Examining *Listeria monocytogenes* Cell Envelope Physiology in Dairy Relevant Environments

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Kathryn Ann Magee
August 2018

Copyright © 2018 by Kathryn A. Magee
All rights reserved.

ACKNOWLEDGEMENTS

I would like to thank my major professor, Thomas Denes, for giving me the opportunity to pursue graduate studies and to push me to be a better student and working professional. He has really made me get the most out of my education and has helped me through the times of struggle and supported me in times of success. I also would like to thank Faith Critzer and Doris D'Souza for serving on my committee and providing a new insight on the project and helping me reach my goals.

I really appreciate the entire Food Science Department at the University of Tennessee. I came to the university as an outsider and they have welcomed and supported me throughout the whole process. I truly enjoyed being able to give back to the department by serving as the Food Science Club President and as a member of the Dairy Products Judging Team. This department cares a lot about their students and provides so many opportunities for them to reach their full potential. I feel very lucky to have been a part of such a great department.

Lastly, I would like to thank my boyfriend, Mark Eddy, and my family and friends. They have been there for me since day one with their love and support. They've pushed me through the hard times and made me smile and laugh when I thought the world was ending. They gave me the confidence to pursue my dreams and I can't thank them enough for how much they've done for me.

ABSTRACT

In the food industry, many interventions used to control *L. monocytogenes* target the cell envelope, the outer barrier of the cell made up of various glycopolymers and wall teichoic acids. The cell envelope functions in protection of the cell and as an entryway for many treatment methods. It's unclear if changes in the environment will affect the physiology of the cell envelope and in turn the cell's sensitivity to antimicrobial interventions. For example, nisin is a common bacteriocin used as an antimicrobial agent against *L. monocytogenes*. However, previous studies have found that cell envelope changes are responsible for the acquisition of nisin resistance. It has also been shown that environmental conditions such as temperature and pH have had significant impact on nisin and bacteriophage susceptibility. The purpose of this study was to determine if typical dairy processing conditions affect the physiological state of *L. monocytogenes* cell envelope. Model laboratory, dairy outbreak, and mutant strains of *L. monocytogenes* were grown on skim milk agar media at different pHs (5.7, 6.0, 6.2, 6.5) and temperatures (6°C, 14°C, 22°C, 30°C) relevant to the dairy industry. The cells were collected, washed, and standardized to a specific optical density (OD_{600}). The cells were then added to either phage binding or cytochrome *c* binding assays. Phages LP-048 and LP-125 were used as they bind to specific cell surface residues (*N*-acetylglucosamine and rhamnose). Cytochrome *c* was used to measure cell wall charge as a negative charge indicator. Significant differences were found between conditions, but results varied by experiment and strain. It was concluded that the physiological state of the *L. monocytogenes* cell envelope is affected by the cell's growth conditions. As the

cell envelope is a major factor in resistance or susceptibility to antimicrobials and other control interventions, these data suggest that control interventions can be targeted to specific pH or temperature conditions for greater impact.

TABLE OF CONTENTS

Chapter One: Literature Review 1

***Listeria monocytogenes*: A Foodborne Pathogen 2**

Biology and Pathogenesis 2

Clinical Manifestations and Epidemiology 5

Food Safety, Outbreaks, and Associated Costs 6

***L. monocytogenes* Cell Envelope Physiology 8**

The Cell Envelope of *L. monocytogenes* 8

Antimicrobial Activity and Environmental Effects on the Cell Envelope 11

Bacteriophages 13

Bacteriophages and Host Interactions 13

Phage Application in Food Processing 16

Listeria-phages 20

Dairy Processing and Safety 21

Chapter Two: *Listeria monocytogenes* Cell Envelope Physiology is Influenced by Growth in Dairy Relevant Conditions 24

Abstract 25

Introduction 26

Materials and Methods 29

Bacterial Strains and Growth Conditions 29

Phage Strains and Preparation of Stocks 30

Cell Collection, Washing, and Standardization 30

Bacteriophage Binding Assays 31

Cytochrome *c* Binding Assays 31

Statistical Analysis 32

Results 33

Growth pH and Temperature Affects Phage and Cytochrome *c* Binding to Wild-type 10403S Strain 33

Mutant Strains Lacking Specific Cell Surface Features Had Significant Differences in Phage and Cytochrome *c* Binding When Subjected to Various Conditions 34

Growth Temperature and pH Influence Phage Binding and Cytochrome *c* Binding on Dairy Related Outbreak Strains and Serotype 4b Lab Strain 36

Discussion 38

Acknowledgements 42

References 43

Appendix 64

Vita 77

LIST OF TABLES

Table 1. Bacterial strains and phages used in the study. **65**

LIST OF FIGURES

Figure 1. Phage binding of (A) LP-048 and (B) LP-125 on wild-type 10403S strains grown at varying dairy pHs. **66**

Figure 2. Phage binding of (A) LP-048 and (B) LP-125 on wild-type strain 10403S grown at varying dairy temperatures. **67**

Figure 3. Cytochrome *c* binding on wild-type 10403S strain grown at varying dairy temperatures. **68**

Figure 4. Phage binding of (A) LP-048 and (B) LP-125 on wild-type 10403S and mutant 10403S strains grown in varying dairy pHs. **69**

Figure 5. Phage binding of (A) LP-048 and (B) LP-125 and on wild-type 10403S and mutant 10403S strains grown in varying dairy temperatures. **70**

Figure 6. Cytochrome *c* binding to wild-type 10403S and mutant 10403S strains grown at varying dairy pHs. **71**

Figure 7. Cytochrome *c* binding to wild-type 10403S and mutant 10403S strains grown in varying dairy temperatures. **72**

Figure 8. Phage binding of (A) LP-048 and (B) LP-125 to dairy related outbreak strains grown at varying dairy pHs. **73**

Figure 9. Phage binding of (A) LP-048 and (B) LP-125 to dairy related outbreak strains grown at varying dairy temperatures. **74**

Figure 10. Cytochrome *c* binding on lab strain F2365 and dairy related outbreak strains grown at varying dairy pHs. **75**

Figure 11. Cytochrome *c* binding on lab strain F2365 and dairy related outbreak strains grown at varying dairy temperatures. **76**

CHAPTER ONE
LITERATURE REVIEW

Listeria monocytogenes: A Foodborne Pathogen

Biology and Pathogenesis

Listeria monocytogenes is a gram-positive facultative anaerobic rod bacterium that can cause a severe disease known as listeriosis. *L. monocytogenes* belongs in the genus *Listeria*. Currently, 17 different species are a part of this genus but only six of them are known as *Listeria* sensu strictu. Species that are a part of this subgroup share common phenotypic characteristics such as the ability to grow at low temperatures, motility at 30°C, and a positive catalase reaction (*98*). In addition to *Listeria monocytogenes*, *Listeria* sensu strictu includes *Listeria marthii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, and *Listeria ivanovii* (*29*). Only two of these species, *L. monocytogenes* and *L. ivanovii*, have been known to be pathogenic to humans and ruminants, respectively (*104*).

L. monocytogenes is ubiquitous in the environment and is tolerant to many environmental stresses. It has the ability to grow at a range of temperatures from -0.4°C to 50°C, making it a psychrotrophic organism (*49*). This pathogen is highly salt tolerant, growing in concentrations of up to 10% NaCl. It's also acid tolerant, surviving at a pH range of 4.6 to 9.5 (*19*, *46*). *L. monocytogenes* contains approximately four to six peritrichous flagella making it a motile bacterium (*82*). However, flagellar motility is temperature dependent (*99*). At 30°C and below, *L. monocytogenes* is motile and can also use flagella as a mechanism for biofilm formation (*82*). At mammalian metabolic

temperature (37°C), it's been found that flagellar motility genes are down regulated and the bacteria does not express flagella (*82*, *95*, *99*).

These resilient characteristics make *L. monocytogenes* persistent in wild and domestic animals, soil, waste water, birds, insects, and vegetation (*46*, *106*). This distribution gives the bacterium ample opportunity to spread through the food system. For example, contamination can be brought into a processing plant through raw products, employees, pests, improper sanitation procedures, or inefficient control strategies (*19*, *38*). Since *L. monocytogenes* can survive and thrive in food processing environments, food contamination is the biggest source of listeriosis infection (*19*, *44*).

The start of a potential listeriosis infection begins upon ingestion of a contaminated product. The infectious dose needed to cause disease is variable based on the individual. Typically, a healthy individual requires a higher dose to cause illness. Those who are young, old, pregnant, or immunocompromised are more susceptible to disease from a lower initial dose (*5*). The gastrointestinal system serves as the pathogen's primary mode of entry into the hosts cells (*130*). As the bacterial cells travel through the gastrointestinal tract, they first reach the intestinal epithelium. It's at this point, the pathogen utilizes receptor mediated endocytosis to enter the host's non-phagocytic cells such as epithelia (*104*). Entry is majorly regulated by two cell surface proteins in the internalin family called Internalin A (InlA) and Internalin B (InlB). These proteins bind to host cell membrane receptors called E-cadherin and Met (receptor of hepatocyte growth factor) (*33*). Once bound, entry into the cell is initiated and the pathogen is absorbed into the cell by a vacuole. Once inside the cell, vacuole rupture is

mediated by secretion of a toxin called listeriolysin O (LLO) and two phospholipases, phospholipase A (PlcA) and phospholipase B (PlcB) (*104*, *130*). This causes the vacuole to break and release the pathogen into the cytoplasm of the cell. The bacteria can then initiate cell-to-cell spread. Intracellular and intercellular motility is regulated by the polymerization of the protein actin. The creation of actin propels the bacteria from cell to cell making them less discoverable to host immune responses (*101*, *104*, *130*). The virulence mechanism described above is controlled by the regulatory protein, PrfA. With PrfA, *L. monocytogenes* is able to switch between extracellular and intracellular life (*35*).

As the pathogen moves from cell to cell, it travels from the intestinal epithelium to the lamina propria. This allows the pathogen to disseminate throughout the body via the lymph into the bloodstream and towards its target organs, the liver and spleen (*32*). In the liver, the hepatocytes are the primary locations of bacterial multiplication (*130*). Once replicated to higher numbers, the bacteria can be spread through the blood causing bacteremia. This allows the pathogen to cross the blood-brain barrier and cause neurological illness such as meningoencephalitis. It also can cross the placental barrier in pregnant women leading to neonatal septicemia or abortion (*104*, *130*).

L. monocytogenes is a deadly pathogen that causes outbreaks, deaths, and many food product recalls each year. Understanding the biology and pathogenicity of *L. monocytogenes* is beneficial to developing pathogen control strategies within the food industry as well as treatment methods for patients infected with listeriosis.

Clinical Manifestations and Epidemiology

L. monocytogenes is infectious to both humans and animals (*67*). It's estimated that approximately 1,455 hospitalizations occur in the United States each year from foodborne listeriosis, resulting in 255 deaths. This makes *L. monocytogenes*, the third leading cause of death due to foodborne illness; behind nontyphoidal *Salmonella* spp., and *Toxoplasma gondii* (*113*). Once an individual is infected, the disease can manifest itself in two different forms. There is non-invasive gastrointestinal listeriosis and invasive listeriosis. Non-invasive listeriosis in immune-competent individuals causes fever and typical gastroenteritis symptoms such as diarrhea, cramps, nausea, and vomiting. These clinical symptoms usually begin about 20 hours after initial ingestion (*3*, *34*). The invasive disease can lead to severe septicemia and meningoencephalitis in immunocompromised individuals and placental infection and abortion in pregnant women (*3*). The symptoms of invasive listeriosis typically start much later than non-invasive, approximately 20 to 30 days after initial ingestion (*109*).

Specific strains of *L. monocytogenes* are differentiated by a variety of mechanisms which is helpful in tracking outbreak data. After a clinical lab reports *Listeria* infection to the public health department, the samples are sent to a state public health lab where they are subjected to pulsed-field gel electrophoresis (PFGE). This method creates a DNA fingerprint for a bacterial isolate which is recorded in the CDC's PulseNet database (*6*). This process can take up to four days to complete. In addition to PFGE, whole genome sequencing (WGS) can also be performed to observe the complete DNA make-up of the isolate. WGS can take 4-5 days to process and up to an

additional three weeks to complete due to logistics (*6*). An older method of differentiation is to serotype based on somatic (O) and flagellar (H) antigens. This method is still used, however, newer techniques such as PFGE and WGS provide a much more discriminatory method (*32*).

Over time, phylogenetic research involving subtyping with these and other methods such as ribotyping and sequence variation divided *L. monocytogenes* into four distinct lineages (*128*). Lineage I includes serotypes 1/2b, 3b, 4ab, 4d, 4b, and 4e (*27*, *133*). Lineage II includes serotypes 1/2a, 1/2c, 3a, and 3c (*133*) . Lineage III is made up of serotypes 4a, 4c, 7 and some strains of 4b (*32*, *97*). Lineage IV, most recently classified, contains serotypes 4a, 4b, and 4c (*97*, *135*). When observing human and food isolates, it's found that most isolates are comprised of Lineage I and II strains (*97*). Most cases of human listeriosis are caused by four serotypes including 1/2a, 1/2b, 1/2c, and 4b (*32*).

Food Safety, Outbreaks, and Associated Costs

Listeriosis is transmitted primarily through contaminated food. As a result, many food processors have made food safety a top priority to prevent these outbreak events. The United States has many regulatory agencies in charge of enforcing food safety policies. The US Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) are generally responsible for meat, poultry, and egg products. The Food and Drug Administration (FDA) is generally responsible for all other foods including seafood. The third main agency, the Center for Disease Control and Prevention (CDC),

conducts epidemiological investigations and disease surveillance for foodborne illness (*117*).

In 1987, the collaboration of these three agencies generated a zero-tolerance policy of *L. monocytogenes* in ready-to-eat (RTE) foods. Ready-to-eat foods are food products that do not need any further processing before consumption. This policy states that a RTE food shall be labeled adulterated if *L. monocytogenes* is detected in either of 2, 25-g samples of product. This is defined in Federal Food, Drug, and Cosmetic Act, 21 U.S.C. 342(a)(1) (*117*, *128*). This principle also applies for meat and poultry products under the Federal Meat Inspection Act and Poultry Inspection Act, 21 U.S.C. 601(m) or 453(g), respectively.

Despite the efforts of these regulatory agencies, outbreaks have still frequently occurred. One of the first outbreaks to be associated with foodborne transmission of *L. monocytogenes* was in Canada in 1981 involving contaminated coleslaw. This outbreak resulted in 17 deaths with a 27% infant mortality rate and 28.6% adult mortality rate (*20*, *31*). In the United States, the first recognized *Listeria* outbreak was with pasteurized milk in Massachusetts in 1983. In this outbreak, 49 patients acquired listeriosis and 14 of those patients died from their illness (*45*).

Perhaps one of the most well-known *Listeria* outbreaks occurred in 2011 from contaminated cantaloupe grown at Jensen Farms in Colorado. This outbreak caused 84 cases from 19 different states. Of these patients, 15 deaths were reported (*4*). Using epidemiological methods, all four of the outbreak strains were traced back to the cantaloupes from Jensen Farms. After this, Jensen Farms issued a voluntary recall of

their cantaloupe products and the damage resulted in millions of dollars in law suits and restitutions. Though the number of cases involved in the cantaloupe outbreak is quite large, there are a few other outbreaks in the United States that have surpassed this. Those include a Mexican-style cheese outbreak with 142 cases (1988) and a frankfurter-associated outbreak with 108 cases (1998-1999) (*84*, *91*).

A recent *Listeria* outbreak (2015) occurred due to contaminated ice cream from Blue Bell Creameries. This event resulted in 10 hospitalizations, 3 deaths, and a recall of all products made by Blue Bell Creameries (*103*). Unfortunately, these outbreaks are only a few of many that have occurred throughout the world in the past decades due to *L. monocytogenes* contamination.

The most common products that have been found contaminated with *L. monocytogenes* are prepared meats, dairy products, unwashed raw vegetables, and seafood (*31*). It is currently estimated that a single recall can cause $160 to $300 thousand in lost product (*70*). It's also estimated that sales of the product decrease significantly by 22% to 27% during the following months after a recall (*124*). With these risks on the line, companies make food safety a top priority and are always re-evaluating their pathogen control strategies to keep up with current measures.

L. monocytogenes Cell Envelope Physiology

The Cell Envelope of L. monocytogenes

Many different components make up a bacterial cell. These include a cell envelope, cytoplasm, ribosomes, and genetic material. These components work

together to create a functioning and living organism and all have crucial responsibilities. The cell envelope itself is the outer layer of the cell composed of several glycopolymers and wall teichoic acids. It serves an integral role in protecting the internal components but also in controlling transport in and out of the cell. In a rapidly changing environment, the response of the cell envelope is most critical in maintaining the essential components of life (*118*).

In the late 1800s, scientist Christian Gram developed a method that differentiated and helped classify bacteria into two different groups based on the cell envelope. These are known as Gram-negative and Gram-positive organisms. Gram-positive organisms could retain the crystal violet stain while Gram-negative could not. This technique changed the way bacteria were identified and classified. It also opened the door to an entire new field of study looking at the fundamental structures that made these two groups so different (*11*).

