyr, a positive regulator of hydrogen peroxide-inducible genes in *escherichia coli* and *salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins. Proceedings of the National Academy of Sciences of the United States of America **86:**3484-3488.
57. **Colburn, K., C. Kaysner, C. Abetya, and M. Wekell.** 1990. *Listeria s*pecies in a California coast estuarine environment. Applied and Environmental Microbiology **56:**2007-2011.

المنارة للاستشارات
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58. **Comi, G., R. Frigerio, and C. Cantoni.** 1992. *Listeria monocytogenes* serotypes in Italian meat products. Letters in Applied Microbiology **15:**168-171.
59. **Cossart, P., and P. Sansonetti.** 2004. Bacterial invasion: The paradigms of enteroinvasive pathogens. Science **304:**242-248.
60. **Cossart, P., and A. Toledo-Arana.** 2008. *Listeria monocytogenes*, a unique model in infection biology: an overview. Microbes and Infection **10:**1041-1050.
61. **Cotter, P., C. Gahan, and C. Hill.** 2001. A glutamate decarboxylase system protects *Listeria monocytogenes* in gastric fluid. Molecular Microbiology**:**465-475.
62. **Cotter, P., C. Gahan, and C. Hill.** 2000. Analysis of the role of the *Listeria monocytogenes* F0F1-ATPase operon in the acid tolerance response. International Journal of Food Microbiology**:**137-146.
63. **Cotter, P., K. O'Reilly, and C. Hill.** 2001. Role of the glutamate decarboxylase acid resistance system in the survival of *Listeria monocytogenes* LO28 in low pH foods. Journal of Food Protection**:**1362-1368.
64. **Cotter, P., S. Ryan, C. Gahan, and C. Hill.** 2005. Presence of GadD1 glutamate decarboxylase in selected *Listeria monocytogenes* strains is associated with an ability to grow at low pH. Applied and Environmental Microbiology**:**2832-2839.
65. **Cressy, H., A. Jerrett, C. Osborne, and P. Bremer.** 2003. A novel method for the reduction of numbers of *Listeria monocytogenes* cells by freezing in combination with an essential oil in bacteriological media. Journal of Food Protection **66:**390-395.
66. **Dantigny, P., A. Guilmart, F. Radoi, M. Bensoussan, and M. Zwietering.** 2005. Modelling the effect of ethanol on growth rate of food spoilage moulds. International Journal of Food Microbiology **98:**261-269.
67. **Database, F. I. O.** 2008. Maple leaf consumer foods of Canada deli meat-Canada 2008.
68. **Database, F. I. O.** 2010. SanGar Produce & Processing Company Chopped Celery 2010.
69. **de Pablo, M., M. Puertollano, A. Galvez, E. Ortega, J. Gaforio, and G. de Cienfuegos.** 2000. Determination of natural resistance of mice fed dietary lipids to experimental infection induced by *Listeria monocytogenes*. Fems Immunology and Medical Microbiology **27:**127-133.
70. **Donnelly, C.** 2001. *Listeria monocytogenes*: A continuing challenge. Nutrition Reviews **59:**183-194.
71. **Duche, O., F. Tremoulet, A. Namane, and J. Labadie.** 2002. A proteomic analysis of the salt stress response of *Listeria monocytogenes*. FEMS Microbiology Letters **215:**183-188.
72. **Duong, T., R. Barrangou, W. Russell, and T. Klaenhammer.** 2006. Characterization of the tre locus and analysis of trehalose cryoprotection in *Lactobacillus acidophilus* NCFM. Applied and Environmental Microbiology **72:**1218-1225.
73. **El-Kest, S., and E. Marth.** 1992. Freezing of *Listeria monocytogenes* and other microorganisms: A review. Journal of Food Protection **55:**639-648.
74. **Elhanafi, D., B. Leenanon, W. Bang, and M. A. Drake.** 2004. Impact of cold and cold-acid stress on poststress tolerance and virulence factor expression of *Escherichia coli* O157:H7. Journal of Food Protection **67:**19-26.

المنارة للاستشارات
www.manaraa.com

75. **El-Kest, S., and E. Marth.** 1991. Injury and death of frozen *Listeria monocytogenes* as affected by glycerol and milk components. Journal of Dairy Science **74:**1201-1208.
76. **El-Kest, S., and E. Marth.** 1991. Strains and suspending menstrua as factors affecting death and injury of *Listeria monocytogenes d*uring freezing and frozen storage. Journal of Dairy Science **74:**1209-1213.
77. **El-Kest, S., A. Yousef, and E. Marth.** 1991. Fate of *Listeria monocytogenes* during freezing and frozen storage. Journal of Food Science **56:**1068-1071.
78. **Ells, T. C., and L. Truelstrup Hansen.** 2011. Increased Thermal and Osmotic Stress Resistance in *Listeria monocytogenes* 568 Grown in the Presence of Trehalose Due to Inactivation of the Phosphotrehalase-Encoding Gene treA. Applied and Environmental Microbiology **77:**6841-6851.
79. **Elson, R., F. Burgess, C. Little, R. Mitchell, and L. A. Coordinators.** 2004. Microbiological examination of ready-to-eat cold sliced meats and pate from catering and retail premises in the UK. Journal of Applied Microbiology **96:**499-509.
80. **Ermolenko, D. N., and G. I. Makhatadze.** 2002. Bacterial cold-shock proteins. Cellular and Molecular Life Sciences **59:**1902-1913.
81. **Ewen, T.** 2007. Listeria, p. 767-812. *In* E. T. Ryser and E. H. Marth (ed.), Listeria, Listeriosis, and Food Safety, 3rd ed. CRC Press, Boca Raton, FL.
82. **Farber, J., and F. Pagotto.** 1992. The effect of acid shock on the heat-resistance of *Listeria monocytogenes*. Letters in Applied Microbiology **15:**197-201.
83. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiology Reviews **55:**476-511.
84. **Faxén, K., J. L. Andersen, P. Gourdon, N. Fedosova, J. P. Morth, P. Nissen, and J. V. Møller.** 2011. Characterization of a *Listeria monocytogenes* Ca(2+) pump: a SERCA-type ATPase with only one Ca(2+)-binding site. Journal of Biological Chemistry **286:**1609-1617.
85. **FDA** October 25, 2011 1999, posting date. 21 CFR 184.1293 Ethyl alcohol. [Online.]
86. **FDA** October 24, 2011 2009, posting date. Food Code. [Online.]
87. **FDA** 2009, posting date. Sprout Recall Due to Potential Contamination with *Listeria*. [Online.]
88. **Ferreira, A., M. Gray, M. Wiedmann, and K. J. Boor.** 2004. Comparative genomic analysis of the sigB operon in *Listeria monocytogenes* and in other gram-positive bacteria. Current Microbiology **48:**39-46.
89. **Fiocco, D., V. Capozzi, M. Collins, A. Gallone, P. Hols, J. Guzzo, S. Weidmann, A. Rieu, T. Msadek, and G. Spano.** 2010. Characterization of the CtsR stress response regulon in *Lactobacillus plantarum*. Journal of Bacteriology **192:**896-900.
90. **Foegeding, P., A. Thomas, D. Pilkington, and T. Klaenhammer.** 1992. Enhanced control of *Listeria monocytogenes b*y insitu-produced pediocin during dry fermented sausage production. Applied and Environmental Microbiology**:**884-890.
91. **Foley, D. M., S. L. Trimboli, J. Lamb, J. Gogley, J. Thompson, F. Caporaso, M. Calicchia, and A. Prakash.** 2005. Acid-adaptation does not increase the resistance of *Listeria monocytogenes* to irradiation in a seafood salad. International Journal of Food Microbiology **99:**147-156.

