Impact of carbohydrates and carnocyclin A on growth and gene expression of *Listeria monocytogenes*

by

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Abstract

L. monocytogenes is the foodborne pathogen that causes listeriosis, which has a high fatality rate of 20 to 30%. *L. monocytogenes* is often associated with ready-to-eat food products. Therefore, techniques to control growth of this organism are needed for the safety of these foods. Biopreservation techniques such as bacteriocins are being researched as an alternative to chemical preservatives to control *L. monocytogenes*. Carnocyclin A is one of the bacteriocins (class 1b) that can inhibit *L. monocytogenes*. The aim of this research was to investigate the impact of carbohydrates on the development of resistance in strains of *L. monocytogenes* exposed to carnocyclin A in cooked ground beef.

To determine the impact of carbohydrates and carnocyclin A, the strains of *L. monocytogenes* were grown in cooked ground beef supplemented with 3 different carbohydrates (fructose, dextrose and sucrose) with or without carnocyclin A and the growth was observed. The *L. monocytogenes* isolates from the cooked ground beef were screened for resistance to carnocyclin A. For the bacteriocin resistant isolates of *L. monocytogenes*, reverse transcription q-PCR was carried out to determine the impact of carbohydrates and carnocyclin A on the expression of genes involved in resistance. To determine the impact of carnocyclin A on the genomes of *L. monocytogenes*, whole genome sequencing was performed to investigate the SNPs (single nucleotide polymorphisms) of the parent strains (carnocyclin A sensitive) and resistant isolates of *L. monocytogenes*.

The resistance of *L. monocytogenes* to carnocyclin A is both strain and carbohydrate dependent, as evidenced by the different growth patterns. The downregulation of the Mannose PTS system in the presence of dextrose for the resistant *L. monocytogenes* J1-177 strain suggests that the

carbohydrate transport systems are used as receptor molecules for carnocyclin A. However, the upregulation of the Mannose PTS system, sucrose phosphorylase, β-glucoside PTS system in other resistant isolates (*L. monocytogenes* J1-177 and *L. monocytogenes* C1-056 isolated from meat supplemented with sucrose) suggests that mechanism of resistance to carnocyclin A is also dependant on carbohydrate and strain. The high number of SNPs present in the resistant isolates from cooked ground beef indicate that there is hypermutation of *L. monocytogenes* in response to carnocyclin A. In addition, as the SNPs occurred not only in genes related to the carbohydrate transport systems, but also in genes associated with cell wall and virulence, this suggests a more general stress response to the presence of bacteriocins.

Overall, the *L. monocytogenes* resistance to carnocyclin A is mediated by factors such as strain individuality and carbohydrate source, and mechanisms of resistance are broad rather than specific. The results presented in this thesis will contribute to a more comprehensive understanding on how carnocyclin A and the carbohydrates available in food products can impact the resistance to bacteriocins and the mechanisms of resistance in *L. monocytogenes*. This can then inform the use of bacteriocins in the food industry, particularly towards more effective control strategies for *L. monocytogenes* in ready-to-eat meat products.

Preface

This thesis is an original work by Man Sun. The partially purified Carnocyclin A was provided by Dr. Lynn McMullen and previously purified by Dr. Danielle Balay and Dr. Janu Teixiera. The whole genome sequencing of strains of *L. monocytogenes* and genome assembly were done by Shaelyn Xu. I did the SNP analysis with assistance of Vi Pham.

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List of Abbreviations

ANOVA- analysis of variance

CFU- colony forming units

CT- threshold cycle

IPA- Isopropyl alcohol

OD- optical density

PALCAM- Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol

PBS- phosphate buffered saline

PCR- Polymerase chain reaction

PTS- phosphotransferase system

qPCR- quantitative polymerase chain reaction

RPM- revolutions per min

RTE- ready-to-eat

SNP- single-nucleotide polymorphism

TSA- tryptic soy broth

TSB- tryptic soy agar

WGS- whole genome sequencing

1 Introduction and Literature Review

1.1 Introduction

Listeria monocytogenes is a gram positive, facultative anaerobic foodborne pathogen that causes listeriosis. *L. monocytogenes* infections have a fatality rate of 20 to 30 % and the risk of infection increases for vulnerable populations such as the elderly, pregnant individuals as well as individuals with weaker immune systems (Farber and Peterkin, 1991). *L. monocytogenes* is able to grow at a wide range of temperatures from -2 to 45 °C, high salt concentrations up to 10% NaCl, aw ≧ 0.92, and a pH ≧ 4.4 (FAO and WHO, 2022; Health Canada, 2023)

In the United States of America, there are on average 1600 cases of listeriosis annually (CDC, 2023). In Canada, the foodborne pathogen causes around 134 cases annually (Health Canada, 2023) When compared to other foodborne pathogens, *L. monocytogenes* presents fewer cases, but due to its high fatality rate, it represents about a third of the deaths due to foodborne bacteria, viruses and parasites in Canada annually (Health Canada, 2023).