The difference between Gram-negative and Gram-positive organisms lies within the cell envelope. A Gram-negative cell envelope is composed of an outer membrane, a peptidoglycan cell wall, and an inner cell membrane. This outer membrane is only found in Gram-negative organisms and is made up of a lipid bilayer to help protect the cell in rugged environments (*118*). The outer membrane also contains glycolipids and lipopolysaccharides that contribute to infectious properties (*73*, *105*, *118*). The peptidoglycan layer is composed of repeating units of disaccharide N-acetyl glucosamine and N-acetylmuramic acid and gives the cell it's characteristic shape (*131*). Finally, the inner membrane is composed of a phospholipid bilayer and the

membrane proteins that contribute to energy production, lipid biosynthesis, and protein secretion and transport (*118*). The inner membrane is negatively charged giving the bacteria higher affinity for positively charged cations (*136*). These structures are present in Gram-negative organisms such as *Escherichia coli*, *Campylobacter jejuni*, and *Salmonella*, all prominent foodborne pathogens that can cause serious disease (*118*).

As this thesis focuses on *Listeria monocytogenes*, the details on Gram-positive cell envelopes will be covered in much more detail. One of the biggest differences between a Gram-negative and Gram-positive cell is that Gram-positive cells do not have an outer membrane. To make up for the lack of outer membrane, Gram-positive cells have a much thicker peptidoglycan layer. Polymers called teichoic acids weave through this peptidoglycan layer. There are two types of teichoic acids. The first are wall teichoic acids (WTA). WTAs are covalently bound to the peptidoglycan layer itself through a phosphodiester bond. The second group of teichoic acids are lipoteichoic acids (LTA). LTAs are amphipathic molecules that are bound to the inner cell membrane (*39*, *118*).

In *L. monocytogenes*, WTAs are mostly composed of polyglycerol-phosphate (GroP) or polyribitol-phosphate (RboP). These WTAs contain repeating RboPs with various carbohydrate compounds such as *N*-acetylglucosamine (GlcNAc), rhamnose (Rha), glucose (Glc), and galactose (Gal) (*39*). Glycosidic substitutions on the polyribitol phosphate backbone account for structural diversity in different serotypes of *L. monocytogenes* (*74*, *126*). Serotype 1/2 typically contains Rha and GlcNAc on the ribitol molecule while serotype 3 contains only GlcNAc. Serotypes 4, 5, and 6 contain GlcNAc on the polyribitol but may have substitutions of Glc or Gal (*39*, *126*).

WTAs have a multitude of functions in *L. monocytogenes*. They have been shown to play a role in biofilm formation, phage binding, cell division, and other interactions (*118*, *136*). The cell envelope can also be a key target for control of *L. monocytogenes* in the food industry. Understanding the key components and physiology of the cell envelope is crucial for establishing the most effective treatment methods in food applications.

Antimicrobial Activity and Environmental Effects on the Cell Envelope

Bacterial cells contribute a significant amount of energy towards the biosynthesis of their cell envelope. Therefore, it's concluded that the cell envelope has an important function when it comes to surviving in a hostile environment such as the gastrointestinal tract (*118*, *136*). As *L. monocytogenes* is a Gram-positive organism, the cell envelope contains a variety of cell-wall glycopolymers that consist of wall teichoic acids. These WTAs have been shown to play a part in protection of the cell by blocking the pores between peptidoglycan strands. They also can modify the physicochemical properties to block the passage of harmful substances such as antibiotics, bacteriocins, surfactants and bacteriophages (*136*).

Several studies have also shown how changes in cell surface features can affect tolerance to antimicrobials (*8*, *52*, *53*, *127*). In previous studies, it's been shown that *L. monocytogenes* cell envelope-acting antimicrobial tolerance is dependent on an alternative sigma factor called SigB (*8*). Alternative sigma factors are proteins that help simultaneously regulate large numbers of genes (*76*). Several loci regulated by SigB have been identified as cell wall related. The disruption of SigB likely plays a role in

membrane characteristics including charge and lipid composition (*8*). These characteristics contribute to the susceptibility of the cell to cell envelope-acting antimicrobials including bacteriocins and antibiotics. Another study, Vadyvaloo et. at (2004) found that *L. monocytogenes* strains that were highly resistant to class IIa bacteriocins had a more positive cell surface and higher alanine:phosphorus ratio in the teichoic acids.

Though the cell envelope can provide protection from various threats, it is still a common target for antimicrobials. The use of cell envelope-acting antimicrobials has great potential to prevent or decrease the growth of *L. monocytogenes* in food products (*72*, *86*, *94*, *138*). However, much research is needed to understand the interactions of these interventions with the cell envelope and how environmental conditions affect these reactions.

An example of a cell envelope-acting antimicrobial is nisin. Nisin is a bacteriocin produced by a Gram-positive bacterium called *Lactococcus lactis*. It can be used as an antimicrobial against many foodborne pathogens including *Staphylococcus aureus*, *Clostridium botulinum*, and *Listeria monocytogenes*. Nisin is known to attach to the cell envelope by binding to a major cell wall component precursor (Lipid II) (*16*). After attachment, the bacteriocin inserts itself into the membrane and forms pores ultimately destroying the cell (*47*, *65*). In some cases, mechanisms have developed over time in *Listeria* that result in nisin resistance. For example, d-alanylation of teichoic acids has been found to cause nisin resistance. D-alanylation or the addition of alanyl groups on

LTA causes an increase in positive charge on the cell surface. Therefore, this makes the cell less susceptible to cationic peptide attacks by nisin (*108*, *111*).

There is also evidence that environmental conditions can affect the cell surface features of a cell these changes have been linked to susceptibility to antimicrobial treatment. Previous studies have found that acid adapted, osmotically stressed, or cells pre-exposed to potassium lactate can select for protection against nisin due to cell surface changes (*10*, *75*, *129*). The success of nisin has also been shown to be affected by environmental pH and temperature (*1*).

Food products can expose pathogens to a wide range of conditions including different acidity levels, temperatures, ingredients, and processing steps. Exposure of bacterial cells to these unique environmental conditions can lead to unexpected changes within the cell population. It is necessary to understand how these changes affect cell envelope composition and therefore susceptibility to interventions. Researching these effects can assist in the development of robust treatment methods against foodborne pathogens.

Bacteriophages

Bacteriophages and Host Interactions

Bacteriophages, or bacterial viruses, are obligate intracellular parasites that lack a metabolism. They require a host cell to replicate and spread (*119*). Bacteriophages are extremely diverse and have been shown to outnumber bacteria in most environments with an estimated global population size of ≥ 10^{30} phage particles (*30*).

They are a major player in bacterial ecosystem dynamics and have significant effects on bacterial populations (*22*). Frederick W. Twort (1915) and Felix d'Herelle (1917) were among the first scientists to describe bacteriophage and begin modern day phage research (*122*).

A bacteriophage particle typically consists of a single-stranded or double-stranded DNA or single stranded RNA molecule which is encapsulated in a protein coat. This protein coat is usually an icosahedral shape where the size is determined by the length of genetic information inside (*61*). The other parts of the phage particle are the sheath and tail fibers. These components all work together to generate a successful infection of a host cell.

Bacteriophages can be divided into two main groups: virulent and temperate phages. Virulent or lytic phages immediately replicate and lyse their host cell within hours after the initial infection. All phages used as antimicrobial agents in the food industry are virulent phages. Temperate or lysogenic phages work much slower and can have a stable relationship with their host cell (*22*).

Temperate phages integrate their genetic material into the host's genome at a specific location and maintain a constant relationship with their host cell. Once the genetic material is integrated, the phage is known as a prophage. The phage genome is then replicated with the host's chromosome and any virus genes that could be harmful to the host are not expressed. These integrated genes have been shown to have varying effects on the host cell including protection from phage infection and increased virulence of a pathogenic host (*15*, *22*). As the host cell encounters different stressors,

the prophage can be stimulated to create virulent phage particles. This process can occur spontaneously and results in the switch from lysogenic to lytic cycle. As more virulent particles are released from the cell, the cycle begins again as the phages look for more host targets (*22*).

The lytic phage infection cycle follows similar steps that must be carried out efficiently for the virus to replicate and spread. The first step is adsorption or attachment to the host cell. This is done by recognition of a specific binding site on the cell surface. In Gram-negative bacteria, many of the proteins in the cell envelope can be used as binding sites (*66*). In Gram-positive cells, however, the process of phage binding is much more complex. Therefore, more specific binding sites are used by bacteriophages infecting these types of cells. For example, substituents of teichoic acids such as GlcNAc and Rha in *L. monocytogenes* are known phage receptors for specific *Listeria*-phages. The bacteriophage utilizes its tail fibers to bind to these specific receptors (*137*).

After initial attachment to the host cell, the genetic material must be transferred through the cell envelope into the cytoplasm for the phage to replicate (*21*). To break through the cell wall barrier, the tail fibers deploy enzymes that break down the peptidoglycan layers and protect the genome from pre-mature extrusion. The genome is then drawn into the cell using metabolic energy and a membrane potential mechanism (*83*). Many phages also avoid exonucleases and restriction enzymes within the cell by circularizing the genetic material through sticky ends or terminal repeats (*22*).

After the bacteriophage genome is internalized into the cell, the host's RNA polymerase recognizes promoters on the phage genome. This leads to transcription of the early genes (*22*). These genes are typically responsible for hijacking the metabolic machinery in the host and creating an optimal environment for phage replication (*22*, *92*). Once this is achieved, the host machinery is used to replicate the genome and create phage particles. The phage particles are then assembled and matured into whole virulent bacteriophages and released from the cell typically by lysing and killing the host (*22*, *139*).

In food applications, lytic phages have been targeted as a biocontrol due to their ability to reduce pathogenic bacterial populations (*69*). As phages are abundantly available and self-replicating, they can work as a natural biocontrol for unwanted pathogens in the food processing facility.

Phage Application in Food Processing

Bacteriophages have recently been explored as a biocontrol for *L. monocytogenes* and many other foodborne pathogens (*69*). Phages are easy to find in the environment and are often incidentally consumed regularly through water and food. No undesirable side effects have occurred from the consumption of bacteriophage laying the groundwork for recognition as a safe treatment for use in food manufacturing and on foods. Bacteriophages have been widely evaluated for efficacy by various segments of the food industry (*88*).

The Food and Drug Administration has approved the use of commercial phage treatments labeling them as GRAS (Generally Recognized as Safe) (*42*). There are

bacteriophage products that currently exist to treat *L. monocytogenes* in a variety of food products. In the United States, a commercial product called ListShield, manufactured by Intralytix (Baltimore, MD, USA), is a phage cocktail consisting of P100-like phages used for the treatment of *L. monocytogenes*. Various studies have reported successful reduction of *L. monocytogenes* in food matrices to as low as undetectable levels (*57*, *60*, *100*). It's also been shown that this phage cocktail can also be effective against *L. monocytogenes* biofilms in food contact surface treatment (*112*).

There are four essential applications of phage in the food processing environment that have shown to be effective in control of foodborne pathogens (*119*). The first is phage therapy which is typically a pre-harvest treatment used in the meat industry. Though there is no oral treatment commercially available, much research has been done to show the effectiveness of phage therapy on live animals (*96*, *107*, *110*). This method is used to treat contamination at the source and reduce mortality and fecal shedding of foodborne pathogens in poultry and livestock (*119*).

The second method of phage application is post-harvest control. This is the reduction of bacterial populations in foods at the food processing level. This is most commonly done by applying the phage directly to the food product on the processing line. The third method is sanitation. Sanitation is disinfecting the food contact surfaces themselves by applying phage (*119*). The last application is preservation. This method is used to prevent contamination in products during storage. Multiple studies have shown the success of applying phage to food packaging materials to keep products free of pathogens during storage and transport (*14*, *50*, *54*, *85*, *119*).

L. monocytogenes is known for its ability to form biofilms on common food manufacturing plant surface materials such as stainless steel, rubber, and plastic. Scratches on food contact surfaces can also lead to biofilm formation due to pits or cracks that are difficult to clean (*9*, *14*). In these cases, phages can also be used on surfaces within the plant to prevent cross contamination. In a study performed by Chaitiemwong et. al (2014), it was found that bacteriophages had better antimicrobial effect in shallow grooves than chemical disinfectants. They concluded that the use of bacteriophages could be beneficial in some cases but use of large quantities could turn out to be costly and the risk of phage resistance is a possibility (*23*). A more recent study showed that phage application to stainless steel coupons and rubber surfaces had a 1-2.4 log reduction of *L. monocytogenes* when in biofilms (*112*). These studies show that phage treatment is not only beneficial for direct food contact but also for treatment of food contact surfaces.

There are many benefits to consider when using bacteriophage as a control strategy. It offers high specificity to a target host while leaving desirable bacteria untouched. Phages are also self-replicating and can grow to large numbers from a small dose. They are always on a continuous cycle with their hosts that allow them to adapt to host defense mechanisms and they also provide a natural antimicrobial control method which has been becoming increasingly popular in food production today. Lastly, they can withstand the stressors found in food processing environments (*69*, *119*).

When creating a phage based treatment method, it's important to understand the desirable properties of phages to select the most efficient method for a specific food

product. As summarized by Hagens and Loessner (*64*), the following key principles must be considered. The selected phage or phages must have a broad host range that is capable of infecting multiple strains of the target organism. This is done to minimize potentially resistant strains from colonizing the product or processing plant. The phages used should also be strictly lytic serving only the purpose of lysing host cells. The genome of the phages must be known and genes associated with any potential allergenic proteins must be absent. All phages used in products must be certified GRAS and should maintain stability over a long period of time. Lastly, it's also beneficial to have a phage treatment that is easily scalable for production (*48*, *63*). The combination of these principles can lead to successful a bacteriophage treatment intervention capable of reducing pathogenic populations in food products.

Despite the many advantages of phage treatment, there are still many drawbacks and much needed research to improve this method of control in food processing facilities. One of the largest concerns with bacteriophage treatment is the accumulation of phage resistance within the target populations. It's been previously shown that phage predation can induce phage resistance in host cells (*40*, *121*) It's also known that environmental factors can play a part in phage infection efficiency. This is especially important to consider when working with a diverse array of food products (*125*). In a constantly changing process it's crucial to understand all aspects of bacteriophage treatment for it to be as effective as possible.

Listeria-phages

Listeria phages are commonly found in sewage, silage, food processing plants, and soil. These are phages that specifically target *L. monocytogenes* either in a lysogenic or lytic matter. Over 500 *Listeria* specific phages have been discovered but only a few have been completely genetically analyzed. All of the known *Listeria* phages belong to the *Caudovirales* order. These viruses contain a tail and double stranded DNA. Though many of the existing *Listeria* phages follow a temperate cycle, there are some virulent *Listeria*-phages that have been well studied especially for their food application purposes (*64*).

Listeria-infecting phages follow the typical virulent infection steps starting with attachment and entry. As previously described, *Listeria*-phages recognize and bind to the wall teichoic acids on the cell envelope of *L. monocytogenes* (*12*, *36*, *62*). This attachment allows for entry into the cell, replication of the DNA, and eventual lysis of the host.

As previously described, it's known that the environmental conditions in which the cell is subjected to can have an effect on cell envelope composition (*1*, *10*, *75*, *108*). Since the cell envelope contains the binding sites for *Listeria*-phages, the success of phage binding can also be altered in these conditions. Thus, the cell's susceptibility to bacteriophage infection can be changed. With more research on the effectiveness of phage based treatment methods, it's necessary to understand the effects environmental conditions can have on phage-host interactions (*37*). This research can help develop better and more effective interventions to prevent deadly foodborne outbreaks.

Dairy Processing and Safety

Dairy products have been around for thousands of years and have created a large industry to this day with a multitude of products including milk, cheese, cream, butter, yogurt, and ice cream. The processing of these products is essential to microbial safety but can be very challenging due to the nature of milk. Raw whole milk consists of fat, protein (casein and whey), lactose, and ash. In the United States, the dairy industry is regulated by the Grade A Pasteurized Milk Ordinance (*116*). This ordinance controls milk production, transportation, and processing to produce high quality and safe products for consumption (*26*).

In the United States, most of the milk comes from farmers' cooperatives and individual contracts. The first step of the dairy process is collecting the raw milk at dairy farms. This milk is then transported to the dairy plant where it is separated into cream and skim milk and mixed with dry ingredients. This product is then sent to the pasteurizer where the most important microbial kill step is located. Pasteurization is used to kill any pathogenic bacteria that may be present in raw milk. Pasteurization is typically conducted through plate heat exchangers and heat time is dependent upon PMO standards. The standards are calculated using thermal death time studies for various pathogens that can found in milk. Typical processing involves high temperature, short time (HTST) processing. For whole, low-fat, and skim milk, this process treats the milk for 15 seconds at 72°C (*26*, *116*).

After pasteurization, milk can either be packaged directly for sale or used to make other dairy products. As the safety of consumers depends on the pasteurization

process, it is crucial that this step is successful or other forms of biocontrol must be implemented. Overall, milk is threatened by many microorganisms including pathogenic and spoilage organisms. The short list of these include *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *L. monocytogenes*, and *Coxiella*, and *Clostridium* species (*2*, *115*). Because of this, milk quality assurance and safety is of utmost importance to dairy processors.