المنارة للاستشارات
www.manaraa.com

92. **Fritsche, K. L., L. M. Shahbazian, C. Feng, and J. N. Berg.** 1997. Dietary fish oil reduces survival and impairs bacterial clearance in C3H/Hen mice challenged with *Listeria monocytogenes*. Clin Sci (Lond) **92:**95-101.
93. **Frye, D., R. Zweig, J. Sturgeon, M. Tormey, M. LeCavalier, I. Lee, L. Lawani, and L. Mascola.** 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes*. Clinical Infectious Diseases **35:**943-949.
94. **Gaidenko, T. A., and C. W. Price.** 1998. General stress transcription factor sigmaB and sporulation transcription factor sigmaH each contribute to survival of *Bacillus subtilis* under extreme growth conditions. Journal of Bacteriology **180:**3730-3733.
95. **Galsworthy, S. B., S. Girdler, and S. F. Koval.** 1990. Chemotaxis in *Listeria monocytogenes*. Acta Microbiol Hung **37:**81-85.
96. **Gao, D., and J. K. Critser.** 2000. Mechanisms of cryoinjury in living cells. ILAR Journal **41:**187-196.
97. **Gardan, R., P. Cossart, J. Labadie, and E. L. G. Consort.** 2003. Identification of *Listeria monocytogenes* genes involved in salt and alkaline-pH tolerance. Applied and Environmental Microbiology **69:**3137-3143.
98. **Garenaux, A., M. Ritz, F. Jugiau, F. Rama, M. Federighi, and R. de Jonge.** 2009. Role of Oxidative Stress in *C jejuni* Inactivation During Freeze-Thaw Treatment. Current Microbiology **58:**134-138.
99. **Geoffroy, C., J. Gaillard, J. Alouf, and P. Berche.** 1987. Purification, characterization, and toxicity of the sulfhydryl-activated hemolysin listeriolysin-o from *Listeria monocytogenes*. Infection and Immunity **55:**1641-1646.
100. **George, S. M., L. C. Richardson, and M. W. Peck.** 1996. Predictive models of the effect of temperature, pH and acetic and lactic acids on the growth of *Listeria monocytogenes*. International Journal of Food Microbiology **32:**73-90.
101. **Gervilla, R., V. Ferragut, and B. Guamis.** 2000. High pressure inactivation of microorganisms inoculated into ovine milk of different fat contents. Journal of Dairy Science **83:**674-682.
102. **Giotis, E., M. Julotok, B. Wilkinson, I. Blair, and D. McDowell.** 2008. Role of sigma B factor in the alkaline tolerance response of *Listeria monocytogenes* 10403S and cross-protection against subsequent ethanol and osmotic stress. Journal of Food Protection **71:**1481-1485.
103. **Giotis, E. S., I. S. Blair, and D. A. McDowell.** 2007. Morphological changes in *Listeria monocytogenes* subjected to sublethal alkaline stress. International Journal of Food Microbiology **120:**250-258.
104. **Giotis, E. S., D. A. McDowell, I. S. Blair, and B. J. Wilkinson.** 2007. Role of branched-chain fatty acids in pH stress tolerance in *Listeria monocytogenes*. Applied and Environmental Microbiology **73:**997-1001.
105. **Giotis, E. S., A. Muthaiyan, I. S. Blair, B. J. Wilkinson, and D. A. McDowell.** 2008. Genomic and proteomic analysis of the Alkali-Tolerance Response (AlTR) in *Listeria monocytogenes* 10403S. BMC Microbiology **8:**102.
106. **Giotis, E. S., A. Muthaiyan, S. Natesan, B. J. Wilkinson, I. S. Blair, and D. A. McDowell.** 2010. Transcriptome analysis of alkali shock and alkali adaptation in *Listeria monocytogenes* 10403S. Foodborne Pathogens and Disease **7:**1147-1157.

المنارة للاستشارات
www.manaraa.com

107. **Goff, H. D., and H. Richard.** 2004. Ice Cream and Frozen Desserts, Handbook of Frozen Foods. CRC Press.
108. **Golden, D., L. Beuchat, and R. Brackett.** 1988. Evaluation of selective direct plating media for their suitability to recover uninjured, heat-injured, and freeze-injured *Listeria monocytogenes* from foods. Applied and Environmental Microbiology **54:**1451-1456.
109. **Gombas, D., Y. Chen, R. Clavero, and V. Scott.** 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. Journal of Food Protection **66:**559-569.
110. **Graham, P., and T. Blumer.** 1972. Effect of pre-freezing hams on quality attributes of dry-cured product. Journal of Milk and Food Technology **35:**98-101.
111. **Graves, L., L. Helsel, A. Steigerwalt, R. Morey, M. Daneshvar, S. Roof, R. Orsi, E. Fortes, S. Milillo, H. den Bakker, M. Wiedmann, B. Swaminathan, and B. Sauders.** 2010. *Listeria marthii* sp nov., isolated from the natural environment, Finger Lakes National Forest. International Journal of Systematic and Evolutionary Microbiology **60:**1280-1288.
112. **Gravesen, A., C. Lekkas, and S. Knøchel.** 2005. Surface attachment of *Listeria monocytogenes* is induced by sublethal concentrations of alcohol at low temperatures. Applied and Environmental Microbiology **71:**5601-5603.
113. **Gray, M. L., and A. H. Killinger.** 1966. *Listeria monocytogenes* and listeric infections. Bacteriological Reviews **30:**309-382.
114. **Gray, M. L., and H. J. Stafseth.** 1948. A new technique for isolating listerellae from the bovine brain. Journal of Bacteriology **55:**471-476.
115. **Gross, M., K. Lehle, R. Jaenicke, and K. Nierhaus.** 1993. Pressure-induced dissociation of ribosomes and elongation cycle intermediates: Stabilizing conditions and identification of the most sensitive functional-state. European Journal of Biochemistry **218:**463-468.
116. **Hain, T., H. Hossain, S. Chatterjee, S. Machata, U. Volk, S. Wagner, B. Brors, S. Haas, C. Kuenne, A. Billion, S. Otten, J. Pane-Farre, S. Engelmann, and T. Chakraborty.** 2008. Temporal transcriptomic analysis of the *Listeria monocytogenes* EGD-e sigma(B) regulon. Bmc Microbiology **8:**20-31.
117. **Haldenwang, W.** 1995. The sigma-factors of *bacillus subtilis*. Microbiological Reviews **59:**1-30.
118. **Hansen, C., B. Vogel, and L. Gram.** 2006. Prevalence and survival of *Listeria monocytogenes* in Danish aquatic and fish-processing environments. Journal of Food Protection **69:**2113-2122.
119. **Hayman, M., R. Anantheswaran, and S. Knabel.** 2007. The effects of growth temperature and growth phase on the inactivation of *Listeria monocytogenes* in whole milk subject to high pressure processing. International Journal of Food Microbiology **115:**220-226.
120. **Hayman, M., G. Kouassi, R. Anantheswaran, J. Floros, and S. Knabel.** 2008. Effect of water activity on inactivation of *Listeria monocytogenes* and lactate dehydrogenase during high pressure processing. International Journal of Food Microbiology **124:**21-26.

المنارة للاستشارات
www.manaraa.com

121. **Hayman, M. M., R. C. Anantheswaran, and S. J. Knabel.** 2008. Heat shock induces barotolerance in *Listeria monocytogenes*. Journal of Food Protection **71:**426-430.
122. **Hecker, M., and U. Volker.** 2004. Towards a comprehensive understanding of *Bacillus subtilis* cell physiology by physiological proteomics. Proteomics **4:**3727-3750.
123. **Heden, C., T. Lindahl, and I. Toplin.** 1964. Stability of deoxyribonucleic acid solutions under high pressure. Acta Chemica Scandinavica **18:**1150-1156.
124. **Hefnawy, Y., S. Moustafa, and E. Marth.** 1993. Behavior of *Listeria monocytogenes* in refrigerated and frozen ground beef and in sausage and broth with and without additives. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie **26:**167-170.
125. **Heinz, V.** 2009. Food preservation by high pressure. Journal für Verbraucherschutz und Lebensmittelsicherheit **5:**73-81.
126. **Helloin, E., L. Jansch, and L. Phan-Thanh.** 2003. Carbon starvation survival of *Listeria monocytogenes* in planktonic state and in biofilm: A proteomic study. Proteomics **3:**2052-2064.
127. **Helmann, J. D., M. F. Wu, A. Gaballa, P. A. Kobel, M. M. Morshedi, P. Fawcett, and C. Paddon.** 2003. The global transcriptional response of *Bacillus subtilis* to peroxide stress is coordinated by three transcription factors. Journal of Bacteriology **185:**243-253.
128. **Herbert, K., and S. Foster.** 2001. Starvation survival in *Listeria monocytogenes*: characterization of the response and the role of known and novel components. Microbiology-Sgm **147:**2275-2284.
129. **Herbig, A. F., and J. D. Helmann.** 2001. Roles of metal ions and hydrogen peroxide in modulating the interaction of the *Bacillus subtilis* PerR peroxide regulon repressor with operator DNA. Molecular Microbiology **41:**849-859.
130. **Hermeslima, M., and K. Storey.** 1993. Antioxidant defenses in the tolerance of freezing and anoxia by garter snakes. American Journal of Physiology **265:**R646-R652.
131. **Higuchi, T., H. Hayashi, and K. Abe.** 1997. Exchange of glutamate and gamma-aminobutyrate in a *Lactobacillus* strain. Journal of Bacteriology **179:**3362-3364.
132. **Hite, B. H.** 1899. The effect of pressure in the preservation of milk. **58:**13-35.
133. **Ho, J., K. Shands, G. Friedland, P. Eckind, and D. Fraser.** 1986. An outbreak of type-4B Listeria *monocytogenes* infection involving patients from 8 Boston hospitals. Archives of Internal Medicine **146:**520-524.
134. **Holah, J., J. Taylor, D. Dawson, and K. Hall.** 2002. Biocide use in the food industry and the disinfectant resistance of persistent strains of *Listeria monocytogenes* and *Escherichia coli*. Journal of Applied Microbiology **92:**111S-120S.
135. **Hoover, D., C. Metrick, A. Papineau, D. Farkas, and D. Knorr.** 1989. Biological effects of high hydrostatic pressure on food microorganisms. Food Technology **43:**99-107.
136. **Hudson, J., S. Mott, and N. Penney.** 1994. Growth of Listeria *monocytogenes, Aeromonas hydrophila,* and *Yersinia enterocolitica* on vacuum and saturated carbon

المنارة للاستشارات
www.manaraa.com

dioxide controlled atmosphere-packaged sliced roast beef. Journal of Food Protection **57:**204-208.

137. **Hunger, K., C. L. Beckering, F. Wiegeshoff, P. L. Graumann, and M. A. Marahiel.** 2006. Cold-induced putative DEAD box RNA helicases CshA and CshB are essential for cold adaptation and interact with cold shock protein B in *Bacillus subtilis*. Journal of Bacteriology **188:**240-248.
138. **Hutkins, R.** 2006. Microbiology and technology of fermented foods. Blackwell Publishing, Ames, IA.
139. **Höper, D., U. Völker, and M. Hecker.** 2005. Comprehensive characterization of the contribution of individual SigB-dependent general stress genes to stress resistance of *Bacillus subtilis*. Journal of Bacteriology **187:**2810-2826.
140. **Igoshin, O., M. Brody, C. Price, and M. Savageau.** 2007. Distinctive topologies of partner-switching signaling networks correlate with their physiological roles. Journal of Molecular Biology **369:**1333-1352.
141. **Inouye, M., and S. Phadtare.** 2004. Cold shock response and adaptation at near-freezing temperature in microorganisms. Sci STKE **2004:**pe26.
142. **Isalan, M., C. Lemerle, K. Michalodimitrakis, C. Horn, P. Beltrao, E. Raineri, M. Garriga-Canut, and L. Serrano.** 2008. Evolvability and hierarchy in rewired bacterial gene networks. Nature **452:**840-U842.
143. **Isom, L., A. Ahmed, and S. Martin.** 1995. Influence of plasmids on catalase and superoxide-dismutase activities in *Listeria monocytogenes*. Journal of Food Protection **58:**955-959.
144. **Isom, L., Z. Khambatta, J. Moluf, D. Akers, and S. Martin.** 1995. Filament formation in *Listeria monocytogenes*. Journal of Food Protection **58:**1031-1033.
145. **Ivanek, R., Y. Grohn, and M. Wiedmann.** 2006. *Listeria monocytogenes* in multiple habitats and host populations: review of available data for mathematical modeling. Foodborne Pathogens and Disease **3:**319-336.
146. **Jackson, K. A., M. Biggerstaff, M. Tobin-D'Angelo, D. Sweat, R. Klos, J. Nosari, O. Garrison, E. Boothe, L. Saathoff-Huber, L. Hainstock, and R. P. Fagan.** 2011. Multistate outbreak of *Listeria monocytogenes* associated with Mexican-style cheese made from pasteurized milk among pregnant, Hispanic women. Journal of Food Protection **74:**949-953.
147. **Jaradat, Z. W., and A. K. Bhunia.** 2002. Glucose and nutrient concentrations affect the expression of a 104-kilodalton Listeria adhesion protein in *Listeria monocytogenes*. Applied and Environmental Microbiology **68:**4876-4883.
148. **Jasson, V., M. Uyttendaele, A. Rajkovic, and J. Debevere.** 2007. Establishment of procedures provoking sub-lethal injury of *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* O157 to serve method performance testing. International Journal of Food Microbiology **118:**241-249.
149. **Jay, J. M., M. J. Loessner, and D. A. Golden.** 2005. Modern Food Microbiology 7th edition. Springer Science and Business Media, New York, NY.
150. **Jenkins, D., J. Schultz, and A. Matin.** 1988. Starvation-induced cross protection against heat or H2o2 challenge in *escherichia coli*. Journal of Bacteriology **170:**3910-3914.

المنارة للاستشارات

www.manaraa.com

151. **Jeong, K., K. Hung, D. Baumler, J. Byrd, and C. Kaspar.** 2008. Acid stress damage of DNA is prevented by Dps binding in *Escherichia coli* O157: H7. Bmc Microbiology **8:**181.
152. **Johnson, J. L., M. P. Doyle, R. G. Cassens, and J. L. Schoeni.** 1988. Fate of *Listeria monocytogenes* in tissues of experimentally infected cattle and in hard salami. Applied and Environmental Microbiology **54:**497-501.
153. **Jorgensen, L., and H. Huss.** 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. International Journal of Food Microbiology **42:**127-131.
154. **Juneja, V.** 2003. A comparative heat inactivation study of indigenous microflora in beef with that of *Listeria monocytogenes*, *Salmonella* serotypes and *Escherichia coli* O157 : H7. Letters in Applied Microbiology **37:**292-298.
155. **Juneja, V.** 2003. Predictive model for the combined effect of temperature, sodium lactate, and sodium diacetate on the heat resistance of *Listeria monocytogenes* in beef. Journal of Food Protection **66:**804-811.
156. **Junttila, J. R., S. I. Niemelä, and J. Hirn.** 1988. Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic *Listeria*. Journal of Applied Bacteriology **65:**321-327.
157. **Kaale, L., T. Eikevik, T. Rustad, and K. Kolsaker.** 2011. Superchilling of food: A review. Journal of Food Engineering **107:**141-146.
158. **Kalchayanand, N., A. Sikes, C. Dunne, and B. Ray.** 1998. Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. Food Microbiology **15:**207-214.
159. **Kato, M., R. Hayashi, T. Tsuda, and K. Taniguchi.** 2002. High pressure-induced changes of biological membrane - Study on the membrane-bound Na+/K+-ATPase as a model system. European Journal of Biochemistry **269:**110-118.
160. **Kayal, S., and A. Charbit.** 2006. Listeriolysin O: a key protein of *Listeria monocytogenes* with multiple functions. Fems Microbiology Reviews **30:**514-529.
161. **Kazmierczak, M. J., S. C. Mithoe, K. J. Boor, and M. Wiedmann.** 2003. *Listeria monocytogenes* sigma B regulates stress response and virulence functions. Journal of Bacteriology **185:**5722-5734.
162. **Kim, S., L. Gorski, J. Reynolds, E. Orozco, S. Fielding, Y. Park, and M. Borucki.** 2006. Role of uvrA in the growth and survival of *Listeria monocytogenes* under UV radiation and acid and bile stress. Journal of Food Protection**:**3031-3036.
163. **Knight, P., J. Jackson, B. Bain, and D. Eldemire-Shearer.** 2003. Household food safety awareness of selected urban consumers in Jamaica. International Journal of Food Sciences and Nutrition **54:**309-320.
164. **Kohn, R.** 2011, posting date. Understanding temperature cycling in lab refrigerators and freezers. TovaTech. [Online.]
165. **Koutsoumanis, K., and J. Sofos.** 2005. Effect of inoculum size on the combined temperature, pH and a(w) limits for growth of *Listeria monocytogenes*. International Journal of Food Microbiology**:**83-91.
166. **Laird, J., F. Bartlett, and R. Mckellar.** 1991. Survival of *Listeria monocytogenes i*n egg washwater. International Journal of Food Microbiology **12:**115-122.

المنارة للاستشارات

www.manaraa.com

167. **Lange, R., and R. Henggearonis.** 1991. Identification of a central regulator of stationary-phase gene-expression in *escherichia coli*. Molecular Microbiology **5:**49-59.