L. monocytogenes is a growing problem in the dairy industry especially as unpasteurized products such as soft cheeses become increasingly popular. It's ability to grow at refrigeration temperatures also makes it a concern for dairy processors as products are often stored or ripened in these temperatures. Though the chances of contamination are low, there have still been several outbreaks in dairy products such as cheese, milk, and ice cream (*71*, *93*, *102*).

Dairy products also have unique environmental conditions including varying storage and ripening temperatures and acidity. As previously discussed, it's known that environmental factors can influence cell surface physiology of *L. monocytogenes*. As these factors in dairy products can be variable, it's important to understand the changes that occur when *L. monocytogenes* is exposed to these conditions and subsequently how they affect susceptibility to antimicrobial treatment. Examining these effects on cell surface physiology can help in the development of improved control strategies for pathogens in dairy products. The purpose of this study is to observe cell envelope changes in *L. monocytogenes* when cells are exposed to various dairy related conditions. This information can be helpful for understanding the physiology of this

pathogen in dairy products and how effectiveness of treatments can change in these conditions.

CHAPTER TWO

LISTERIA MONOCYTOGENES CELL ENVELOPE PHYSIOLOGY IS INFLUENCED BY GROWTH IN DAIRY RELEVANT CONDITIONS

Abstract

Listeria monocytogenes is a recurring problem in the dairy industry causing a multitude of recalls and unfortunately some outbreaks in a variety of products including milk, cheese, and ice cream. Its ability to tolerate and thrive in a wide range of conditions makes it even more of a concern for dairy manufacturers. Many industry interventions such as antimicrobials work by targeting the cell envelope. There is clear evidence, however, that the cell envelope components are affected by exposure to different environmental conditions. Here, we examined and characterized *L. monocytogenes* cell envelope changes when cells were exposed to varying dairy relevant pH (5.7, 6, 6.2, 6.5) and temperature (6°, 14°, 22°, 30°C) conditions. Two known *Listeria*-phages (LP-048 and LP-125) and cytochrome *c* were used in binding assays to determine cell surface changes in selected laboratory, dairy outbreak, and mutant *L. monocytogenes* strains. The results of these assays showed significant differences in binding efficiency within strains grown in a range of dairy pHs and temperatures. These data suggest that growth pH and temperature influence binding efficiency of *Listeria*-phages and cytochrome *c* and thus affect specific cell envelope characteristics. The results found were strain-dependent meaning that similar strains did not always show similar effects. The data presented here clearly show that cell envelope composition is affected by environmental conditions and thus cell envelope-acting treatment methods may not always be as effective in these environments. This should be considered when designing effective treatment methods in the prevention of *L. monocytogenes* in dairy products.

Introduction

L. monocytogenes is a Gram-positive rod bacterium that is found ubiquitously throughout the environment (*41*). It can cause a severe disease known as listeriosis. The fecal-oral route is the most common mode of transmission of *L. monocytogenes* which makes contaminated food one of the greatest sources of *Listeria* infection in both humans and animals (*67*). As a psychrotrophic organism, it can tolerate and grow at temperatures as low as -0.4°C (*134*). It also can grow in a wide pH range of 4.6 to 9.5, low moisture content or high salt conditions (*46*, *90*). Bacterial cells can be exposed to many stressors in a food processing environment such as different temperatures and acidity, chemical sanitizers, and antimicrobials (*18*, *28*). Due to its resilient nature, *L. monocytogenes* is able to persist in food processing environments causing deadly foodborne outbreaks (*19*).

Listeriosis can be a severe disease resulting in neurological illness such as meningoencephalitis or neonatal listeriosis (*41*). Clinical listeriosis mainly occurs in young, old, pregnant, or immunocompromised individuals (*87*). In 2010, it was estimated that over 23,000 illnesses were caused by *L. monocytogenes* worldwide. Of these cases, approximately 5,500 deaths were estimated to occur (*87*). In the United States alone, an annual estimated 1,455 cases are predicted to be caused by *L. monocytogenes* with a 94% hospitalization rate and 15.9% death rate of those infected. This makes *L. monocytogenes* the third most leading cause of death due to foodborne illness behind *Salmonella* spp. and *T. gondii* (*114*).

In 1987, the U.S. Food and Drug Administration created a zero-tolerance policy for *L. monocytogenes* in ready-to-eat (RTE) products to overcome this burden of foodborne illness. This means that if any *L. monocytogenes* is detected in these types of products, then the product is labeled as adulterated (*117*, *128*). Thus, the presence of *L. monocytogenes* can be particularly detrimental to a food company. It's estimated that a single product recall can cost $160 to $300 thousand in damages (*70*). In the United States, it's estimated that the annual cost of *L. monocytogenes* is around $2.3 to $22 billion (*70*). The strict no tolerance policy and heavy costs of *L. monocytogenes* has made efforts to prevent this pathogen from entering a food system a high priority by manufacturers (*128*).

In recent times, the dairy industry specifically has experienced several *Listeria* outbreaks in a variety of products including milk, cheese, and ice cream (*71*, *93*, *102*). The use of cell envelope-acting antimicrobials in dairy processing has shown great potential in decreasing or preventing the growth of *L. monocytogenes* in many situations (*57*, *58*, *78*, *86*). However, dairy products have unique characteristics that may change the effectiveness of these interventions.

The cell envelope serves many purposes including the first line of contact and protection for the cell. The cell envelope of *L. monocytogenes*, a Gram-positive organism, consists of an inner cell membrane and a thick peptidoglycan layer containing wall teichoic acids (WTA), and lipoteichoic acids (LTA) (*118*). These components interact with many antimicrobials and therefore contribute to the susceptibility of the cell to antimicrobial treatment (*89*).

It has been previously shown that effectiveness of nisin treatment, a cell envelope-acting bacteriocin, is affected by environmental growth conditions including pH and temperature (*1*). Other interventions including phage-based applications, are also affected by the conditions in which *L. monocytogenes* is exposed (*37*). However, there are still many unknown mechanisms involved in this field of research. For the dairy industry specifically, understanding how these product's unique conditions influence the cell envelope composition and subsequent antimicrobial susceptibility is crucial for developing the most effective control strategies.

In the dairy industry, there is a wide range of temperatures used for ripening and storage of products. This can range anywhere from refrigeration to above room temperature (*13*). There are also differences in acidity between products (*81*). The goal of this study was to observe cell surface changes that occur when *L. monocytogenes* strains are exposed to varying dairy relevant temperatures and pHs. These cell surface changes were observed using cell envelope binding bacteriophages and a cell wall charge indicator cytochrome *c* as measurement tools. A variety of *L. monocytogenes* strains were used including lab model strains (serotype 1/2a and 4b), dairy related outbreak strains (CDC), and mutant strains lacking various cell surface features. The key objective of the study was to characterize expression of *L. monocytogenes* cell envelope constituents under varying dairy relevant environmental conditions.

Materials and Methods

Bacterial Strains and Growth Conditions

Bacterial strains were stored in glycerol stocks at -80°C. Strains were streaked from 15% glycerol stocks onto BHI agar plates and incubated for 16±1 h at 37°C. Single colonies were inoculated into 5mL liquid BHI and incubated in a shaking water bath at 30°C for 16±1. These overnight cultures were then spread plated on skim milk agar (SMA) plates comprised of 10% (wt/vol) skim milk powder (Becton Dickinson Co., Sparks, MD), 0.1% (wt/vol) glucose (Fisher Scientific, Fair Lawn, NJ), 0.25% (wt/vol) yeast extract (Oxoid Ltd, Hampshire, UK), and 1% (wt/vol) agar (Fisher Scientific). *L. monocytogenes* cells were subjected to two different sets of environmental conditions (pH and temperature). The pH experiments were conducted using SMA plates buffered with 0.5 M 2-(N-morpholino)ethanesulfonic (MES) acid buffer (Fisher Scientific) adjusted to pH 5.7, 6.0, 6.2, and 6.5 ± 0.1. *Listeria* strains were incubated on these pH adjusted plates for 48 hours at 30°C. The temperature experiments were conducted by incubating the SMA plates at 6, 14, 22, 30 ± 0.5°C for 11, 5, and 2 days (22°C and 30°C), respectively. Incubation periods were set to obtain a lawn of bacterial cells. Bacterial strains used in this study can be found in Table 1[1].

[1] All figures and tables shown in the Appendix.

Phage Strains and Preparation of Stocks

Phage lysates were prepared as previously described (*132*) and stored in the dark at 4°C. Phage enumeration was conducted after serial dilution with SM Buffer (100mM NaCl (Fisher Scientific), 8 mM $MgSO_4 \cdot 7H_2O$ (Acros Organics, Fair Lawn, NJ), 0.002% [wt/vol] gelatin (Fisher Scientific), and 50 mM Tris-Cl adjusted to a pH of 7.5 (Fisher Scientific)) followed by a double-agar overlay plaque assay (*79*) using modified LB-MOPS media (LB medium buffered with 50 mM morpholinepropanesulfonic acid [MOPS] at pH of 7.6 (Fisher Scientific)). Agar overlays were made with 0.7% (wt/vol) LB-MOPS agar supplemented with glucose (0.1% wt/vol) and 10 mM each $MgCl_2$ and $CaCl_2$. Agar underlays were made with 1.5% LB-MOPS also supplemented with glucose and salts as previously described (*36*). Plated phage samples were incubated at 25°C for 16±2 hours. Phages used in this study can be found in Table 1.

Cell Collection, Washing, and Standardization

After incubation, cells were collected to carry out two different assays to detect differences in cell surface composition. To collect the cells, they were gently scraped from the plate surface using an inoculating loop. The cells were then washed by spinning and re-suspending 3 times (8,000 × *g* for 5 min at 4°C, Thermo Scientific Sorvall Legend Micro21R model, Waltham, MA) with either SM buffer for phage binding assays or MOPS buffer (20mM at pH 7.0) for cytochrome *c* binding assays. After the last suspension, the optical density was measured at 600nm (Thermo Scientific GENESYS 30 Visible Light Spectrophotometer, Waltham, MA). The phage binding

cultures were standardized using SM buffer to OD_{600} = 0.05. The cytochrome *c* cultures were standardized using MOPS buffer to OD_{600} = 0.1.

Bacteriophage Binding Assays

Bacteriophage stocks of LP-048 and LP-125 were diluted to a concentration of 1 × 10^9 PFU/mL. Concentrated phage was added with the standardized cell culture (OD_{600} = 0.05) to obtain a phage concentration of approximately 2 × 10^7 PFU/mL for each experimental condition. Tubes were gently inverted to evenly disperse the sample. Assays were incubated at 25°C for 15 min. Samples were then centrifuged (16,000 × *g* for 2 min at 4°C) and the supernatant was collected and serially diluted in SM buffer for enumeration of phage in the supernatant. Dilutions were plated via double agar overlay plaque assay using modified LB-MOPS. Agar overlays and underlays were prepared in the same manner as described above. *L. monocytogenes* Mack strain was used for enumeration. Plates were inverted and incubated at 25°C for 16±2 hours. PFU/mL was calculated for each sample and log_{10} reduction was calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control (no cells).

Cytochrome c Binding Assays

Master stocks of cytochrome *c* from bovine heart (Sigma-Aldrich, St. Louis, MO) were made at a concentration of 5 mg/mL in sterile water. Working stocks of 500 µg/mL were made from dilutions of the master stock. All cytochrome *c* stocks were stored at -20°C. Working stock cytochrome *c* was added to standardized cells (OD_{600} = 0.1) for

each condition to obtain a final cytochrome *c* concentration of 50 µg/mL. Tubes were gently inverted and incubated at room temperature (18±1°C) for 10 min. Samples were centrifuged (16,200 × *g* for 5 min at room temperature) and the supernatant was collected. The optical density of the supernatant at 410nm was measured and recorded. The reduction OD_{410} of cytochrome *c* was calculated by subtracting the OD_{410} of the supernatant from the OD_{410} of the negative control (no cells).

Statistical Analysis

Experiments were all replicated 3 times and values reported for each experiment were the average of two duplicate samples. The log_{10} reduction and reduction OD_{410} of cytochrome *c* for each condition were analyzed using JMP (Version 13. SAS Institute Inc., Cary, NC).

Linear models were constructed using the factors strain or condition. Biological replicate was also added to the model as a random factor. Log_{10} reduction was the model response for phage binding experiments, whereas reduction OD_{410} of cytochrome *c* was the model response for cytochrome *c* binding experiments. Pairwise comparisons were made using lsmeans with Dunnett's test (mutant experiments) and Tukey's range test (outbreak experiments). The cutoff for significant was set at $p < 0.05$.

Results

Growth pH and Temperature Affects Phage and Cytochrome c Binding to Wild-type 10403S Strain

Phage binding experiments using LP-048, a rhamnose binding phage, with laboratory wild-type strain 10403S grown in a range of dairy relevant pHs found that wild-type exhibited a positive linear trend with the highest known value at a pH of 6.5. Log reduction or approximate phage bound at pH 6.5 was significantly different than phage binding at 5.7 and 6. This showed that as pH increased from 5.7 to 6.5, phage binding also increased (Figure 1A).

Phage binding experiments using LP-125, a rhamnose and N-acetylglucosamine binding phage, with wild-type 10403S grown in various pH showed a similar result. With this phage, a positive linear trend was also seen with significant differences in phage binding occurring between pH 5.7 and 6.5. This showed that phage binding increased as the growth pH increased (Figure 1B). Overall these experiments showed that pH influences binding efficiency of LP-048 and LP-125 suggesting that rhamnose or N-acetylglucosamine are changing on the cell surface in these different conditions.

L. monocytogenes strain 10403S showed negative quadratic trends in phage binding for both LP-048 and LP-125 as temperature increased. For LP-048, significant differences were found between 14°C and 22°C from 6°C and 30°C (Figure 2A). A similar result was found using LP-125 with significant differences between 14°C and 22°C from 6°C (Figure 2B). This means that the growth temperature had significant influence on phage binding for both P100-like phages on wild-type strain. This suggests

that changes in rhamnosylation or *N*-acetylglucosamine regulation are occurring when cells are exposed to different temperatures.

To further show the significance of environmental conditions and cell surface features, cytochrome *c* was used to show changes in cell envelope charge. Wild-type 10403S strain showed significant differences in cytochrome *c* binding as growth temperature changed. Significant differences were seen between 14°C and 30°C from 22°C (Figure 3). As cytochrome *c* is a negative charge indicator, it is inferred that more cytochrome *c* adsorption would mean a higher negative charge on those cells. These results show that less cytochrome *c* is adsorbed to cells grown at 14°C and 30°C than those grown at 22°C. Electronegativity of the cell is influenced by the structures that make up the cell envelope (*136*). Since cytochrome *c* measures the cell surface charge, these data suggest that cell envelope composition is affected by the cell's growth conditions.

Mutant Strains Lacking Specific Cell Surface Features Had Significant Differences in Phage and Cytochrome c Binding When Subjected to Various Conditions

A specific set of *L. monocytogenes* 10403S mutant strains were tested in both phage binding and cytochrome *c* binding assays. These strains were selected as they contain mutations that affect specific cell envelope characteristics. Strain ΔdltA contains a mutation in the *dlt* operon which is responsible for incorporating D-alanine in the teichoic acids of the cell envelope. With this mutation, the net negative charge of the cell is reduced which influences the binding of cationic compounds (*77*). Strains 10403S 541_M and 542_M both contain nonsense mutations affecting wall teichoic acid

decorations. 10403S 541_M is deficient in *N*-acetylglucosamine (GlcNAc), a major receptor for LP-125. 10403S 542_M is deficient in rhamnose (Rha), a major receptor for LP-048 and LP-125. These WTA decorations are sites for phage binding receptors for LP-048 and LP-125 (*36*). Lastly, the mutants ΔmprF and ΔmprFΔdltA contain deletions in the mprF gene resulting in loss of lyslphosphatidylglycerol (L-PG), a cell envelope compound. L-PG is responsible for a net positive cell membrane charge and repulsion of cationic peptides. The loss of this compound makes the cell more negatively charged and susceptible to cationic antimicrobials (*120*).

The mutant 10403S strains were tested under the same growth conditions, temperature and pH, and compared to wild-type 10403S within each condition. For phage binding of LP-048, strain 10403S ΔdltA showed significant difference in phage binding from 10403S at a pH of 5.7. Strains 10403S (541_M) and ΔmprF showed significantly higher phage binding than 10403S at a pH of 6.5 (Figure 4A). No significant differences were seen when testing pH and LP-125 binding (Figure 4B).

For phage binding using LP-048, a significant difference was found between the ΔmprF mutant and wild-type at 22°C and 30°C with higher phage binding occurring in the mutant strain at these temperatures (Figure 5A). No other significant differences were seen when testing pH with this phage. LP-125 phage binding also found differences in the ΔdltAΔmprF and ΔmprF strains. ΔdltAΔmprF showed significantly higher phage binding of LP-125 at 6°C, 22°C, and °30C. ΔmprF mutant showed significantly higher phage binding at 6°C and 30°C (Figure 5B).