168. **Lawrie, R., and D. Ledward.** 2006. The storage and preservation of meat: I temperature control, p. 189-234, Lawrie's Meat Science, 7th ed. CRC Press, Boca Raton, FL.

169. **Leclercq, A., D. Clermont, C. Bizet, P. A. Grimont, A. Le Fleche-Mateos, S. M. Roche, C. Buchrieser, V. Cadet-Daniel, A. Le Monnier, M. Lecuit, and F. Allerberger.** 2010. *Listeria rocourtiae* sp. nov, p. 2210-2214, International Journal of Systematic and Evolutionary Microbiology, vol. 60, England.

170. **Lehto, T., A. Brosinsky, H. Heinonen-Tanski, and T. Repo.** 2008. Freezing tolerance of ectomycorrhizal fungi in pure culture. Mycorrhiza **18:**385-392.

171. **Lengeler, J. W., G. Drews, and H. G. Schlegel (ed.).** 1999. Biology of the prokaryotes. Thieme ; Distributed in the USA by Blackwell Science, Stuttgart; New York; Malden, MA.

172. **Lianou, A., and K. Koutsoumanis.** 2009. Evaluation of the effect of defrosting practices of ground beef on the heat tolerance of *Listeria monocytogenes* and *Salmonella* Enteritidis. Meat Science **82:**461-468.

173. **Lin, C., S. Fernando, and C. Wei.** 1996. Occurrence of *Listeria monocytogenes*, *Salmonella* spp, *Escherichia coli* and E-coli O157:H7 in vegetable salads. Food Control **7:**135-140.

174. **Little, C., and I. Gillespie.** 2008. Prepared salads and public health. Journal of Applied Microbiology **105:**1729-1743.

175. **Little, C., J. Rhoades, S. Sagoo, J. Harris, M. Greenwood, V. Mithani, K. Grant, and J. McLauchlin.** 2008. Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK. Food Microbiology **25:**304-312.

176. **Little, C., S. Sagoo, I. Gillespie, K. Grant, and J. McLauchlin.** 2009. Prevalence and Level of *Listeria monocytogenes* and Other *Listeria* Species in Selected Retail Ready-to-Eat Foods in the United Kingdom. Journal of Food Protection **72:**1869-1877.

177. **Little, C., F. Taylor, S. Sagoo, I. Gillespie, K. Grant, and J. McLauchlin.** 2007. Prevalence and level of *Listeria monocytogenes* and other *Listeria* species in retail pre-packaged mixed vegetable salads in the UK. Food Microbiology **24:**711-717.

178. **Liu, S., D. Bayles, T. Mason, and B. Wilkinson.** 2006. A cold-sensitive *Listeria monocytogenes* mutant has a transposon insertion in a gene encoding a putative membrane protein and shows altered (p)ppGpp levels. Applied and Environmental Microbiology **72:**3955-3959.

179. **Liu, S., J. Graham, L. Bigelow, P. Morse, and B. Wilkinson.** 2002. Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Applied and Environmental Microbiology **68:**1697-1705.

180. **Liu, Y., and A. Ream.** 2008. Gene expression profiling of *Listeria monocytogenes* strain F2365 during growth in ultrahigh-temperature-processed skim milk. Applied and Environmental Microbiology **74:**6859-6866.

المنارة للاستشارات

www.manaraa.com

181. **Loncarevic, S., M. Danielsson-Tham, and W. Tham.** 1995. Occurrence of *Listeria monocytogenes* in soft and semi-soft chesses in retail outlets in Sweden. International Journal of Food Microbiology **26:**245-250.
182. **Longhi, C., M. Conte, M. Penta, A. Cossu, G. Antonini, F. Superti, and L. Seganti.** 2004. Lactoferricin influences early events of *Listeria monocytogenes* infection in THP-1 human macrophages. Journal of Medical Microbiology **53:**87-91.
183. **Lou, W., and A. Yousef.** 1996. Resistance of *Listeria monocytogenes* to heat after adaptation to environmental stresses. Journal of Food Protection **59:**465-471.
184. **Lou, Y., and A. Yousef.** 1997. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. Applied and Environmental Microbiology **63:**1252-1255.
185. **Louise, W.** 2004. Frozen Citrus Juices, Handbook of Frozen Foods. CRC Press.
186. **Lund, B.** 2000. Freezing, p. 122–145. *In* B. Lund, T. Baird Parker, and G. Gould (ed.), The Microbiological Safety and Quality of Food, vol. 1. Aspen Publishers, Gaithersburg, MD.
187. **Lyautey, E., D. Lapen, G. Wilkes, K. McCleary, F. Pagotto, K. Tyler, A. Hartmann, P. Piveteau, A. Rieu, W. Robertson, D. Medeiros, T. Edge, V. Gannon, and E. Topp.** 2007. Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the South Nation River watershed, Ontario, Canada. Applied and Environmental Microbiology **73:**5401-5410.
188. **MacGowan, A., K. Bowker, J. Mclauchlin, P. Bennett, and D. Reeves.** 1994. The occurence and seasonal changes in the isolation of *Listeria* spp. in shop bought food stuffs, human feces, sewage and soil from urban sources. International Journal of Food Microbiology **21:**325-334.
189. **Maddox, I. S.** 1994. Practical sanitation in the food industry. Gordon and Breach Science Publishers, Grey, Switzerland.
190. **Martín, M. S., G. Barbosa-Cánovas, and B. Swanson.** 2004. Food processing by high hydrostatic pressure. Critical Reviews in Food Science and Nutrition **42:**627-645.
191. **Mateo-Oyague, J., and M. Perez-Chabela.** 2004. Frozen Meat, Handbook of Frozen Foods. CRC Press.
192. **Matser, A., B. Krebbers, R. van den Berg, and P. Bartels.** 2004. Advantages of high pressure sterilisation on quality of food products. Trends in Food Science & Technology **15:**79-85.
193. **Mazur, P., and K. W. Cole.** 1989. Roles of unfrozen fraction, salt concentration, and changes in cell volume in the survival of frozen human erythrocytes. Cryobiology **26:**1-29.
194. **Mazzotta, A.** 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157 : H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. Journal of Food Protection**:**315-320.
195. **McClements, J. M., M. F. Patterson, and M. Linton.** 2001. The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. Journal of Food Protection **64:**514-522.
196. **McKellar, R. C.** 1994. Use of the CAMP test for identification of *Listeria monocytogenes*. Applied and Environmental Microbiology **60:**4219-4225.

المنارة للاستشارات
www.manaraa.com

197. **McKinney, J., R. Williams, G. Boardman, J. Eifert, and S. Sumner.** 2009. Effect of Acid Stress, Antibiotic Resistance, and Heat Shock on the Resistance of *Listeria monocytogenes* to UV Light When Suspended in Distilled Water and Fresh Brine. Journal of Food Protection**:**1634-1640.
198. **Mendonca, A., T. Amoroso, and S. Knabel.** 1994. Destruction of gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. Applied and Environmental Microbiology**:**4009-4014.
199. **Mendonca, A. F., M. G. Romero, M. A. Lihono, R. Nannapaneni, and M. G. Johnson.** 2004. Radiation resistance and virulence of *Listeria monocytogenes* Scott A following starvation in physiological saline. Journal of Food Protection **67:**470-474.
200. **Mengaud, J., J. Chenevert, C. Geoffroy, J. Gaillard, and P. Cossart.** 1987. Identification of the structural gene encoding the SH-activated hemolysin of L*isteria monocytogenes* - listeriolysin-o is homologous to strptolysin-o and pneumolysin. Infection and Immunity **55:**3225-3227.
201. **Mengaud, J., H. Ohayon, P. Gounon, R. Mege, and P. Cossart.** 1996. E-cadherin is the receptor for internalin, a surface protein required for entry of *L monocytogenes* into epithelial cells. Cell **84:**923-932.
202. **Metrick, C., D. Hoover, and D. Farkas.** 1989. Effects of high hydrostatic-pressure on heat-resistant and heat-sensitive strains of *Salmonella*. Journal of Food Science **54:**1547.
203. **Midelet-Bourdin, G., G. Leleu, S. Copin, S. M. Roche, P. Velge, and P. Malle.** 2006. Modification of a virulence-associated phenotype after growth of *Listeria monocytogenes* on food. J Appl Microbiol **101:**300-308.
204. **Miettinen, H., A. Arvola, T. Luoma, and G. Wirtanen.** 2003. Prevalence of *Listeria monocytogenes* in, and mircobiological and sensory quality of, rainbow trout, whitefish, and vendance roes from Finnish retail markets. Journal of Food Protection **66:**1832-1839.
205. **Miller, A.** 1992. Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. Journal of Food Protection **55:**414-418.
206. **Minor, T., and E. Marth.** 1972. Loss of viability by *Staphylococcus aureus* in acidified media. Journal of Milk and Food Technology **35:**548-555.
207. **Molle, V., Y. Nakaura, R. P. Shivers, H. Yamaguchi, R. Losick, Y. Fujita, and A. L. Sonenshein.** 2003. Additional targets of the *Bacillus subtilis* global regulator CodY identified by chromatin immunoprecipitation and genome-wide transcript analysis. Journal of Bacteriology **185:**1911-1922.
208. **Monfort, P., J. Minet, J. Rocourt, G. Piclet, and M. Cormier.** 1998. Incidence of *Listeria* spp. in Breton live shellfish. Letters in Applied Microbiology **26:**205-208.
209. **Moorman, M., W. Nettleton, E. Ryser, J. Linz, and J. Pestka.** 2005. Altered sensitivity to a quaternary ammonium sanitizer in stressed *Listeria innocua*. Journal of Food Protection**:**1659-1663.
210. **Moorman, M., C. Thelemann, S. Zhou, J. Pestka, J. Linz, and E. Ryser.** 2008. Altered Hydrophobicity and membrane composition in stress-adapted *Listeria innocua*. Journal of Food Protection**:**182-185.