For cytochrome *c* binding to mutant strains grown in different pHs, only the double mutant ΔdltAΔmprF showed a significantly higher reduction OD_{410} at a pH of 5.7 from the wild-type (Figure 6). The same mutant ΔdltAΔmprF showed significantly higher cytochrome *c* binding from the wild-type when grown at 14°C and 30°C. 10403S (541M) also showed significantly higher cytochrome *c* binding at 30°C (Figure 7). Overall, these data suggest that cell surface features are changing when cells are exposed to varying dairy relevant conditions. The specific mutations of these strains involve cell envelope components and the differences seen suggest changes in expression during exposure to environmental conditions.

Growth Temperature and pH Influence Phage Binding and Cytochrome c Binding on Dairy Related Outbreak Strains and Serotype 4b Lab Strain

When testing phage binding with dairy related outbreak strains, different results were found using LP-048 for each strain. FSL R9-5621 (Ricotta cheese 2012, serotype 1/2a) showed significantly higher phage binding at a pH of 6 than 5.7. FSL R9-5623 (Semi soft fresh style cheese 2013, serotype 4b) showed significantly lower phage binding of LP-048 at pH 6.5 than pH 6. There was also a significantly lower phage binding in FSL R9-5624 (Queso fresco 2013, serotype 1/2b) at pH 6 than pH 5.7 which is the opposite of FSL R9-5621, the other serotype 1/2 strain. Overall, both serotype 1/2 strains, showed higher phage binding of LP-048 in all pH conditions than both serotype 4b strains (Figure 8A). No significant differences were seen using LP-125 on these strains testing pH (Figure 8B).

Different results were seen in phage binding experiments using LP-048 and growth temperatures. FSL R9-5621 (serotype 1/2a) showed significant differences in binding at 22°C and 30°C while FSL R9-5625 (serotype 4b) showed differences between 6°C and 30°C (Figure 9A). However, when testing phage binding of LP-125, FSL R9-5621 did not show any significant differences while FSL R9-5623 (serotype 4b), FSL R9-5624 (serotype 1/2b), and FSL R9-5625 (serotype 4b) did. FSL R9-5623 (serotype 4b) had significantly higher binding of LP-125 at 22°C and 30°C than at 6°C and 14°C. FSL R9-5624 (serotype 1/2b) had differences between 14°C and 30°C while FSL R9-5625 (serotype 4b) had differences between 6°C and 30°C (Figure 9B). These results suggest that higher and lower temperatures change the surface features that affect phage binding of LP-125 and that the patterns can be different among similarly serotyped strains.

Cytochrome *c* binding experiments using dairy outbreak strains also strengthened this result. Growth pH showed significant influence on reduction OD_{410} for one outbreak strain, FSL R9-5625. There was significantly lower cytochrome *c* binding at pH 6.5 than at pH 6. No significant differences were found for FSL R9-5621, FSL R9-5623, and FSL R9-5624. To further examine these differences, model lab strain F2365 was also tested and significant differences between pH 5.7, 6, 6.2, and 6.5 were found (Figure 10).

When testing the effect of temperature on cytochrome *c* binding, both serotype 1/2 strains showed significant differences between conditions. FSL R9-5621 had significantly lower binding occur in cells grown at 22°C and 30°C than at 6°C. FSL R9-

5624 showed the opposite with significantly higher binding occurring in cells grown at 30°C than at 6°C and 14°C (Figure 11). Overall, these results suggest that cell surface changes are occurring when cells are exposed to varying dairy relevant environmental conditions. It also suggests that differences in binding efficiency are variable between strains even if they are similar in nature.

Discussion

In this study, we examined the effect of growth temperature and pH on the efficiency of phage binding and cytochrome *c* binding to a variety of *L. monocytogenes* strains. The use of bacteriophages and cytochrome *c* as two cell envelope binding tools helped measure the changes in cell surface composition in each environmental condition. Overall, this study showed that (i) growth pH and temperature significantly influence binding efficiency of both phages and cytochrome *c*, (ii) significant trends in binding occur within strains but are not always the same between similar strains and conditions, (iii) it is difficult to predict the effects of temperature and pH due to the strain-specific changes that occurred. The results of this study are also important as it's been previously shown that cell envelope changes can affect sensitivity to antimicrobial treatment. Understanding the effects on *Listeria* cell surface properties are important to the dairy industry as *L. monocytogenes* contamination has occurred in these products. Dairy products also expose bacteria to a wide range of environmental conditions which could affect their cell envelope characteristics and thus their tolerance to antimicrobial treatment.

Our study found that the binding efficiencies of *Listeria*-phages and cytochrome *c* to *L. monocytogenes* strains were influenced by the environmental growth conditions. Listeria-phages and cytochrome *c* were used as they bind to cell envelope components specifically so therefore could assess the cell envelope changes occurring in cells grown in different environmental conditions. These results are consistent with other studies that have shown that environmental conditions affect *L. monocytogenes* cell envelope charge and composition. For example, one study (*56*) showed that cell wall modifications occurred in *L. monocytogenes* strain 10403S exposed to bile conditions at a pH of 5.5. They found that the *dltABCD* operon, which is responsible for D-alanylation of LTAs, was induced when cells were exposed to bile acidic conditions (*77*, *108*).

It has also been shown that genes affecting the cell surface features such as *dlt*, *ami*, and *mur*, are transcriptionally regulated by alternative sigma factor σ^B (*8*). We know that many factors such as acid and temperature stress cause σ^B mediated shifts in global gene expression essentially affecting the cell surface components expressed by transcription of σ^B (*7*, *25*, *43*). A study observing the effects of environmental stress on σ^B expression showed that active expression was induced in random patterns when *L. monocytogenes* cells were exposed to high heat and salt conditions (*59*). Heat and salt stress were found to activate σ^B but only under certain conditions. Similar results showing environmental effects on σ^B have also been found in other species such as *Staphylococcus aureus* and *Bacillus subtilis* (*17*, *24*). This is relevant to our study because it shows how specific cell surface features are affected when the cells are exposed to different environments.

Cell envelope effects have also been seen using similar phage binding experiments. For example, *Escherichia coli*, was found to down regulate the phage receptor λ (LamB) when exposed to quorum sensing signals (*37*, *68*, *80*). These phage-host interactions have not been as well studied in Gram-positive organisms nor have they addressed specific cell growth conditions as a factor. However, one study did find that temperature influenced phage adsorption in *L. monocytogenes* that was likely to be caused by temperature dependent regulation of WTA decorations rhamnose and *N*-acetylglucosamine (*125*). These studies also relate to this study as they show how environmental growth conditions can have different effects on cell envelope components which supports our idea that dairy relevant conditions influence cell envelope composition.

This study also showed that significant differences and trends were not consistent across strains. This can clearly be seen in the dairy related outbreak strains and the dissimilarity in binding efficiency and trends between strains. Another study found similar strain-specific effects in the development of nisin resistance when *L. monocytogenes* cells were exposed to different environmental stressors (*51*). The same study previously mentioned (*56*) on effects of bile stimulon on *L. monocytogenes* also found strain-specific patterns in their results. These data support our findings that strain-specific results are likely to occur due to differences in expression of cell envelope components. It also suggests how it is difficult to predict environmental effects on cell envelope composition due to strain variance.

Lastly, these changes in cell envelope components have also been found to affect the bacterial sensitivity to cationic antimicrobial peptide (CAMP) treatment. For example, *B. subtilis* was found to achieve lysozyme resistance with modification of its cell envelope through O-acetylation and D-alanylation of teichoic acids (*55*). Another study (*77*), showed that alteration of *dltA* led to increased sensitivity to CAMPs nisin and gallidermin in *Streptococcus pneumoniae,* a Gram-positive bacteria. Cationic antimicrobial sensitivity has also been observed in MprF-deficient strains. MprF, multiple peptide resistant factor, contributes to the synthesis of lysylphosphatidylglycerol, a phospholipid that contributes to a net positive charge on the cell surface giving resistance to CAMPs. A study (*120*) showed that inactivation of mprF in *S. aureus* gave increased sensitivity to CAMPs and attenuated virulence. MprF has also been found in *Listeria* species with the same function of lysinylation of phospholipids on the cell surface also contributing to CAMP resistance (*123*). These studies show the different effects that cell envelope features have on antimicrobial sensitivity which is important to note when developing control strategies in the food industry. This is important to know in relation to our study in which we show significant differences in binding efficiency of bacteriophage and cytochrome *c* to *L. monocytogenes* grown in dairy product conditions which could affect the susceptibility to antimicrobial treatment.

Overall, the data collected from this study shows the significant influence that dairy related environmental conditions have on cell surface composition in *L. monocytogenes*. Understanding the cell surface and its effect on antimicrobial susceptibility is important in industry to develop the best control strategies. Our data

show the complexity of a single species of *Listeria* and how effects cannot always be predicted even between similar strains. This implies that susceptibility to interventions may be different between these strains even though they were subjected to the same conditions. It supports the idea that dairy companies must remain vigilant in their food safety programs. They must continue to take environmental samples and not always rely on the same treatment methods in all their plant processes. Our study shows how complex the mechanisms for antimicrobial susceptibility and resistance are and how environmental conditions can impact those mechanisms. This research shows a future need for studies to understand the specific cell surface changes that affect susceptibility to current antimicrobial interventions, in addition to designing control interventions that can be targeted for specific conditions for greater impact.

Acknowledgements

We would like to thank Leticia Orellana (Universidad Zamorano, Honduras) for her assistance in data collection and Robert Pellegrino (University of Tennessee, Knoxville) and Dr. Xiaojuan Zhu (University of Tennessee, Knoxville) for statistical consulting on the project. This project was supported by the National Dairy Council (Rosemont, IL).

REFERENCES

1. Abee, T., F. M. Rombouts, J. Hugenholtz, G. Guihard, and L. Letellier. 1994. Mode of Action of Nisin against Listeria monocytogenes Grown at High and Low Temperatures. *Appl. Environ. Microbiol.* 60:1962–8.
2. Al, O. E. T., B. M. Jayarao, and R. A. Almeida. 2005. Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications. *Foodborne Pathog. Dis.* 2:115–129.
3. Allerberger, F., and M. Wagner. 2010. Listeriosis: A resurgent foodborne infection. *Clin. Microbiol. Infect.* European Society of Clinical Infectious Diseases 16:16–23.
4. Anonymous. 2011. Multistate Outbreak of Listeriosis Associated with Jensen Farms Cantaloupe - United States, August-September 2011. *MMWR Morb. Mortal. Wkly. Rep.* 60:1357–1358.
5. Anonymous. 1991. Recommendations by The National Advisory Committee on Microbiological Criteria for Foods: The human infectious dose of Listeria monocytogenes. *Int. J. Food Microbiol.* 14:216–219.
6. Anonymous. 2016. Timeline for Linking a Case of Listeria Infection to an Outbreak. *Centers Dis. Control Prev.*
7. Becker, L. A., S. N. Evans, R. W. Hutkins, and A. K. Benson. 2000. Role of sigma(B) in Adaptation of Listeria monocytogenes to Growth at Low Temperature. *J. Bacteriol.* 182:7083–7087.
8. Begley, M., C. Hill, and R. P. Ross. 2006. Tolerance of Listeria monocytogenes to Cell Envelope-Acting Antimicrobial Agents Is Dependent on SigB. *Appl. Environ. Microbiol.* 72:2231–2234.

9. Beresford, M. R., P. W. Andrew, and G. Shama. 2001. Listeria monocytogenes adhers to many materials found in food-processing environments. *J. Appl. Microbiol.* 90:1000–1005.

10. Bergholz, T. M., S. Tang, M. Wiedmann, and K. J. Boor. 2013. Nisin resistance of listeria monocytogenes is increased by exposure to salt stress and is mediated via liar. *Appl. Environ. Microbiol.* 79:5682–5688.

11. Beveridge, T. J. 2001. Use of the Gram stain in microbiology. *Biotech. Histochem.* 76:111–118.

12. Bielmann, R., M. Habann, M. R. Eugster, R. Lurz, R. Calendar, J. Klumpp, and M. J. Loessner. 2014. Receptor binding proteins of Listeria monocytogenes bacteriophages A118 and P35 recognize serovar-speci fi *c* teichoic acids. *Virology*. Elsevier 477:110–118.

13. Bishop, J. A. Y. R., and M. Smukowski. 2006. Storage Temperatures Necessary to Maintain Cheese Safety. *Food Prot. Trends* 29–33.

14. Blackman, I. C., J. F. Frank, and F. Frank. 1996. Growth of Listeria monocytogenes as a Biofilm on Various Food-Processing Surfaces. *J. Food Prot.* 8:827–831.

15. Boyd, E. F., and H. Brüssow. 2002. Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. *Trends Microbiol.* 10:521–529.

16. Breukink, E., and B. de Kruijff. 2006. Lipid II as a target for antibiotics. *Nat. Rev. Drug Discov.* 5:321–323.

17. Brigulla, M., T. Hoffmann, A. Krisp, A. Völker, E. Bremer, and U. Völker. 2003. Chill induction of the SigB-dependent general stress response in Bacillus subtilis and its contribution to low-temperature adaptation. *J. Bacteriol.* 185:4305–4314.

18. Capozzi, V., D. Fiocco, M. L. Amodio, and A. Gallone. 2009. Bacterial Stressors in Minimally Processed Food. *Int. J. Mol. Sci.* 10:3076–3105.

19. Carpentier, B., and O. Cerf. 2011. Review - Persistence of Listeria monocytogenes in food industry equipment and premises. *Int. J. Food Microbiol.* Elsevier B.V. 145:1–8.

20. Cartwright, E. J., K. A. Jackson, S. D. Johnson, L. M. Graves, B. J. Silk, and B. E. Mahon. 2013. Listeriosis Outbreaks and Associated Food Vehicles , United States , 1998 – 2008. *Emerg. Infect. Dis.* 19:1–10.

21. Ceyssens, P., and R. Lavigne. 2010. Introduction to Bacteriophage Biology and Diversity, p. 11–29. *In* P.M. Sabour, and M.W. Griffiths (eds.), Bacteriophage in the Control of Food- and Waterborne Pathogens. ASM Press.

22. Ceyssens, P.-J., and R. Lavigne. 2010. Introduction to Bacteriophage Biology and Diversity, p. 11–29. *In* P.M. Sabour, and M.W. Griffiths (eds.), Bacteriophage in the Control of Food- and Waterborne Pathogens. ASM Press.

23. Chaitiemwong, N., W. C. Hazeleger, and R. R. Beumer. 2014. Inactivation of Listeria monocytogenes by Disinfectants and Bacteriophages in Suspension and Stainless Steel Carrier Tests. *J. Food Prot.* 77:2012–2020.

24. Chan, P. F., S. Foster, E. Ingham, and M. O. Clements. 1998. The Staphylococcus aureus alternative sigma factor sB controls the environmental

stress response but not starvation survival or pathogenicity in a mouse model. *J. Bacteriol.* 180:6082–6089.

25. Chan, Y. C., and M. Wiedmann. 2009. Physiology and genetics of listeria monocytogenes survival and growth at cold temperatures. *Crit. Rev. Food Sci. Nutr.* 49:237–253.

26. Chandan, R. C. 2015. Dairy Processing and Quality Assurance: An Overview. *Dairy Process. Qual. Assur.* 1–40.

27. Chatterjee, S. S., S. Otten, T. Hain, A. Lingnau, U. D. Carl, J. Wehland, E. Domann, and T. Chakraborty. 2006. Invasiveness is a variable and heterogeneous phenotype in Listeria monocytogenes serotype strains. *Int. J. Med. Microbiol.* 296:277–286.

28. Chen, Z. 2017. Stress Responses of Foodborne Pathogens and Food Safety Implications. *J. Food Microbiol. Saf. Hyg.* 2.

29. Chiara, M., M. Caruso, A. M. D'Erchia, C. Manzari, R. Fraccalvieri, E. Goffredo, L. Latorre, A. Miccolupo, I. Padalino, G. Santagada, D. Chiocco, G. Pesole, D. S. Horner, and A. Parisi. 2015. Comparative genomics of Listeria Sensu Lato: Genus-wide differences in evolutionary dynamics and the progressive gain of complex, potentially pathogenicity-related traits through lateral gene transfer. *Genome Biol. Evol.* 7:2154–2172.

30. Chibani-chennoufi, S., A. Bruttin, H. Brüssow, M. Dillmann, and H. Bru. 2004. Phage-Host Interaction : an Ecological Perspective. *J. Bacteriol.* 186:3677–3686.

31. Conly, J. M., and B. L. Johnston. 2008. Listeria : A persistent food-borne

pathogen. *Can. J. Infect. Dieases Med. Microbiol.* 19:327–328.

32. Cossart, P. 2011. Illuminating the landscape of host-pathogen interactions with the bacterium Listeria monocytogenes. *Proc. Natl. Acad. Sci.* 108:19484–19491.