المنارة للاستشارات
www.manaraa.com

211. **Morales, P., J. Calzada, and M. Nunez.** 2006. Effect of high-pressure treatment on the survival of *Listeria monocytogenes* Scott A in sliced vacuum-packaged Iberian and Serrano cured hams. Journal of Food Protection **69:**2539-2543.
212. **Morales, P., J. Calzada, B. Rodriguez, M. de Paz, P. Gaya, and M. Nunez.** 2006. Effect of cheese water activity and carbohydrate content on the barotolerance of *Listeria monocytogenes* Scott A. Journal of Food Protection **69:**1328-1333.
213. **Moss, C. W., and M. L. Speck.** 1966. Release of biologically active peptides from *Escherichia coli* at subzero temperatures. Journal of Bacteriology **91:**1105-1111.
214. **Muriana, P.** 1997. Effect of pH and hydrogen peroxide on heat inactivation of *Salmonella* and *Listeria* in egg white. Food Microbiology **14:**11-19.
215. **Murray, E., R. Webb, and M. Swann.** 1926. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). Journal of Pathology and Bacteriology **29:**407-439.
216. **Nakamoto, H., and L. Vigh.** 2007. The small heat shock proteins and their clients. Cellular and Molecular Life Sciences **64:**294-306.
217. **Niebuhr, S. E., and J. S. Dickson.** 2003. Impact of pH enhancement on populations of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in boneless lean beef trimmings. Journal of Food Protection **66:**874-877.
218. **Niven, G., C. Miles, and B. Mackey.** 1999. The effects of hydrostatic pressure on ribosome conformation in *Escherichia coli*: an in vivo study using differential scanning calorimetry. Microbiology-Uk **145:**419-425.
219. **Nolan, D. A., D. C. Chamblin, and J. A. Troller.** 1992. Minimal water activity levels for growth and survival of *Listeria monocytogenes* and *Listeria innocua*. International Journal of Food Microbiology **16:**323-335.
220. **Norrung, B., J. Andersen, and J. Schlundt.** 1999. Incidence and control of *Listeria monocytogenes* in foods in Denmark. International Journal of Food Microbiology **53:**195-203.
221. **Novak, J., and V. Juneja.** 2003. Effects of refrigeration or freezing on survival of *Listeria monocytogenes* Scott A in under-cooked ground beef. Food Control **14:**25-30.
222. **Novak, J. S., and J. T. Yuan.** 2003. Viability of *Clostridium perfringens*, *Escherichia coli*, and *Listeria monocytogenes* surviving mild heat or aqueous ozone treatment on beef followed by heat, alkali, or salt stress. Journal of Food Protection **66:**382-389.
223. **Nychas, G., E. Panagou, M. Parker, K. Waldron, and C. Tassou.** 2002. Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. Letters in Applied Microbiology **34:**173-177.
224. **O'Bryan, C. A., M. L. Sostrin, R. Nannapaneni, S. C. Ricke, P. G. Crandall, and M. G. Johnson.** 2009. Sensitivity of *Listeria monocytogenes* Scott A to nisin and diacetyl after starvation in sodium phosphate buffered saline. Journal of Food Science **74:**M493-498.
225. **O'Byrne, C. P., and K. A. G. Karatzas.** 2008. Chapter 5 The Role of Sigma B (σB) in the Stress Adaptations of *Listeria monocytogenes*: Overlaps Between Stress Adaptation and Virulence, p. 115-140. *In* S. S. a. G. M. G. Allen I. Laskin (ed.), Advances in Applied Microbiology, vol. Volume 65. Academic Press.

المنارة للاستشارات
www.manaraa.com

226. **Okada, Y., N. Okada, S. Makino, H. Asakura, S. Yamamoto, and S. Igimi.** 2006. The sigma factor RpoN (sigma 54) is involved in osmotolerance in *Listeria monocytogenes*. Fems Microbiology Letters **263:**54-60.
227. **Osaili, T., C. Griffis, E. Martin, B. Beard, A. Keener, and J. Marcy.** 2007. Thermal inactivation of *Escherichia coli* O157 : H7, *Salmonella*, and *Listeria monocytogenes* in breaded pork patties. Journal of Food Science **72:**M56-M61.
228. **Osaili, T., C. Griffis, E. Martin, B. Beard, A. Keener, and J. Marcy.** 2006. Thermal inactivation studies of Escherichia coli O157 : H7, Salmonella, and Listeria monocytogenes in ready-to-eat chicken-fried beef patties. Journal of Food Protection **69:**1080-1086.
229. **Padan, E., E. Bibi, M. Ito, and T. Krulwich.** 2005. Alkaline pH homeostasis in bacteria: New insights. Biochimica Et Biophysica Acta-Biomembranes **1717:**67-88.
230. **Palumbo, S., and A. Williams.** 1991. Resistance of *Listeria monocytogenes* to freezing in foods. Food Microbiology **8:**63-68.
231. **Park, J., C. Grant, P. Attfield, and I. Dawes.** 1997. The freeze-thaw stress response of the yeast *Saccharomyces cerevisiae* is growth phase specific and is controlled by nutritional state via the RAS-cyclic AMP signal. Applied and Environmental Microbiology **63:**3818-3824.
232. **Park, J., C. Grant, M. Davies, and I. Dawes.** 1998. The cytoplasmic Cu,Zn superoxide dismutase of *Saccharomyces cerevisiae* is required for resistance to freeze-thaw stress - Generation of free radicals during freezing and thawing. Journal of Biological Chemistry **273:**22921-22928.
233. **Park, S., K. Sohn, J. Shin, and H. Lee.** 2001. High hydrostatic pressure inactivation of *Lactobacillus viridescens* and its effects on ultrastructure of cells. International Journal of Food Science and Technology **36:**775-781.
234. **Patil, S., S. Cates, and R. Morales.** 2005. Consumer food safety knowledge, practices, and demographic differences: Findings from a meta-analysis. Journal of Food Protection **68:**1884-1894.
235. **Patterson, M., M. Quinn, R. Simpson, and A. Gilmour.** 1995. Sensitivity of vegetative pathogens to high hydrostatic-pressure treatment in phosphate-buffered saline and foods. Journal of Food Protection **58:**524-529.
236. **Perez-Rodriguez, F., R. Castro, G. Posada-Izquierdo, A. Valero, E. Carrasco, R. Garcia-Gimeno, and G. Zurera.** 2010. Evaluation of hygiene practices and microbiological quality of cooked meat products during slicing and handling at retail. Meat Science **86:**479-485.
237. **Perrier-Cornet, J., P. Marechal, and P. Gervais.** 1995. A new design intended to relate high-pressure treatment to yeast-cell mass-transfer. Journal of Biotechnology **41:**49-58.
238. **Persson, P., and D. Londahl.** 1993. Freezing technology, p. 20–58. *In* C. Mallett (ed.), Frozen Food Technology. Blackie Academic and Professional, London.
239. **Petran, R., and E. Zottola.** 1989. A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A Journal of Food Science **54:**458-460.
240. **Phan-Thanh, L., and F. Mahouin.** 1999. A proteomic approach to study the acid response in *Listeria monocytogenes*. Electrophoresis**:**2214-2224.

المنارة للاستشارات
www.manaraa.com

241. **Phan-Thanh, L., and A. Montagne.** 1998. Physiological and biochemical aspects of the acid survival of *Listeria monocytogenes*. Journal of General and Applied Microbiology**:**183-191.
242. **Phang, H. S., and C. M. Bruhn.** In Press. Burger Preparation: What consumers say and do in the home. Journal of Food Protection.
243. **Phan-Thanh, L., and T. Gormon.** 1995. Analysis of heat and cold shock proteins in *Listeria b*y 2-dimensional electrophoresis. Electrophoresis **16:**444-450.
244. **Pichereau, V., A. Hartke, and Y. Auffray.** 2000. Starvation and osmotic stress induced multiresistances. Influence of extracellular compounds. International Journal of Food Microbiology **55:**19-25.
245. **Pirie, H.** 1927. The problem of plague in South Africa. The British Medical Journal **2:**350-351.
246. **Pirie, J. H.** 1940. The Genus Listerella Pirie. Science **91:**383.
247. **Porsby, C., B. Vogel, M. Mohr, and L. Gram.** 2008. Influence of processing steps in cold-smoked salmon production on survival and growth of persistent and presumed non-persistent *Listeria monocytogenes*. International Journal of Food Microbiology **122:**287-295.
248. **Porto, A., J. Call, and J. Luchansky.** 2004. Effect of reheating on viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters following refrigerated or frozen storage. Journal of Food Protection **67:**71-76.
249. **Presser, K. A., T. Ross, and D. A. Ratkowsky.** 1998. Modelling the growth limits (growth/no growth interface) of *Escherichia coli* as a function of temperature, pH, lactic acid concentration, and water activity. Applied and Environmental Microbiology **64:**1773-1779.
250. **Prestamo, G., P. Sanz, M. Fonberg-Broczek, and G. Arroyo.** 1999. High pressure response of fruit jams contaminated with *Listeria monocytogenes*. Letters in Applied Microbiology **28:**313-316.
251. **Prevention, C. f. D. C. a.**, posting date. CDC - September 21, 2011 - CDC - Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms, Colorado. [Online.]
252. **Pérez-Palacios, T., J. Ruiz, J. M. Barat, M. C. Aristoy, and T. Antequera.** 2010. Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process. Meat Science **85:**121-126.
253. **Raengpradub, S., M. Wiedmann, and K. Boor.** 2008. Comparative analysis of the sigma(B)-dependent stress responses in *Listeria monocytogenes* and *Listeria innocua* strains exposed to selected stress conditions. Applied and Environmental Microbiology **74:**158-171.
254. **Raimann, E., B. Schmid, R. Stephan, and T. Tasara.** 2009. The alternative sigma factor sigma(L) of *L monocytogenes* promotes growth under diverse environmental stresses. Foodborne Pathogens and Disease **6:**583-591.
255. **Rajkovic, A., N. Smigic, M. Uyttendaele, H. Medic, L. de Zutter, and F. Devlieghere.** 2009. Resistance of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and Campylobacter jejuni after exposure to repetitive cycles of mild bactericidal treatments. Food Microbiology **26:**889-895.

المنارة للاستشارات
www.manaraa.com

256. **Ramaswamy, V., V. M. Cresence, J. S. Rejitha, M. U. Lekshmi, K. S. Dharsana, S. P. Prasad, and H. M. Vijila.** 2007. Listeria--review of epidemiology and pathogenesis. Journal of Microbiology, Immunology, and Infection **40:**4-13.
257. **Ray, B., and A. Bhunia.** 2008. Fundamental Food Microbiology, 3rd ed. CRC Press, Boca Raton, Florida.
258. **Rea, R., C. Gahan, and C. Hill.** 2004. Disruption of putative regulatory loci in Listeria monocytogenes demonstrates a significant role for fur and PerR in virulence. Infection and Immunity **72:**717-727.
259. **Rea, R., C. Hill, and C. G. Gahan.** 2005. *Listeria monocytogenes* PerR mutants display a small-colony phenotype, increased sensitivity to hydrogen peroxide, and significantly reduced murine virulence. Applied and Environmental Microbiology **71:**8314-8322.
260. **Ritz, M., M. Freulet, N. Orange, and M. Federighi.** 2000. Effects of high hydrostatic pressure on membrane proteins of *Salmonella typhimurium*. International Journal of Food Microbiology **55:**115-119.
261. **Ritz, M., F. Jugiau, M. Federighi, N. Chapleau, and M. de Lamballerie.** 2008. Effects of high pressure, subzero temperature, and pH on survival of *Listeria monocytogenes* in buffer and smoked salmon. Journal of Food Protection **71:**1612-1618.
262. **Ritz, M., J. Tholozan, M. Federighi, and M. Pilet.** 2002. Physiological damages of *Listeria monocytogenes* treated by high hydrostatic pressure. International Journal of Food Microbiology **79:**47-53.
263. **Rivalain, N., J. Roquain, and G. Demazeau.** 2010. Development of high hydrostatic pressure in biosciences: Pressure effect on biological structures and potential applications in Biotechnologies. Biotechnology Advances **28:**659-672.
264. **Rocourt, J., P. BenEmbarek, H. Toyofuku, and J. Schlundt.** 2003. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: the FAO/WHO approach. Fems Immunology and Medical Microbiology **35:**263-267.
265. **Rohrbach, B., F. Draughon, P. Davidson, and S. Oliver.** 1992. Prevalence of *Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica,* and *Salmonella* in bulk tank milk: risk factors and risk of human exposure. Journal of Food Protection **55:**93-97.
266. **Rosmini, M., J. Fernandez-Lopez, and J. Perez-Alvarez.** 2004. Chemical and Physical Aspects of Color in Frozen Muscle-Based Foods, Handbook of Frozen Foods. CRC Press.
267. **Rosmini, M., J. Perez-Alvarez, and J. Fernandez-Lopez.** 2004. Operational Processes for Frozen Red Meat, Handbook of Frozen Foods. CRC Press.
268. **Ross, T., P. Dalgaard, and S. Tienungoon.** 2000. Predictive modelling of the growth and survival of *Listeria* in fishery products. International Journal of Food Microbiology **62:**231-245.
269. **Ryan, S., M. Begley, C. Gahan, and C. Hill.** 2009. Molecular characterization of the arginine deiminase system in *Listeria monocytogenes*: regulation and role in acid tolerance. Environmental Microbiology **11:**432-445.

المنارة للاستشارات
www.manaraa.com

270. **Ryan, S., M. Begley, C. Hill, and C. G. Gahan.** 2010. A five-gene stress survival islet (SSI-1) that contributes to the growth of *Listeria monocytogenes* in suboptimal conditions. Journal of Applied Microbiology **109:**984-995.

271. **Ryser, E. T., and E. H. Marth.** 1988. Growth of *Listeria monocytogenes* at different pH values in uncultured whey or whey cultured with Penicillium camemberti. Canadian Journal of Microbiology **34:**730-734.

272. **Salyers, A., and D. Whitt.** 2002. Bacterial pathogenesis: A molecular approach, 2 ed. American Society for Microbiology, Washington DC.

273. **Sauders, B., M. Durak, E. Fortes, K. Windham, Y. Schukken, A. Lembo, B. Akey, K. Nightingale, and M. Wiedmann.** 2006. Molecular characterization of *Listeria monocytogenes* from natural and urban environments. Journal of Food Protection **69:**93-105.

274. **Schaffter, N., and A. Parriaux.** 2002. Pathogenic-bacterial water contamination in mountainous catchments. Water Research **36:**131-139.

275. **Schaffter, N., J. Zumstein, and A. Parriaux.** 2004. Factors influencing the bacteriological water quality in mountainous surface and groundwaters. Acta Hydrochimica Et Hydrobiologica **32:**225-234.

276. **Scheyhing, C., S. Hormann, M. Ehrmann, and R. Vogel.** 2004. Barotolerance is inducible by preincubation under hydrostatic pressure, cold-, osmotic- and acid-stress conditions in *Lactobacillus sanfranciscensis* DSM 20451(T). Letters in Applied Microbiology **39:**284-289.

277. **Schlech, W., D. Chase, and A. Badley.** 1993. A model of food-borne *Listeria monocytogenes* infection in the Sprague-Dawley rat using gastric inoculation: Development and effect of gastric-acidity on infective dose. International Journal of Food Microbiology **18:**15-24.