33. Cossart, P., and A. Helenius. 2014. Endocytosis of viruses and bacteria. *Cold Spring Harb. Perspect. Biol.* 6.

34. Dalton, C. B., C. C. Austin, J. Sobel, P. S. Hayes, W. F. Bibb, L. M. Graves, B. Swaminathan, M. E. Proctor, and P. M. Griffin. 1997. An Outbreak of Gastroenteritis and Fever Due to Listeria Monocytogenes in Milk. *N. Engl. J. Med.* 336:100–105.

35. de las Heras, A., R. J. Cain, M. K. Bielecka, and J. A. Vázquez-Boland. 2011. Regulation of Listeria virulence: PrfA master and commander. *Curr. Opin. Microbiol.* 14:118–127.

36. Denes, T., H. C. den Bakker, J. I. Tokman, C. Guldimann, and M. Wiedmann. 2015. Selection and Characterization of Phage-Resistant Mutant Strains of Listeria monocytogenes Reveal Host Genes Linked to Phage Adsorption. *Appl. Environ. Microbiol.* 81:4295–305.

37. Denes, T., and M. Wiedmann. 2014. Environmental responses and phage susceptibility in foodborne pathogens : implications for improving applications in food safety. *Curr. Opin. Biotechnol.* Elsevier Ltd 26:45–49.

38. Eklund, M. W., F. T. Poysky, R. N. Paranjpye, L. C. Lashbrook, M. E. Peterson, and G. A. Pelroy. 1995. Incidence and sources of Listeria monocytogenes in cold-smoked fishery products and processing plants. *J. Food Prot.* 58:502–508.

39. Eugster, M. R., and M. J. Loessner. 2011. Rapid analysis of Listeria monocytogenes cell wall Teichoic Acid Carbohydrates by ESI-MS/MS. *PLoS One* 6.

40. Eugster, M. R., L. S. Morax, V. J. Huls, S. G. Huwiler, A. Leclercq, M. Lecuit, and M. J. Loessner. 2015. Bacteriophage predation promotes serovar diversification in Listeria monocytogenes. *Mol. Microbiol.* 97:33–46.

41. Farber, J. M., and P. I. Peterkin. 1991. Listeria monocytogenes, a Food-Borne Pathogen. *Microbiol. Rev.* 55:476–511.

42. Federal Register. 2006. Food Additives Permitted for Direct Addition to Food for Human Consumption; Bacteriophage Preparation. *21 CFR part 172.*

43. Ferreira, A., D. Sue, C. P. O'Byrne, and K. J. Boor. 2003. Role of Listeria monocytogenes sigma(B) in survival of lethal acidic conditions and in the acquired acid tolerance response. *Appl Env. Microbiol* 69:2692–2698.

44. Ferreira, V., M. Wiedmann, P. Teixeira, and M. J. Stasiewicz. 2014. Listeria monocytogenes Persistence in Food-Associated Environments: Epidemiology, Strain Characteristics, and Implications for Public Health. *J. Food Prot.* 77:150–170.

45. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized Milk as Vehicle of Infection in an Outbreak of Listeriosis. *N. Engl. J. Med.* 312:404–407.

46. Gandhi, M., and M. L. Chikindas. 2007. Listeria: A foodborne pathogen that

knows how to survive. *Int. J. Food Microbiol.* 113:1–15.

47. Garg, N., T. J. Oman, T.-S. A. Wang, C. G. de Gonzalo, S. Walker, and W. A. van der Donk. 2014. Mode of Action and Struture-Activity Relationship Studies of Geobacillin I. *J. Antibiot. (Tokyo).* 67:133–136.

48. Goodridge, L. D., and B. Bisha. 2011. Phage-based biocontrol strategies to reduce foodborne pathogens in foods. *Bacteriophage* 1:130–137.

49. Gounot, A. M. 1986. Psychrophilic and psychrotrophie microorganisms. *Experientia* 42:1192–1197.

50. Gouvêa, D. M., R. C. S. Mendonça, M. E. S. Lopez, and L. S. Batalha. 2016. Absorbent food pads containing bacteriophages for potential antimicrobial use in refrigerated food products. *LWT - Food Sci. Technol.* 67:159–166.

51. Gravesen, A., A.-M. Jydegaard Axelson, J. Mendes da Silva, T. B. Hansen, and S. Knøchel. 2002. Frequency of Bacteriocin Resistance Development and Associated Fitness Costs in. *Appl. Environ. Microbiol.* 68:756–764.

52. Gravesen, A., B. Kallipolitis, K. Holmstrøm, E. Høiby, M. Ramnath, S. Knøchel, A. Gravesen, B. Kallipolitis, K. Holmstrøm, P. E. Høiby, M. Ramnath, and S. Knøchel. 2004. pbp2229 -Mediated Nisin Resistance Mechanism in Listeria monocytogenes Confers Cross-Protection to Class IIa Bacteriocins and Affects Virulence Gene Expression pbp2229 -Mediated Nisin Resistance Mechanism in Listeria monocytogenes Confers Cross-Protection 70:1669–1679.

53. Gravesen, A., M. Ramnath, K. B. Rechinger, N. Andersen, L. Jansch, Y. Hechard, J. W. Hastings, and S. Knochel. 2002. High-level resistance to class IIa

bacteriocins is associated with one general mechanism in *Listeria monocytogenes*. *Microbiology* 148:2361–2369.

54. Greer, G. G. 2005. Bacteriophage Control of Foodborne Bacteria. *J. Food Prot.* 68:1102–1111.

55. Guariglia-Oropeza, V., and J. D. Helmann. 2011. Bacillus subtilis confers lysozyme resistance by activation of two cell wall modification pathways, peptidoglycan O-Acetylation and D-alanylation of teichoic acids. *J. Bacteriol.* 193:6223–6232.

56. Guariglia-Oropeza, V., R. H. Orsi, C. Guldimann, M. Wiedmann, and K. J. Boor. 2018. The Listeria monocytogenes Bile Stimulon under Acidic Conditions Is Characterized by Strain-Specific Patterns and the Upregulation of Motility , Cell Wall Modification Functions , and the PrfA Regulon. *Front. Microbiol.* 9:1–18.

57. Guenther, S., D. Huwyler, S. Richard, and M. J. Loessner. 2009. Virulent bacteriophage for efficient biocontrol of listeria monocytogenes in ready-to-eat foods. *Appl. Environ. Microbiol.* 75:93–100.

58. Guenther, S., and M. J. Loessner. 2011. Bacteriophage biocontrol of Listeria monocytogenes on soft ripened white mold and red-smear cheeses. *Bacteriophage* 1:94–100.

59. Guldimann, C., V. Guariglia-Oropeza, S. Harrand, D. Kent, K. J. Boor, and M. Wiedmann. 2017. Stochastic and Differential Activation of σB and PrfA in Listeria monocytogenes at the Single Cell Level under Different Environmental Stress Conditions. *Front. Microbiol.* 8:1–14.

60. Gutiérrez, D., L. Rodríguez-Rubio, L. Fernández, B. Martínez, A. Rodríguez, and P. García. 2017. Applicability of commercial phage-based products against Listeria monocytogenes for improvement of food safety in Spanish dry-cured ham and food contact surfaces. *Food Control* 73:1474–1482.

61. Guttman, B., R. Raya, and E. Kutter. 2005. Basic Phage Biology, p. 29–66. *In* E. Kutter, and A. Sulakvelidze (eds.), Bacteriophages: Biology and Applications. CRC Press, Boca Raton, FL.

62. Habann, M., P. G. Leiman, K. Vandersteegen, A. Van Den Bossche, R. Lavigne, M. M. Shneider, R. Bielmann, M. R. Eugster, M. J. Loessner, and J. Klumpp. 2014. Listeria phage A511 , a model for the contractile tail machineries of SPO1-related bacteriophages. *Mol. Microbiol.* 92:84–99.

63. Hagens, S., and M. Loessner. 2010. Bacteriophage for Biocontrol of Foodborne Pathogens: Calculations and Considerations. *Curr. Pharm. Biotechnol.* 11:58–68.

64. Hagens, S., and M. J. Loessner. 2014. Phages of Listeria offer novel tools for diagnostics and biocontrol. *Front. Microbiol.* 5:1–6.

65. Hasper, H. E., N. E. Kramer, J. L. Smith, J. D. Hillman, C. Zachariah, O. P. Kuipers, B. De Kruijff, and E. Breukink. 2006. An Alternative Bactericidal Mechanism of Action for Lantibiotic Peptides that Target Lipid II. *Science (80-.).* 313:1636–1637.

66. Heller, K. J. 1992. Molecular interaction between bacteriophage and the gram-negative cell envelope. *Arch. Microbiol.* 158:235–248.

67. Hof, H. 2003. History and epidemiology of listeriosis. *FEMS Immunol. Med.*

Microbiol. Blackwell Publishing Ltd 35:199–202.

68. Høyland-kroghsbo, N. M., R. B. Mærkedahl, and S. Lo Svenningsen. 2013. A Quorum-Sensing-Induced Bacteriophage Defense Mechanism 1–8.

69. Hudson, J. A., L. Mcintyre, and C. Billington. 2010. Application of Bacteriophages to Control Pathogenic and Spoilage Bacteria in Food Processing and Distribution, p. 119–136. *In* P.M. Sabour, and M.W. Griffiths (eds.), Bacteriophage in the Control of Food- and Waterborne Pathogens. ASM Press.

70. Ivanek, R., Y. T. Gröhn, L. W. Tauer, and M. Wiedmann. 2004. The cost and benefit of listeria monocytogenes food safety measures. *Crit. Rev. Food Sci. Nutr.* 44:513–523.

71. 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. *J. Food Prot.* 74:949–953.

72. Jiang, Z., H. Neetoo, and H. Chen. 2011. Control of Listeria Monocytogenes on Cold-Smoked Salmon Using Chitosan-Based Antimicrobial Coatings and Films. *J. Food Sci.* 76:22–26.

73. Kamio, Y., and H. Nikaido. 1976. Outer Membrane of Salmonella Typhimurium: Accessibility of Phospholipid Head Groups to Phospholipase *c* and Cyanogen Bromide Activated Dextran in the External Medium. *Biochemistry* 15:2561–2570.

74. Kamisango, K., H. Fujii, H. Okumura, I. Saiki, Y. Araki, Y. Yamamura, and I.

Azuma. 1983. Structural and immunochemical studies of teichoic acid of *Listeria monocytogenes*. *J. Biochem.* 93:1401–1409.

75. Kang, J., M. Wiedmann, K. J. Boor, and T. M. Bergholz. 2015. VirR-mediated resistance of listeria monocytogenes against food antimicrobials and cross-protection induced by exposure to organic acid salts. *Appl. Environ. Microbiol.* 81:4553–4562.

76. Kazmierczak, M. J., M. Wiedmann, and K. J. Boor. 2005. Alternative sigma factors and their roles in bacterial virulence. *Microbiol. Mol. Biol. Rev.* 69:527–543.

77. Kovács, M., A. Halfmann, I. Fedtke, M. Heintz, A. Peschel, W. Vollmer, R. Hakenbeck, and R. Brückner. 2006. A functional dlt operon, encoding proteins required for incorporation of D-alanine in teichoic acids in gram-positive bacteria, confers resistance to cationic antimicrobial peptides in Streptococcus pneumoniae. *J. Bacteriol.* 188:5797–5805.

78. Kozak, S. M., S. R. B. Brown, Y. Bobak, and D. J. D'Amico. 2017. Control of Listeria monocytogenes in whole milk using antimicrobials applied individually and in combination. *J. Dairy Sci.* American Dairy Science Association 101:1889–1900.

79. Kropinski, A. M., A. Mazzocco, T. E. Waddell, E. Lingohr, and R. P. Johnson. 2009. Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay, p. 69–76. *In* M.R.J. Clokie, and A.M. Kropinski (eds.), Bacteriophages: Methods and ProtocolsVolume 1: Humana Press, Totowa, NJ.

80. Labrie, S. J., J. E. Samson, and S. Moineau. 2010. Bacteriophage resistance

mechanisms. *Nat. Rev. Microbiol.* 8:317–327.

81. Lampert, L. M. 1970. Modern dairy products; composition, food value, processing, chemistry, bacteriology, testing, imitation dairy products. New York, Chemical Publishing, New York.

82. Lemon, K. P., D. E. Higgins, and R. Kolter. 2007. Flagellar motility is critical for Listeria monocytogenes biofilm formation. *J. Bacteriol.* 189:4418–4424.

83. Letellier, L., P. Boulanger, L. Plancon, P. Jacquot, and M. Santamaria. 2004. Main Features on Tailed Phage: Host Recognition and DNA Uptake. *Front. Biosci.* 9:1228–1239.

84. Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V Broome. 1988. Epidemic Listeriosis Associated with Mexican-Style Cheese. *N. Engl. J. Med.* Massachusetts Medical Society 319:823–828.

85. Lone, A., H. Anany, M. Hakeem, L. Aguis, A. C. Avdjian, M. Bouget, A. Atashi, L. Brovko, D. Rochefort, and M. W. Griffiths. 2016. Development of prototypes of bioactive packaging materials based on immobilized bacteriophages for control of growth of bacterial pathogens in foods. *Int. J. Food Microbiol.* 217:49–58.

86. Lourenço, A., M. B. Kamnetz, C. Gadotti, and F. Diez-Gonzalez. 2017. Antimicrobial treatments to control Listeria monocytogenes in queso fresco. *Food Microbiol.* 64:47–55.

87. Maertens de Noordhout, C., B. Devleesschauwer, F. J. Angulo, G. Verbeke, J. Haagsma, M. Kirk, A. Havelaar, and N. Speybroeck. 2014. The global burden of

listeriosis: a systematic review and meta-analysis. *Lancet Infect Dis* 14:1073–1082.

88. Mahony, J., O. M. Auliffe, R. P. Ross, and D. Van Sinderen. 2011. Bacteriophages as biocontrol agents of food pathogens. *Curr. Opin. Biotechnol.* 22:157–163.

89. Malanovic, N., and K. Lohner. 2016. Antimicrobial peptides targeting Gram-positive bacteriaPharmaceuticals.

90. McClure, P. J., T. M. Kelly, and T. A. Roberts. 1991. The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of Listeria monocytogenes. *Int. J. Food Microbiol.* 14:77–91.

91. Mead, P. S., E. F. Dunne, L. Graves, M. Wiedmann, M. Patrick, S. Hunter, E. Salehi, F. Mostashari, A. Craig, P. Mshar, T. Bannerman, B. D. Sauders, P. Hayes, W. Dewitt, P. Sparling, P. Griffin, D. Morse, L. Slutsker, B. Swaminathan, K. Boor, W. Bibb, T. Cote, T. Donkar, S. de Fitjer, R. Etzel, E. Koch, S. Kondracki, J. Miller, F. Smith, R. Taylor, and S. Zansky. 2006. Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiol. Infect.* 134:744–751.

92. Miller, E. S., E. Kutter, G. Mosig, T. Kunisawa, W. Rüger, F. Arisaka, and W. Ru. 2003. Bacteriophage T4 Genome. *Microbiol. Mol. Biol. Rev.* 67:86–156.

93. Mungai, E. A., C. B. Behravesh, and L. H. Gould. 2015. Increased outbreaks associated with nonpasteurized Milk, United States, 2007-2012. *Emerg. Infect. Dis.* 21:119–122.

94. Ndoti-Nembe, A., K. D. Vu, N. Doucet, and M. Lacroix. 2015. Antimicrobial Effects

of Essential Oils, Nisin, and Irradiation Treatments against Listeria monocytogenes on Ready-to-Eat Carrots. *J. Food Sci.* 80:M795–M799.

95. O'Neil, H. S., and H. Marquis. 2006. Listeria monocytogenes flagella are used for motility, not as adhesins, to increase host cell invasion. *Infect. Immun.* 74:6675–6681.

96. Oliveira, A., R. Sereno, and J. Azeredo. 2004. In vivo efficiency evaluation of a phage cocktail in controlling severe colibacillosis in confined conditions and experimental poultry houses. *Vet. Microbiol.* Elsevier B.V. 146:303–308.

97. Orsi, R. H., H. C. de. Bakker, and M. Wiedmann. 2011. Listeria monocytogenes lineages: Genomics, evolution, ecology, and phenotypic characteristics. *Int. J. Med. Microbiol.* Elsevier GmbH. 301:79–96.

98. Orsi, R. H., and M. Wiedmann. 2016. Characteristics and distribution of Listeria spp., including Listeria species newly described since 2009. *Appl. Microbiol. Biotechnol.* Applied Microbiology and Biotechnology 100:5273–5287.

99. Peel, M., W. Donachie, and A. Shaw. 1988. Temperature-dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and western blotting. *J. Gen. Microbiol.* 134:2171–2178.

100. Perera, M. N., T. Abuladze, M. Li, J. Woolston, and A. Sulakvelidze. 2015. Bacteriophage cocktail significantly reduces or eliminates Listeria monocytogenes contamination on lettuce, apples, cheese, smoked salmon and frozen foods. *Food Microbiol.* Elsevier Ltd 52:42–48.

101. Pizarro-cerda, J., and A. Ku. 2012. Entry of Listeria monocytogenes in

Mammalian. *Cold Spring Harb. Perspect. Med.* 1–18.