278. **Schlech, W. F., III.** 2000. Foodborne Listeriosis. Clinical Infectious Diseases **31:**770-775.

279. **Schmid, B., J. Klumpp, E. Raimann, M. J. Loessner, R. Stephan, and T. Tasara.** 2009. Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. Applied and Environmental Microbiology **75:**1621-1627.

280. **Scouten, A., and L. Beuchat.** 2002. Combined effects of chemical, heat and ultrasound treatments to kill *Salmonella* and *Escherichia coli* O157 : H7 on alfalfa seeds. Journal of Applied Microbiology **92:**668-674.

281. **Shabala, L., S. H. Lee, P. Cannesson, and T. Ross.** 2008. Acid and NaCl limits to growth of *Listeria monocytogenes* and influence of sequence of inimical acid and NaCl levels on inactivation kinetics. Journal of Food Protection **71:**1169-1177.

282. **Sharma, M., B. Adler, M. Harrison, and L. Beuchat.** 2005. Thermal tolerance of acid-adapted and unadapted *Salmonella*, *Escherichia coli* O157 : H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. Letters in Applied Microbiology**:**448-453.

283. **Shearer, A. E. H., H. S. Neetoo, and H. Chen.** 2010. Effect of growth and recovery temperatures on pressure resistance of *Listeria monocytogenes*. International Journal of Food Microbiology **136:**359-363.

المنارة للاستشارات
www.manaraa.com

284. **Siegele, D.** 1993. *Escherichia coli* functions required for resuming growth after starvation. Journal of Cellular Biochemistry**:**282-282.

285. **Simpson Beauchamp, C., O. A. Byelashov, I. Geornaras, P. A. Kendall, J. A. Scanga, K. E. Belk, G. C. Smith, and J. N. Sofos.** 2010. Fate of *Listeria monocytogenes* during freezing, thawing and home storage of frankfurters. Food Microbiology **27:**144-149.

286. **Simpson, R. K., and A. Gilmour.** 1997. The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. Journal of Applied Microbiology **83:**181-188.

287. **Singh, R., X. Jiang, and F. Luo.** 2010. Thermal Inactivation of Heat-Shocked *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Dairy Compost. Journal of Food Protection **73:**1633-1640.

288. **Singh, V. K., D. S. Hattangady, E. S. Giotis, A. K. Singh, N. R. Chamberlain, M. K. Stuart, and B. J. Wilkinson.** 2008. Insertional inactivation of branched-chain alpha-keto acid dehydrogenase in *Staphylococcus aureus* leads to decreased branched-chain membrane fatty acid content and increased susceptibility to certain stresses. Applied and Environmental Microbiology **74:**5882-5890.

289. **Skandamis, P., Y. Yoon, J. Stopforth, P. Kendall, and J. Sofos.** 2008. Heat and acid tolerance of *Listeria monocytogenes* after exposure to single and multiple sublethal stresses. Food Microbiology **25:**294-303.

290. **Smiddy, M., L. O'Gorman, R. Sleator, J. Kerry, M. Patterson, A. Kelly, and C. Hill.** 2005. Greater high-pressure resistance of bacteria in oysters than in buffer. Innovative Food Science & Emerging Technologies **6:**83-90.

291. **Smith, M., K. Takeuchi, R. Brackett, H. McClure, R. Raybourne, K. Williams, U. Babu, G. Ware, J. Broderson, and M. Doyle.** 2003. Nonhuman primate model for *Listeria monocytogenes*-induced stillbirths. Infection and Immunity **71:**1574-1579.

292. **Soni, K. A., R. Nannapaneni, and T. Tasara.** 2011. The contribution of transcriptomic and proteomic analysis in elucidating stress adaptation responses of *Listeria monocytogenes*. Foodborne Pathogens and Disease **8:**843-852.

293. **Soultos, N., P. Koidis, and R. Madden.** 2003. Presence of *Listeria* and *Salmonella* spp. in retail chicken in Northern Ireland. Letters in Applied Microbiology **37:**421-423.

294. **Souzu, H., M. Sato, and T. Kojima.** 1989. Changes in chemical-structure and function in escherichia-coli cell membranes caused by freeze-thawing .2. Membrane lipid state and response of cells to dehydration. Biochimica Et Biophysica Acta **978:**112-118.

295. **Spector, M., and C. Cubitt.** 1992. Starvation-inducible loci of *Salmonella typhimurium*: Regulation and roles in starvation-survival. Molecular Microbiology **6:**1467-1476.

296. **Stanley, D., A. Bandara, S. Fraser, P. J. Chambers, and G. A. Stanley.** 2010. The ethanol stress response and ethanol tolerance of *Saccharomyces cerevisiae*. Journal of Applied Microbiology **109:**13-24.

المنارة للاستشارات
www.manaraa.com

297. **Stead, D., and S. F. Park.** 2000. Roles of Fe superoxide dismutase and catalase in resistance of *Campylobacter coli* to freeze-thaw stress. Applied and Environmental Microbiology **66:**3110-3112.
298. **Storz, G., and R. Hengge.** 2011. Bacterial Stress Responses, 2nd ed. ASM Press, Washington, DC.
299. **Stres, B., L. Philippot, J. Faganeli, and J. M. Tiedje.** 2010. Frequent freeze-thaw cycles yield diminished yet resistant and responsive microbial communities in two temperate soils: a laboratory experiment. FEMS Microbiology Ecology **74:**323-335.
300. **Styles, M., D. Hoover, and D. Farkas.** 1991. Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic-pressure. Journal of Food Science **56:**1404-1407.
301. **Tam, l. T., H. Antelmann, C. Eymann, D. Albrecht, J. Bernhardt, and M. Hecker.** 2006. Proteome signatures for stress and starvation in *Bacillus subtilis* as revealed by a 2-D gel image color coding approach. Proteomics **6:**4565-4585.
302. **Taormina, P., and L. Beuchat.** 2002. Survival and growth of alkali-stressed *Listeria monocytogenes* on beef frankfurters and thermotolerance in frankfurter exudates. Journal of Food Protection **65:**291-298.
303. **Taormina, P., and L. Beuchat.** 2001. Survival and heat resistance of *Listeria monocytogenes* after exposure to alkali and chlorine. Applied and Environmental Microbiology **67:**2555-2563.
304. **Taormina, P., and L. Beuchat.** 2002. Survival of *Listeria monocytogenes* in commercial food-processing equipment cleaning solutions and subsequent sensitivity to sanitizers and heat. Journal of Applied Microbiology **92:**71-80.
305. **Tapia de Daza, M. S., Y. Villegas, and A. Martinez.** 1991. Minimal water activity for growth of *Listeria monocytogenes* as affected by solute and temperature. International Journal of Food Microbiology **14:**333-337.
306. **Tasara, T., and R. Stephan.** 2006. Cold stress tolerance of *Listeria monocytogenes*: A review of molecular adaptive mechanisms and food safety implications. Journal of Food Protection **69:**1473-1484.
307. **Thimothe, J., J. Walker, V. Suvanich, K. Gall, M. Moody, and M. Wiedmann.** 2002. Detection of *Listeria* in crawfish processing plants and in raw, whole crawfish and processed crawfish (Procambarus spp.). Journal of Food Protection **65:**1735-1739.
308. **Tienungoon, S., D. A. Ratkowsky, T. A. McMeekin, and T. Ross.** 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. Applied and Environmental Microbiology **66:**4979-4987.
309. **Tiganitas, A., N. Zeaki, A. Gounadaki, E. Drosinos, and P. Skandamis.** 2009. Study of the effect of lethal and sublethal pH and a(w) stresses on the inactivation or growth of *Listeria monocytogenes* and *Salmonella* Typhimurium. International Journal of Food Microbiology **134:**104-112.
310. **Tompkin, R.** 2002. Control of *Listeria monocytogenes* in the food-processing environment. Journal of Food Protection **65:**709-725.
311. **Trusca, D., S. Scott, C. Thompson, and D. Bramhill.** 1998. Bacterial SOS checkpoint protein SulA inhibits polymerization of purified FtsZ cell division protein. Journal of Bacteriology **180:**3946-3953.

المنارة للاستشارات
www.manaraa.com

312. **Tsuyuki, S., and J. Weisgerber.** 2010. Best Practices: Managing the control of *Listeria* in a wet processing environment, meatingplace.com. meatingplace.com, meatingplace.com.

313. **USDA-FSIS.** 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products, http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=a24c144e2f67563c721cab3f5205ecd4&rgn=div8&view=text&node=9:2.0.2.4.43.0.70.2&idno=9.