102. Pouillot, R., K. C. Klontz, Y. Chen, L. S. Burall, D. Macarisin, M. Doyle, K. M. Bally, E. Strain, A. R. Datta, T. S. Hammack, and J. M. Van Doren. 2016. Infectious Dose of *Listeria monocytogenes* in Outbreak Linked to Ice Cream, United States, 2015. *Emerg. Infect. Dis.* 22:2113–2119.

103. Prevention, C. for D. C. and. 2015. Multistate outbreak of listeriosis linked to Blue Bell creameries products (final update). *Centers Dis. Control Prev. Atlanta, GA http//www. cdc. gov/listeria/outbreaks/ice-cream-03-15*.

104. Radoshevich, L., and P. Cossart. 2018. Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis. *Nat. Rev. Microbiol.* Nature Publishing Group 16:32–46.

105. Raetz, C. R. H., and C. Whitfield. 2002. Lipopolysaccharide Endotoxins. *Annu. Rev. Biochem.* 71:635–700.

106. Ramaswamy, V., V. Cresence, J. Rejitha, M. Lekshmi, K. Dharsana, S. Prasad, and H. Vijila. 2007. Listeria--review of epidemiology and pathogenesis. *J. Microbiol. Immunol. Infect.* 40:4–13.

107. Raya, R. R., R. A. Oot, B. Moore-maley, S. Wieland, T. R. Callaway, E. M. Kutter, and A. D. Brabban. Naturally resident and exogenously applied T4-like and T5-like bacteriophages can reduce Escherichia coli O157 : H7 levels in sheep guts 15–24.

108. Reichmann, N. T., C. P. Cassona, and A. Gru. 2013. Revised mechanism of D - alanine incorporation into cell wall polymers in Gram-positive bacteria.

Microbiology 159:1868–1877.

109. Riedo, F. X., R. W. Pinner, M. D. L. Tosca, L. Matthew, L. M. Graves, M. W. Reeves, R. E. Weaver, B. D. Plikaytis, C. V Broome, S. The, I. Diseases, N. Sep, F. X. Riedo, R. W. Pinner, M. De Lourdes, and C. V Broome. 1994. A Point-Source Foodborne Listeriosis Outbreak : Documented Incubation Period and Possible Mild Illness. *J. Infect. Dis.* 170:693–696.

110. Rivas, L., B. Coffey, O. Mcauliffe, M. J. Mcdonnell, C. M. Burgess, A. Coffey, R. P. Ross, and G. Duffy. In Vivo and Ex Vivo Evaluations of Bacteriophages e11 / 2 and e4 / 1c for Use in the Control of Escherichia coli O157 : H7 □ 7210–7216.

111. Saar-Dover, R., A. Bitler, R. Nezer, L. Shmuel-Galia, A. Firon, E. Shimoni, P. Trieu-Cuot, and Y. Shai. 2012. D-Alanylation of Lipoteichoic Acids Confers Resistance to Cationic Peptides in Group B Streptococcus by Increasing the Cell Wall Density. *PLoS Pathog.* 8.

112. Sadekuzzaman, M., S. Yang, M. F. R. Mizan, H. S. Kim, and S. Do Ha. 2017. Effectiveness of a phage cocktail as a biocontrol agent against L. monocytogenes biofilms. *Food Control* 78:256–263.

113. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States-Major pathogens. *Emerg. Infect. Dis.* 17:7–15.

114. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V Tauxe, M. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne Illness Acquired in the United States — Major Pathogens. *Emerg. Infect. Dis.* 1–21.

115. Schmidt, R. H. 2009. Microbiological Considerations Related to Dairy Processing. *Dairy Process. Qual. Assur.* 105–143.

116. Services, U. S. D. of H. and H., and F. and D. Administration. 2015. Grade A Pasteurized Milk OrdinanceGrade “A” Pasteurized Milk Ordinance.

117. Shank, F. R., E. L. Elliot, I. K. Wachsmuth, and M. E. Losikoff. 1996. US position on Listeria monocytogenes in foods. *Food Control* 7:229–234.

118. Silhavy, T. J., D. Kahne, and S. Walker. 2010. The Bacterial Cell Envelope. *Cold Spring Harb. Perspect. Biol.* 2:1–16.

119. Sillankorva, S. M., H. Oliveira, and J. Azeredo. 2012. Bacteriophages and their role in food safety. *Int. J. Microbiol.* 1–14.

120. Staubitz, P., H. Neumann, T. Schneider, I. Wiedemann, and A. Peschel. 2004. MprF-mediated biosynthesis of lysylphosphatidylglycerol, an important determinant in staphylococcal defensin resistance. *FEMS Microbiol. Lett.* 231:67–71.

121. Sulakvelidze, A., Z. Alavidze, and G. J. Morris Jr. 2001. The challenges of bacteriophage therapy. *Ind. Pharm.* 45:14–18.

122. Summers, W. C. 2001. Bacteriophage Therapy. *Annu. Rev. Microbiol.* 655:437–451.

123. Thedieck, K., T. Hain, W. Mohamed, B. J. Tindall, M. Nimtz, T. Chakraborty, J. Wehland, and L. Jänsch. 2006. The MprF protein is required for lysinylation of phospholipids in listerial membranes and confers resistance to cationic antimicrobial peptides (CAMPs) on Listeria monocytogenes. *Mol. Microbiol.*

62:1325–1339.

124. Thomsen, M. R., R. Shiptsova, and S. J. Hamm. 2006. Sales Responses to Recalls for Listeria monocytogenes : Evidence from Branded Ready-to-Eat Meats. *Rev. Agric. Econ.* 28:482–494.

125. Tokman, J. I., D. J. Kent, M. Wiedmann, and T. Denes. 2016. Temperature Significantly Affects the Plaquing and Adsorption Efficiencies of Listeria Phages. *Front. Microbiol.* 7:1–10.

126. Uchikawa, K., I. Seikawa, and I. Azuma. 1986. Structural studies on teichoic acids in cell walls of several serotypes of *Listeria monocytogenes*. *J. Biochem.* 99:315–327.

127. Vadyvaloo, V., S. Arous, A. Gravesen, Y. Héchard, R. Chauhan-Haubrock, J. W. Hastings, and M. Rautenbach. 2004. Cell-surface alterations in class IIa bacteriocin-resistant Listeria monocytogenes strains. *Microbiology* 150:3025–3033.

128. Valderrama, W. B., and C. N. Cutter. 2013. An Ecological Perspective of Listeria monocytogenes Biofilms in Food Processing Facilities. *Crit. Rev. Food Sci. Nutr.* 53:801–817.

129. van Schaik, W., C. G. M. Gahan, and C. Hill. 1999. Acid-Adapted Listeria monocytogenes Displays Enhanced Tolerance against the Lantibiotics Nisin and Lacticin 3147. *J. Food Prot.* 62:536–539.

130. Vázquez-boland, J. a, M. Kuhn, P. Berche, T. Chakraborty, G. Domi, B. González-zorn, and J. Wehland. 2001. *Listeria* Pathogenesis and Molecular

Virulence Determinants *Listeria* Pathogenesis and Molecular Virulence Determinants. *Clin. Microbiol. Rev.* 14:584–640.

131. Vollmer, W., D. Blanot, and M. A. De Pedro. 2008. Peptidoglycan structure and architecture. *FEMS Microbiol. Rev.* 32:149–167.
132. Vongkamjan, K., A. M. Switt, H. C. den Bakker, E. D. Fortes, and M. Wiedmann. 2012. Silage collected from dairy farms harbors an abundance of listeriaphages with considerable host range and genome size diversity. *Appl. Environ. Microbiol.* 78:8666–75.
133. Wagner, M., and J. McLauchlin. 2008. Biology, p. 3–25. *In* D. Liu (ed.), Handbook of Listeria monocytogenes. Boca Raton : Taylor & Francis, Boca Raton.
134. Walker, S. J., P. Archer, and J. G. Banks. 1990. Growth of Listeria monocytogenes at refrigeration temperatures. *J. Appl. Bacteriol.* 157–162.
135. Ward, T. J., T. F. Ducey, T. Usgaard, K. A. Dunn, and J. P. Bielawski. 2008. Multilocus genotyping assays for single nucleotide polymorphism-based subtyping of Listeria monocytogenes isolates. *Appl. Environ. Microbiol.* 74:7629–7642.
136. Weidenmaier, C., and A. Peschel. 2008. Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nat. Rev. Microbiol.* 6:276–287.
137. Wendlinger, G., M. J. Loessner, and S. Scherer. 1996. Bacteriophage receptors on Listeria monocytogenes cells are the N-acetylglucosamine and rhamnose substituents of teichoic acids or the pept idog lycan itself. *Microbiology* 142:985–992.

138. Xi, Y., G. A. Sullivan, A. L. Jackson, G. H. Zhou, and J. G. Sebranek. 2012. Effects of natural antimicrobials on inhibition of Listeria monocytogenes and on chemical, physical and sensory attributes of naturally-cured frankfurters. *Meat Sci.* 90:130–138.

139. Young, R., I. N. Wang, and W. D. Roof. 2000. Phages will out: Strategies of host cell lysis. *Trends Microbiol.* 8:120–128.

APPENDIX

Table 1. Bacterial Strains and Phages Used in the Study

Strain or phage	Description	Reference
L. monocytogenes strains		
10403S	Lineage II, serotype 1/2a	Bishop and Hinrichs, 1987
Mack	Lineage II, Serotype 1/2a	Hodgson, 2000
F2365	Lineage I, serotype 4b	Wesley and Ashton, 1991
FSL R9-5621	Outbreak strain, 2012 ricotta cheese, CDC	
FSL R9-5623	Outbreak strain, 2013 semi soft fresh style cheese, CDC	
FSL R9-5624	Outbreak strain, 2013 queso fresco, CDC	
FSL R9-5625	Outbreak strain, 2014 soft cheese, CDC	
ΔdltA	Deletion mutation (Δ) of LMRG_02073 (dltA); 10403S background	
10403S (541_M)	Nonsense mutation (NM) in LMRG_00541; 10403S background; GlcNAc^{-+}	Denes et al., 2015
10403S (542_M)	Nonsense mutation (NM) in LMRG_00542; 10403S background; Rha$^{-‡}$	Denes et al., 2015
ΔmprFΔdltA	Deletion mutation (Δ) of (genes) (mprF and dltA); 10403S background	
ΔmprF	Deletion mutation (Δ) of (gene) (mprF); 10403S background	
Phages		
LP-048	P100-like *Listeria* phage, shown to infect serotype 1/2, 4a, 4b, and 4c strains, binds WTA decoration rhamnose	Denes et al., 2014
LP-125	P100-like *Listeria* phage shown to infect serotype 1/2, 3a, 3b, 4a, and 4b strains, binds WTA decorations rhamnose and GlcNAc	Denes et al., 2014

$^{+}$*The wall teichoic acids of the indicated strain lack N-acetylglucosamine (GlcNAc).* ‡*The wall teichoic acids of the indicated strain lack rhamnose (Rha).*

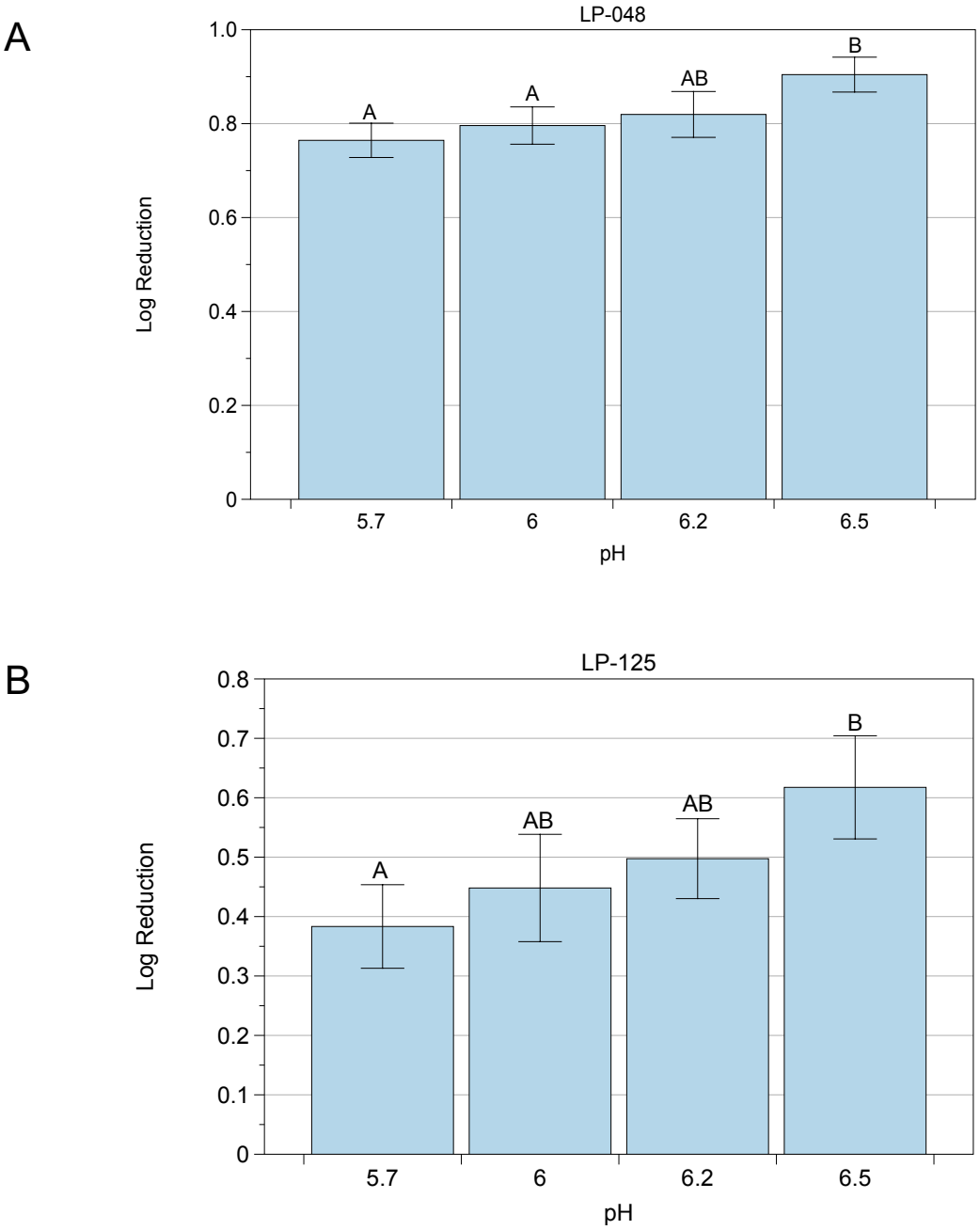


Figure 1. Phage binding of (A) LP-048 and (B) LP-125 on wild-type 10403S strains grown at varying dairy pHs.
Values shown are the $\log_{10}$ reduction of phage in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Bars that do not share letters show significant difference ($p < 0.05$) and bars with no letters did not show significant difference. Error bars show the standard error of the mean.

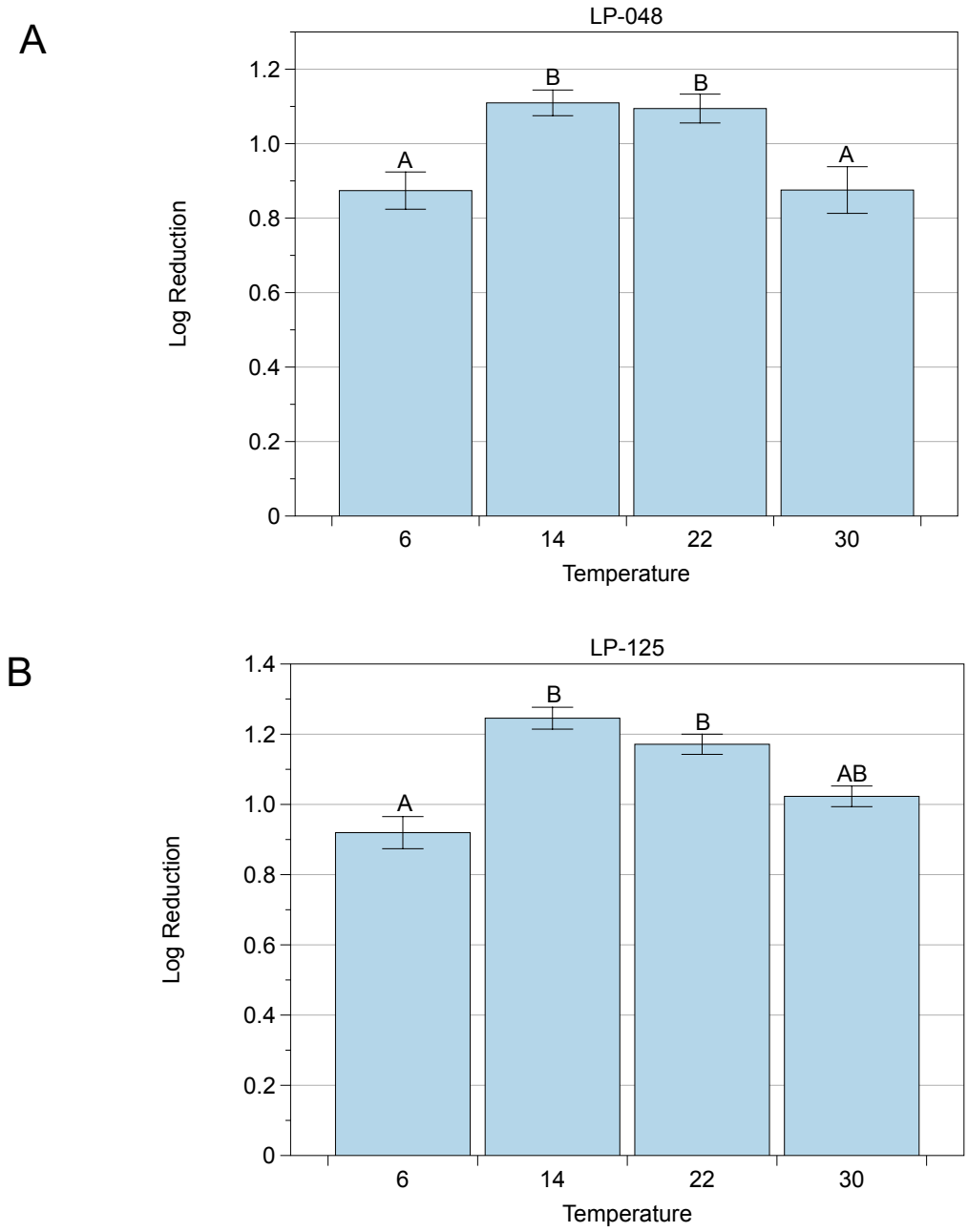


Figure 2. Phage binding of (A) LP-048 and (B) LP-125 on wild-type strain 10403S grown at varying dairy temperatures.
Values shown are the $\log_{10}$ reduction of phage in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Bars that do not share letters show significant difference ($p < 0.05$) and bars with no letters did not show significant difference. Error bars show the standard error of the mean.

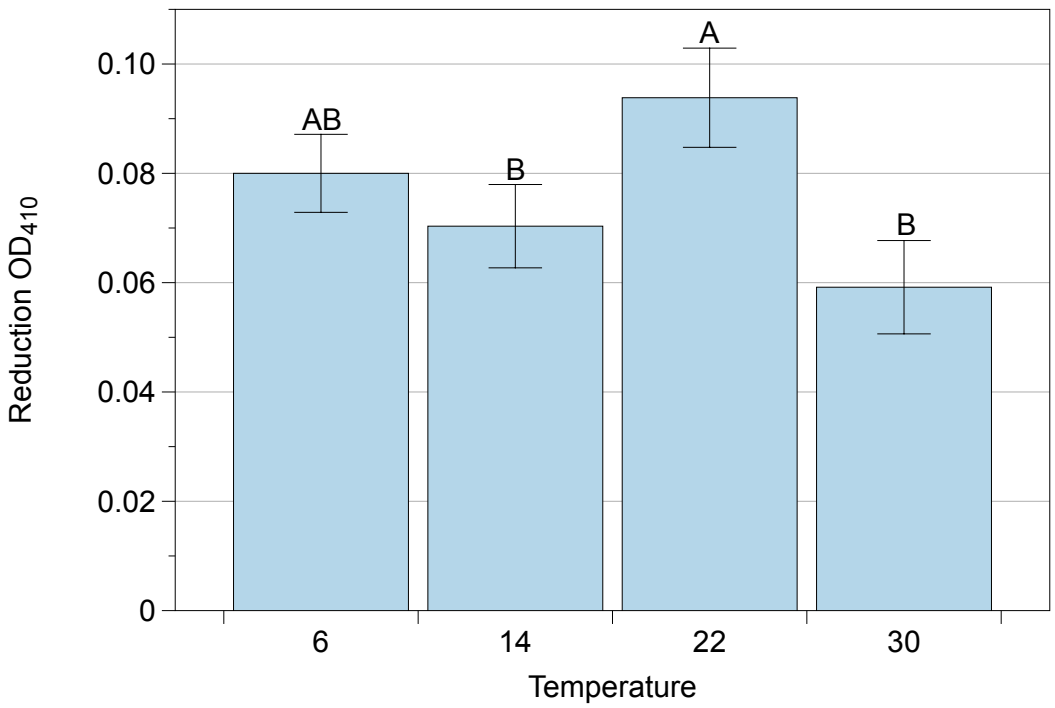


Figure 3. Cytochrome *c* binding on wild-type 10403S strain grown at varying dairy temperatures.
Values shown are the reduction OD_{410} of cytochrome *c* in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Bars that do not share letters show significant difference ($p < 0.05$) and bars with no letters did not show significant difference. Error bars show the standard error of the mean.

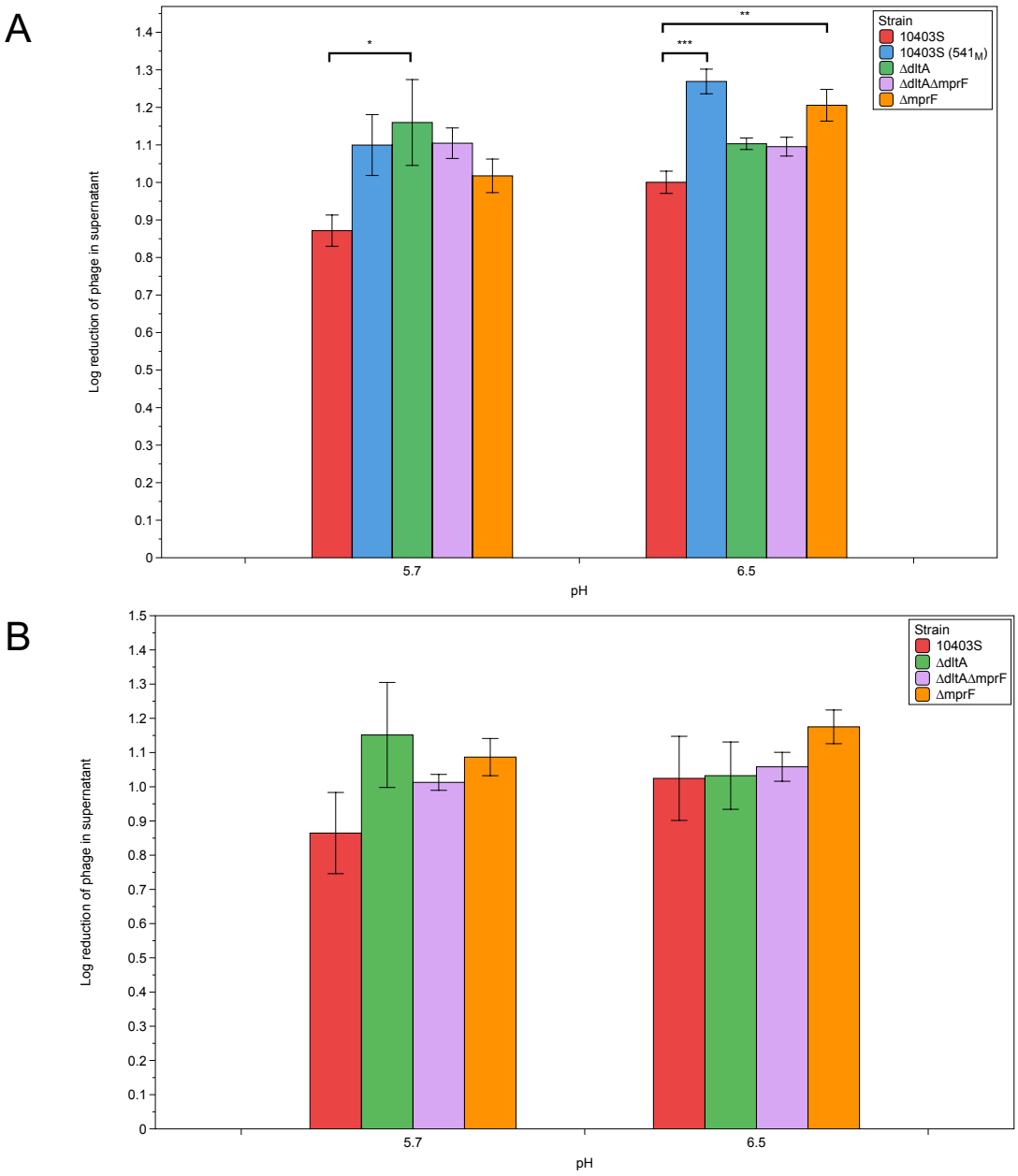


Figure 4. Phage binding of (A) LP-048 and (B) LP-125 on wild-type 10403S and mutant 10403S strains grown in varying dairy pHs.
Values shown are the $\log_{10}$ reduction of phage in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Significant difference is represented by * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.0001$). Error bars show the standard error of the mean.

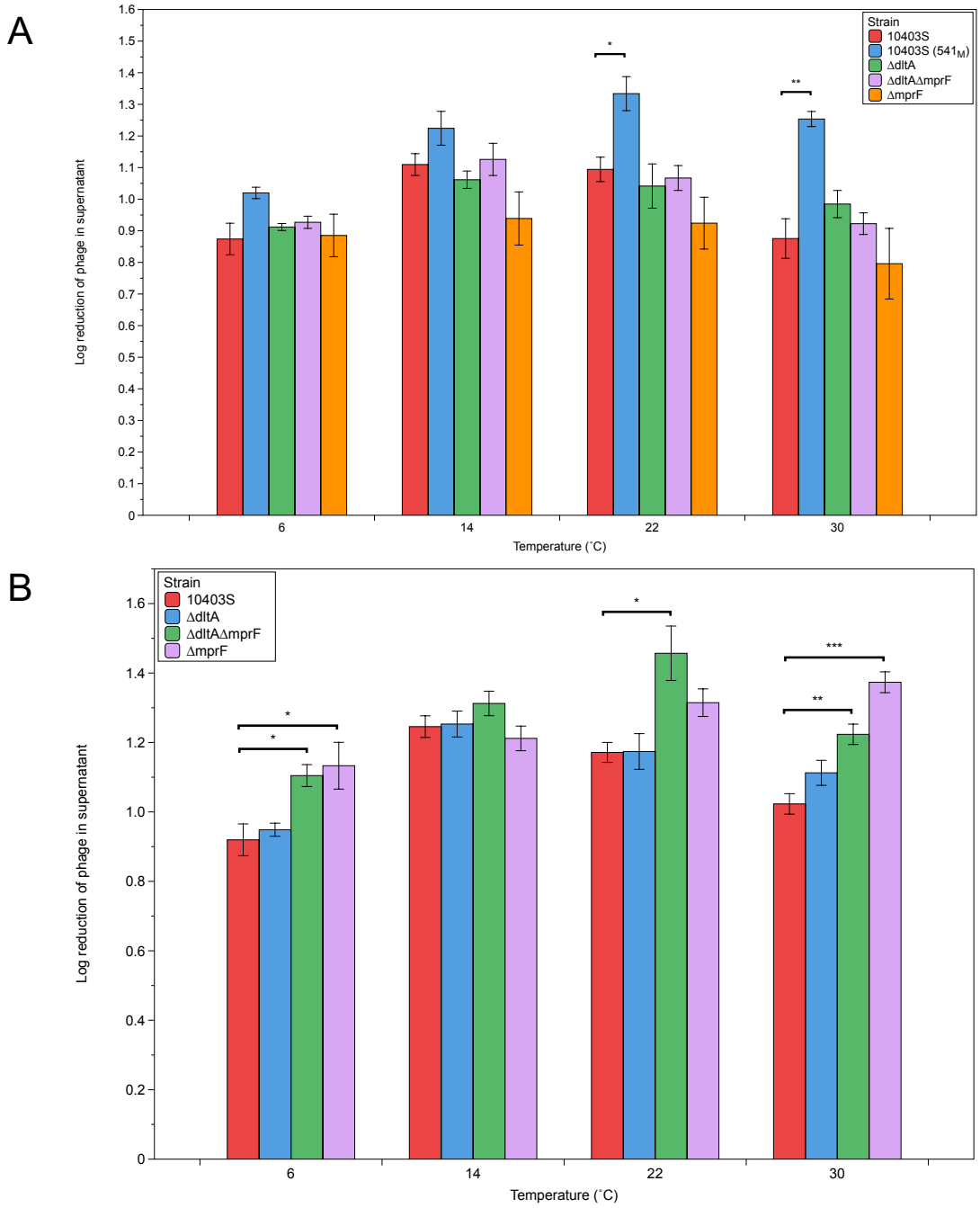


Figure 5. Phage binding of (A) LP-048 and (B) LP-125 and on wild-type 10403S and mutant 10403S strains grown in varying dairy temperatures.
Values shown are the $\log_{10}$ reduction of phage in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Significant difference is represented by * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.0001$). Error bars show the standard error of the mean.

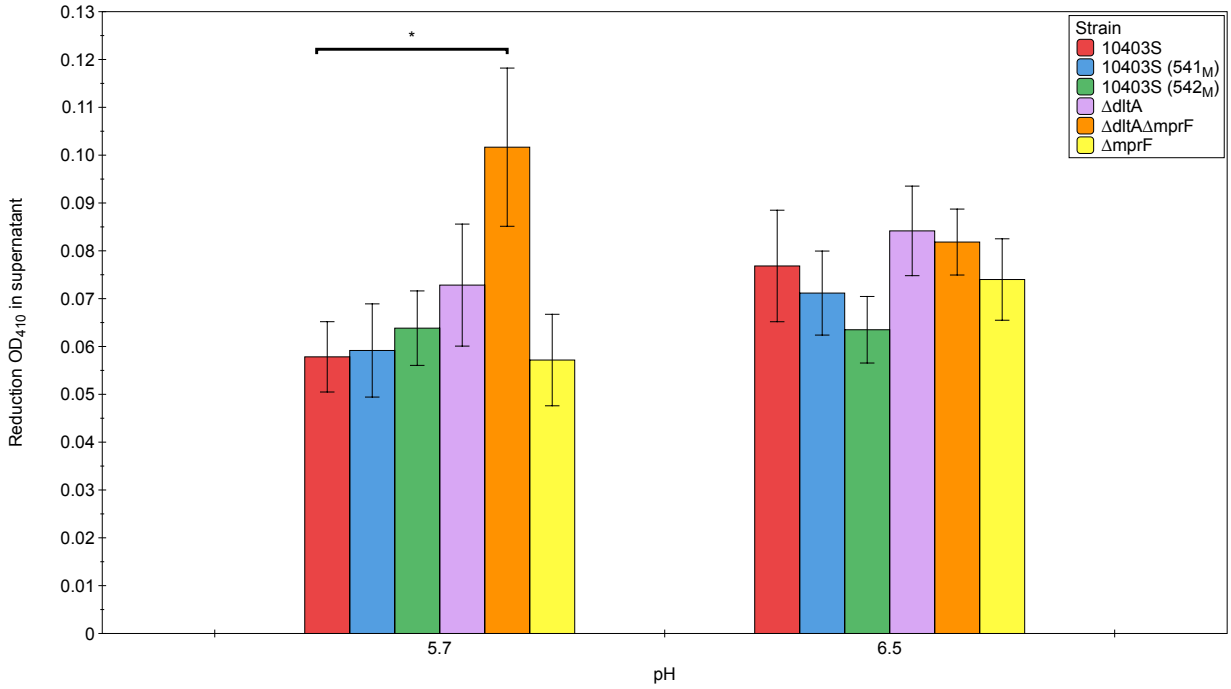


Figure 6. Cytochrome *c* binding to wild-type 10403S and mutant 10403S strains grown at varying dairy pHs.
Values shown are the reduction OD_{410} of cytochrome *c* in the supernatant. This is calculated by subtracting the OD_{410} of the supernatant from the OD_{410} of the negative control. Significant difference is represented by * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.0001$). Error bars show the standard error of the mean.