314. **Uyttendaele, M., P. De Troy, and J. Debevere.** 1999. Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. International Journal of Food Microbiology **53:**75-80.

315. **van Boeijen, I., C. Francke, R. Moezelaar, T. Abee, and M. Zwietering.** 2011. Isolation of Highly Heat-Resistant *Listeria monocytogenes* Variants by Use of a Kinetic Modeling-Based Sampling Scheme. Applied and Environmental Microbiology **77:**2617-2624.

316. **van Bokhorst-van de Veen, H., T. Abee, M. Tempelaars, P. A. Bron, M. Kleerebezem, and M. L. Marco.** 2011. Short- and long-term adaptation to ethanol stress and its cross-protective consequences in *Lactobacillus plantarum*. Applied and Environmental Microbiology **77:**5247-5256.

317. **van der Veen, S., and T. Abee.** 2011. Contribution of *Listeria monocytogenes* RecA to acid and bile survival and invasion of human intestinal Caco-2 cells. International Journal of Medical Microbiology.

318. **van der Veen, S., S. van Schalkwijk, D. Molenaar, W. de Vos, T. Abee, and M. Wells-Bennik.** 2010. The SOS response of *Listeria monocytogenes* is involved in stress resistance and mutagenesis. Microbiology**:**374-384.

319. **van Bogelen, R. A., P. M. Kelley, and F. C. Neidhardt.** 1987. Differential induction of heat shock, SOS, and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli*. Journal of Bacteriology **169:**26-32.

320. **Varma, J., M. Samuel, R. Marcus, R. Hoekstra, C. Medus, S. Segler, B. Anderson, T. Jones, B. Shiferaw, N. Haubert, M. Megginson, P. McCarthy, L. Graves, T. Van Gilder, and F. Angulo.** 2007. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: A case-control study of potential sources of sporadic illness in the United States. Clinical Infectious Diseases **44:**521-528.

321. **Vishnivetskaya, T., R. Siletzky, N. Jefferies, J. Tiedje, and S. Kathariou.** 2007. Effect of low temperature and culture media on the growth and freeze-thawing tolerance of *Exiguobacterium* strains. Cryobiology **54:**234-240.

322. **Vogel, B., L. Jorgensen, B. Ojeniyi, H. Huss, and L. Gram.** 2001. Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smokehouses as assessed by Random Amplified Polymorphic DNA analyses. International Journal of Food Microbiology **65:**83-92.

323. **Vurma, M., Y. K. Chung, T. H. Shellhammer, E. J. Turek, and A. E. Yousef.** 2006. Use of phenolic compounds for sensitizing *Listeria monocytogenes* to high-pressure processing, p. 263-269, Int J Food Microbiol, vol. 106, Netherlands.

المنارة للاستشارات

www.manaraa.com

324. **Walker, S., and M. Stringer.** 1987. Growth of *Listeria monocytogenes and Aeromonas hydrophila* at chill temperatures, Technical Memorandum, vol. 462. CCFRA Chipping Campden, United Kingdom.
325. **Watson, K.** 1990. Microbial stress proteins. Adv Microb Physiol **31:**183-223.
326. **Watson, S., M. Antonio, and S. Foster.** 1998. Isolation and characterization of *Staphylococcus aureus* starvation-induced, stationary-phase mutants defective in survival or recovery. Microbiology-Sgm **144:**3159-3169.
327. **Watson, S., M. Clements, and S. Foster.** 1998. Characterization of the starvation-survival response of *Staphylococcus aureus*. Journal of Bacteriology **180:**1750-1758.
328. **Weber, F. J., and J. A. de Bont.** 1996. Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. Biochim Biophys Acta **1286:**225-245.
329. **Weis, J., and H. Seeliger.** 1975. Incidence of *Listeria monocytogenes* in nature. Applied Microbiology **30:**29-32.
330. **Welshimer, H.** 1960. Survival of *Listeria monocytogenes* in soil. Journal of Bacteriology **80:**316-320.
331. **Welshimer, H. J.** 1968. Isolation of *Listeria monocytogenes* from vegetation. Journal of Bacteriology **95:**300-303.
332. **Wemekamp-Kamphuis, H., A. Karatzas, J. Wouters, and T. Abee.** 2002. Enhanced levels of cold shock proteins in *Listeria monocytogenes* LO28 upon exposure to low temperature and high hydrostatic pressure. Applied and Environmental Microbiology **68:**456-463.
333. **Wemekamp-Kamphuis, H. H., J. A. Wouters, P. P. de Leeuw, T. Hain, T. Chakraborty, and T. Abee.** 2004. Identification of sigma factor sigma B-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria monocytogenes* EGD-e. Applied and Environmental Microbiology **70:**3457-3466.
334. **Wemekamp-Kamphuis, H. H., J. A. Wouters, R. D. Sleator, C. G. Gahan, C. Hill, and T. Abee.** 2002. Multiple deletions of the osmolyte transporters BetL, Gbu, and OpuC of *Listeria monocytogenes* affect virulence and growth at high osmolarity. Applied and Environmental Microbiology **68:**4710-4716.
335. **Wen, J., R. Anantheswaran, and S. Knabel.** 2009. Changes in Barotolerance, Thermotolerance, and Cellular Morphology throughout the Life Cycle of *Listeria monocytogenes*. Applied and Environmental Microbiology **75:**1581-1588.
336. **Wesche, A., J. Gurtler, B. Marks, and E. Ryser.** 2009. Stress, Sublethal Injury, Resuscitation, and Virulence of Bacterial Foodborne Pathogens. Journal of Food Protection **72:**1121-1138.
337. **Wiedmann, M., T. J. Arvik, R. J. Hurley, and K. J. Boor.** 1998. General stress transcription factor sigmaB and its role in acid tolerance and virulence of *Listeria monocytogenes*. Journal of Bacteriology **180:**3650-3656.
338. **Winter, J., M. Ilbert, P. C. Graf, D. Ozcelik, and U. Jakob.** 2008. Bleach activates a redox-regulated chaperone by oxidative protein unfolding. Cell **135:**691-701.
339. **Winter, R., and C. Jeworrek.** 2009. Effect of pressure on membranes. Soft Matter **5:**3157-3173.

المنارة للاستشارات

www.manaraa.com

340. **Wu, V.** 2008. A review of microbial injury and recovery methods in food. Food Microbiology **25:**735-744.
341. **Yordanov, D. G.** 2010. High Pressure Processi ng for Foods Preserving. Biotechnology and Biotechnology Eq. **24:**1940-1945.
342. **Zhang, C., J. Nietfeldt, M. Zhang, and A. Benson.** 2005. Functional consequences of genome evolution in *Listeria monocytogenes*: the lmo0423 and lmo0422 genes encode sigma(C) and LstR, a lineage II-specific heat shock system. Journal of Bacteriology **187:**7243-7253.
343. **Zhang, J., X. Peng, A. Jonas, and J. Jonas.** 1995. NMR-study of the cold, heat, and pressure unfolding of ribonuclease A. Biochemistry **34:**8631-8641.

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ACKNOWLEDGMENTS

Much appreciation for the support, feedback and assessment of this thesis research and writing is given to my major professor Dr. Aubrey Mendonca. I would also like to acknowledge the guidance that was given during research and preparation to my committee members Dr. Byron Brehm-Stecher and Dr. James Dickson. Without the support from my major professor and committee members this thesis would not have been possible.

Consideration and thanks is given to the Microbial Food Safety Laboratory research team which has been an integral part of this research. I would like to thank Dr. Stephanie Jung and Devin Maurer for support throughout high hydrostatic pressure related research and analysis. Statistical support from Karl Pazdernik in Department of Statistics and Statistics Laboratory at Iowa State University was much appreciated during the analysis and interpretation of data. I am also very grateful for support and research guidance from the Department of Food Science and Human Nutrition (FSHN) faculty along the way. Gracious thanks are also given to all the opportunities that were made possible by Dr. Aubrey Mendonca, the College of Agriculture and Life Sciences, the Department of Food Science and Human Nutrition, the Graduate College and Iowa State University over the years. Research funding from the Graduate Minority Assistantship Program (GMAP) contributed fully and/or partially to the research objectives investigated.

I would like to finally thank my family and friends for all the support given to me during this process. The patience and love from my mom and brothers, Ed and Erik, has meant the world to me. Thank you to all my friends near and far that helped make this process possible, including friend and mentor Dr. Dave.

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