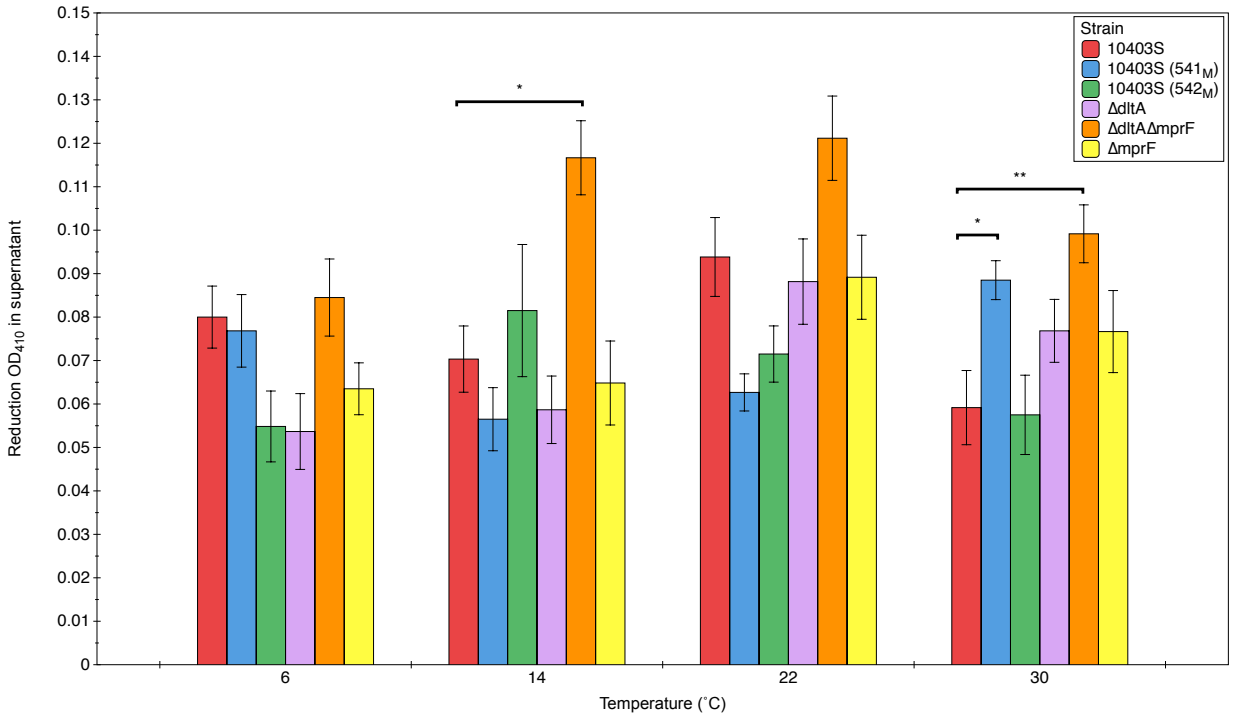


Figure 7. Cytochrome *c* binding to wild-type 10403S and mutant 10403S strains grown in varying dairy temperatures.
Values shown are the reduction OD_{410} of cytochrome *c* in the supernatant. This is calculated by subtracting the OD_{410} of the supernatant from the OD_{410} of the negative control. Significant difference is represented by * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.0001$). Error bars show the standard error of the mean.

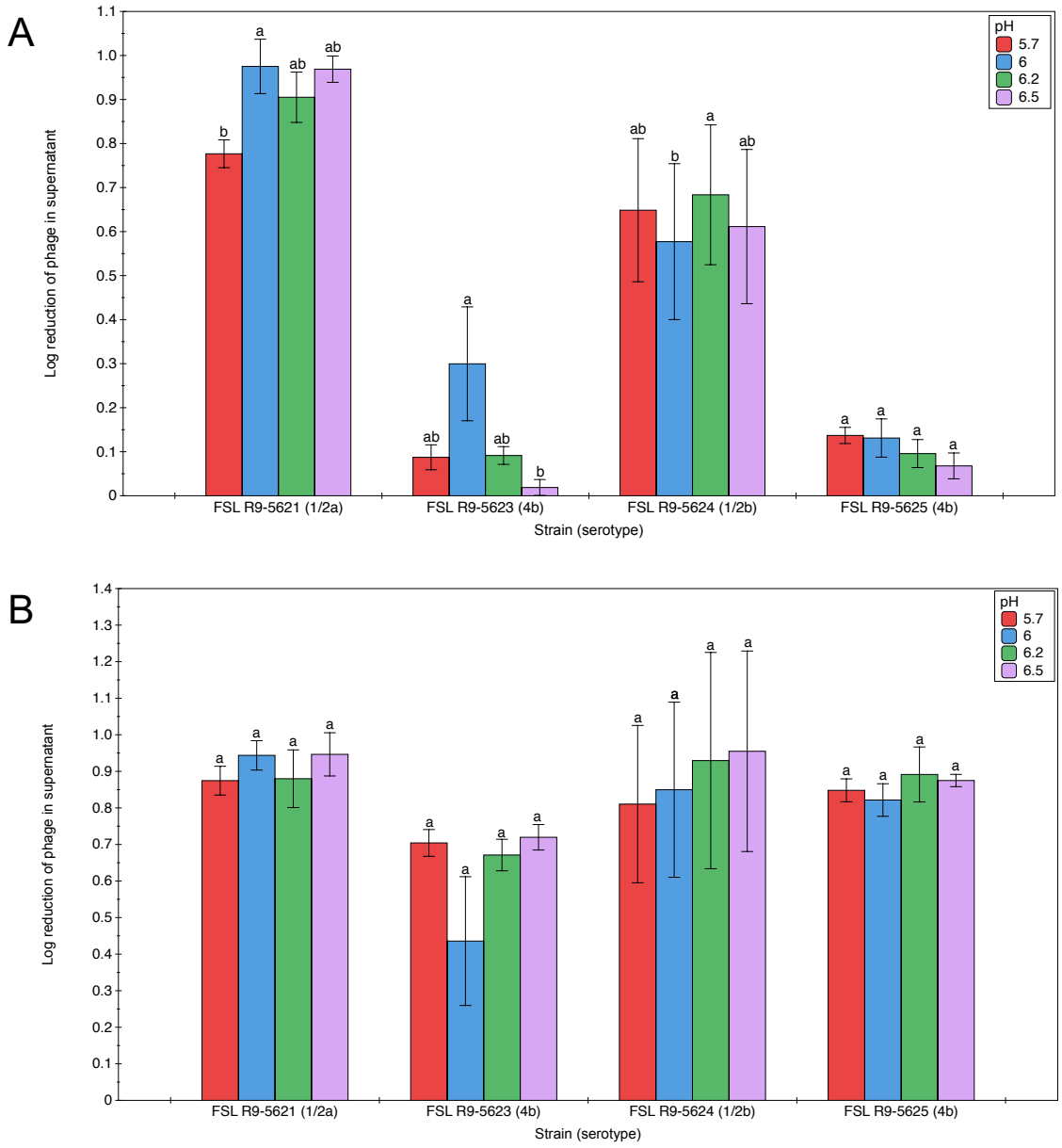


Figure 8. Phage binding of (A) LP-048 and (B) LP-125 to dairy related outbreak strains grown at varying dairy pHs.
Values shown are the $\log_{10}$ reduction of phage in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Bars that do not share letters show significant difference ($p < 0.05$) and bars with no letters did not show significant difference. Error bars show the standard error of the mean.

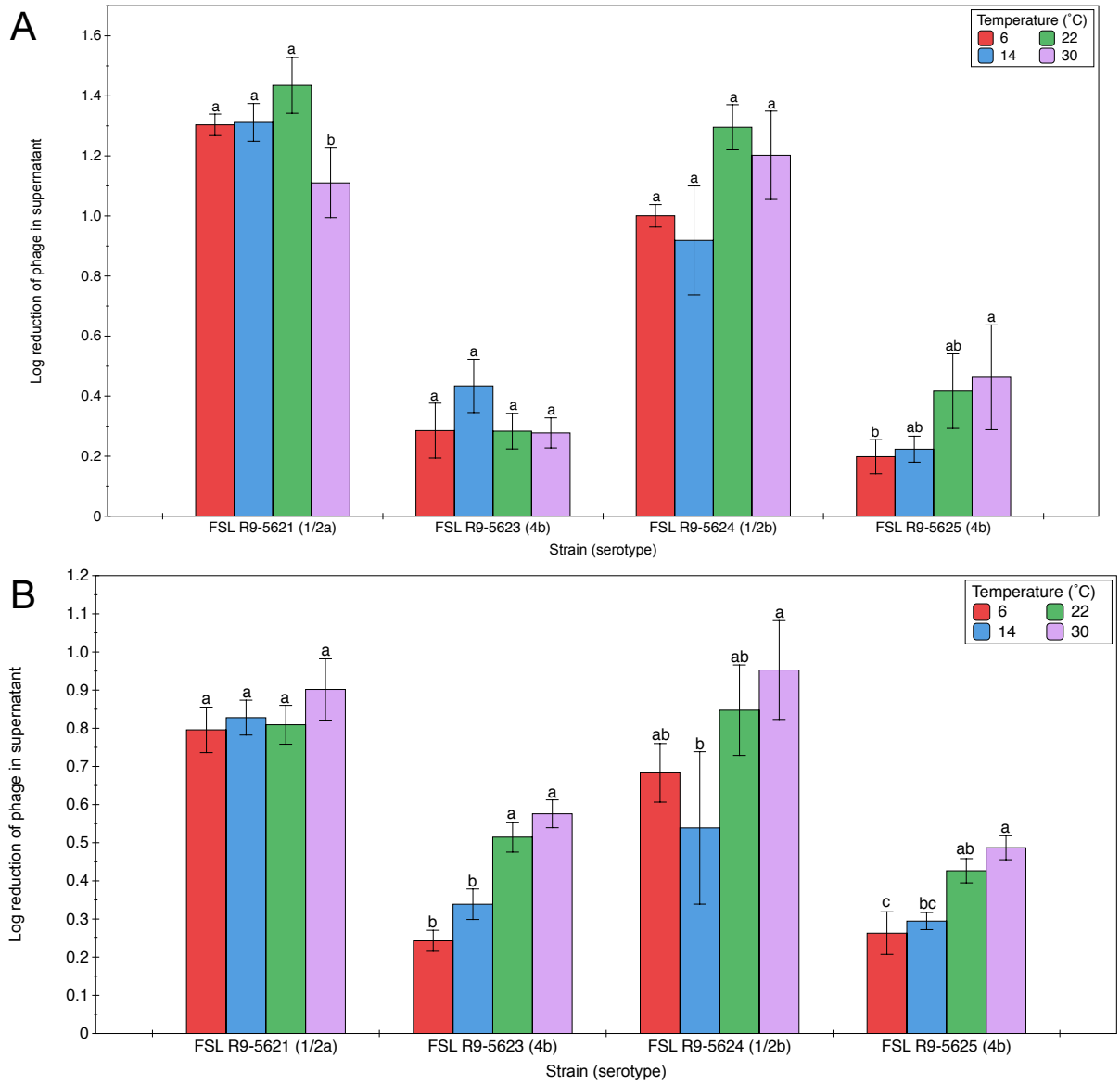


Figure 9. Phage binding of (A) LP-048 and (B) LP-125 to dairy related outbreak strains grown at varying dairy temperatures.

Values shown are the $\log_{10}$ reduction of phage in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Bars that do not share letters show significant difference ($p < 0.05$) and bars with no letters did not show significant difference. Error bars show the standard error of the mean.

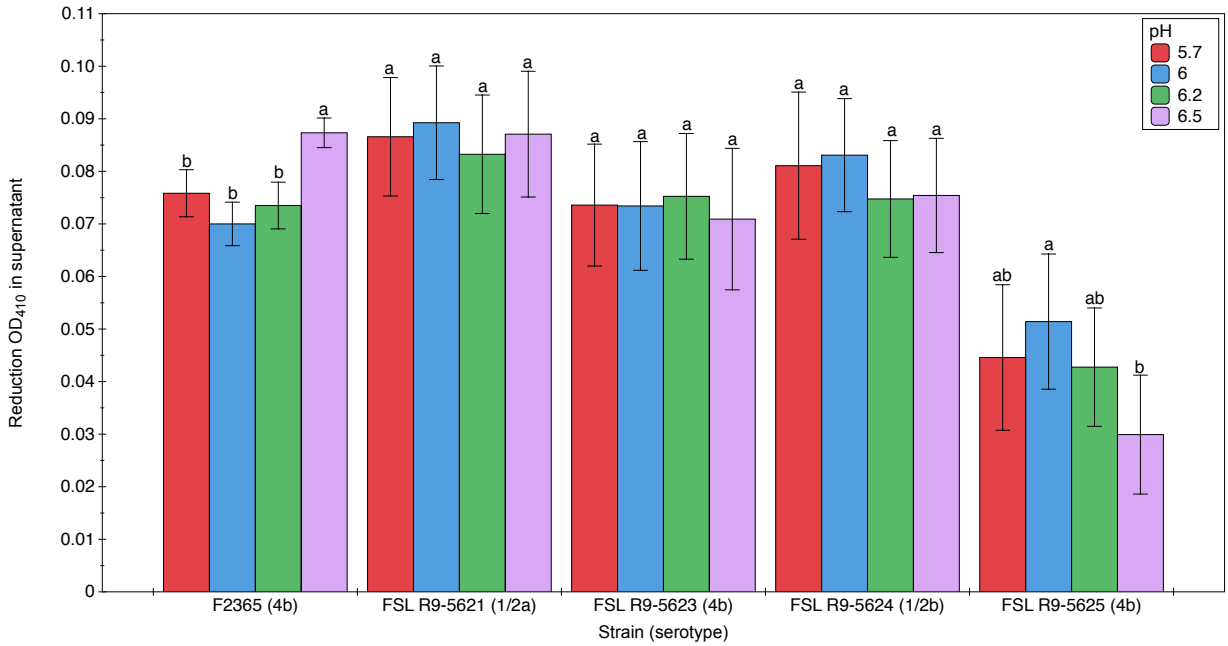


Figure 10. Cytochrome *c* binding on lab strain F2365 and dairy related outbreak strains grown at varying dairy pHs.
Values shown are the reduction OD_{410} of cytochrome *c* in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Bars that do not share letters show significant difference ($p < 0.05$) and bars with no letters did not show significant difference. Error bars show the standard error of the mean.

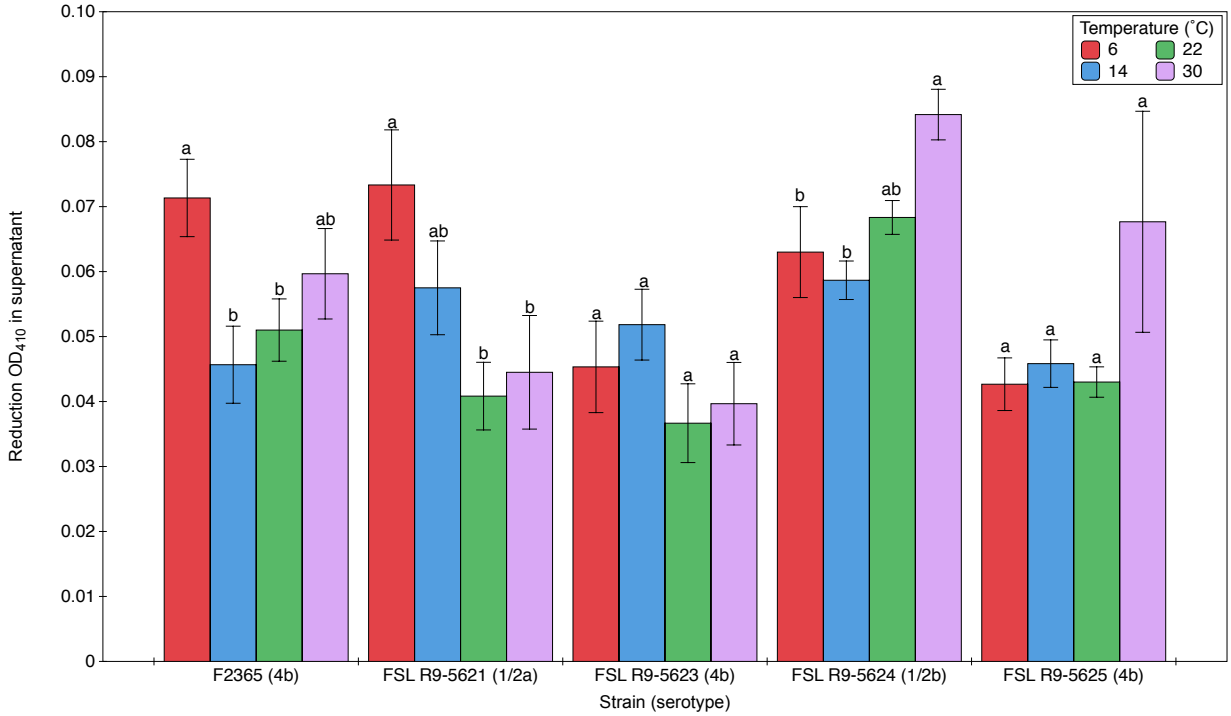


Figure 11. Cytochrome *c* binding on lab strain F2365 and dairy related outbreak strains grown at varying dairy temperatures.
Values shown are the reduction OD_{410} of cytochrome *c* in the supernatant. This is calculated by subtracting the log-transformed number of phage in the supernatant from the log-transformed number of phage in the negative control. Bars that do not share letters show significant difference ($p < 0.05$) and bars with no letters did not show significant difference. Error bars show the standard error of the mean.

VITA

Kathryn A. Magee was born on October 7, 1994 in Buffalo, NY to parents Dan Magee and Jennie Rybarczyk. Kathryn grew up in Kenmore, New York with her younger sister Jenna Rybarczyk. She graduated from Kenmore West Senior High School in 2012. She then continued her studies at the Rochester Institute of Technology receiving a B.S. degree in Biology in 2016. During this time, she completed a research internship at Cornell University in the Department of Food Science. After graduation, she then began the M.S. program in Food Science at the University of Tennessee Knoxville graduating this program in August 2018. Kathryn will begin working in the food industry in August 2018 as a Sanitation/Food Safety Resource at Frito-Lay.