L. monocytogenes is frequently linked to consumption of ready-to-eat food products (RTE) such as refrigerated RTE foods with a long shelf life, such as deli meats and dairy products (Health Canada, 2023). Notably, post-processing contamination is the predominant route in which *L. monocytogenes* contaminates RTE meat products (Ricci et al., 2018). In processing plants, *L. monocytogenes* can exist in multispecies biofilms in places such as tables, equipment, drains and

the floor and demonstrate resistance to commonly used sanitizers (Ricci et al., 2018). Furthermore, there is also evidence for the long-term persistence of individual strains of *L. monocytogenes* in meat processing plants for years (Alvarez-Molina et al., 2021a). These factors together pose serious risks and concerns for these RTE food products because although these products are typically stored at refrigeration temperatures after processing, *L. monocytogenes* can still grow during storage if present. Additionally, consumers do not usually subject these RTE food products to any additional heating steps before consumption, further increasing the infection risk if the product has been contaminated with *L. monocytogenes*.

In Canada, strict guidelines have been implemented by Health Canada to control the foodborne pathogen, as outlined in the policy on *L. monocytogenes* in Ready-to-Eat Foods (Health Canada, 2023) to control the growth of *L. monocytogenes* in these RTE foods. Under this policy, RTE foods are categorized based on the health risks and each category has different sampling methods and criteria for *L. monocytogenes* in the food product. The 2011 policy was updated in October 2023 and includes more details on what are considered RTE foods, the categorization of RTE food products and food products made specifically for vulnerable populations (Health Canada, 2023).

In more recent years, consumers have shown preferences for more minimally processed and convenient food products without the addition of chemical preservatives. To control the growth of *L. monocytogenes* in RTE meats, combinations of sodium lactate or potassium lactate with diacetates are currently used as antimicrobials (Glass et al., 2002; Mbandi and Shelef, 2001). Sodium nitrite, in addition to its role in preventing the outgrowth of *Clostridium botulinum* endospores, can also inhibit the growth of *L. monocytogenes* but the impact is limited (Golden et

al., 2014; McDonnell et al., 2013). However, due to consumer demands, novel methods such as non-thermal processing technologies (high hydrostatic pressure processing, atmospheric cold plasma), essential oils or natural sources of nitrates (such as fermented celery powder) are being investigated as opposed to the use traditional chemical preservatives in RTE foods (Li et al., 2020; Sebranek et al., 2012).

Biopreservation (the use of microorganisms and their metabolites) methods, such as the use of bacteriocins, have been evaluated as a strategy to control the growth of *L. monocytogenes* (Barcenilla et al., 2023). Bacteriocins are antimicrobial peptides that typically have a narrow spectrum of activity (Alvarez-Sieiro et al., 2016). They are ribosomally synthesized and can have inhibitory activity against certain foodborne pathogens (Alvarez-Sieiro et al., 2016). Bacteriocins produced by lactic acid bacteria are usually safe to use in food products (Alvarez-Sieiro et al., 2016). Some commercially available biopreservatives include either the bacteriocin-producing culture or the culture supernatant with bacteriocins but some include both the culture and the culture supernatant. Nisaplin® (Danisco Canada) and Chrisin® (Chr Hansen, Denmark) are culture fermentates that contain nisin, a class I lanthipeptide. Safepro® B-2, B-LC-20 and B-LC-48 are freeze dried bacteriocin-producing cultures of *Latilactobacillus sakei* (sakacin producer), *Pediococcus acidilactici* (pediocin producer) or *Latilactobacillus curvatus* (curvacin producer), respectively. These are sold by Chr Hansen (Denmark) to control the growth of *L. monocytogenes* and indigenous lactic acid bacteria in meat products. Sakacin, curvacin and pediocin are all class II, unmodified bacteriocins (Alvarez et al., 2016). Micocin II® (Griffith Foods, USA) contains both the freeze-dried culture and the culture supernatant of *Carnobacterium maltaromaticum* UAL307. This strain produces two class II bacteriocins (carnobacteriocin BM1 and piscicolin JG126) and one class Ib cyclized peptide (carnocyclin A).

This research focused on carnocyclin A, one of the three bacteriocins produced by *C. maltaromaticum* UAL 307. According to classification of bacteriocins produced by lactic acid bacteria proposed by Alvarez-Sieiro et al (2016), carnocyclin A is a class 1b, head to tail cyclized (circular) bacteriocin made up of 60 amino acids. Class 1 bacteriocins consists of peptides that are modified during synthesis are and are less than 10kDa (Alvarez-Sieiro et al., 2016). Class 1 bacteriocins also include a leader peptide, which is responsible for the peptide's inactivity until use (when it is cleaved), transportation and recognition by enzymes. Due to its circular shape, carnocyclin A demonstrates great stability against a wide range of temperatures (-80°C to 75 °C), pHs (pH 2 to 12), and a variety of proteases (Martin-Visscher et al., 2008a).

The mode of action for carnocyclin A is thought to be due to its ability to form pores in the bacterial membranes (dependent on voltage), which then leads to ion dissipation, leakage of cell compounds, loss of the proton motive force and eventually cell death (Gong et al., 2009). However, recent research findings suggests that there are additional modes of action for carnocyclin A which includes the use of docking molecules for the bacteriocin. Additionally, recent research also found that *L. monocytogenes* is able to develop resistance to bacteriocins, such as carnocyclin A, and that different carbohydrates can impact its resistance to carnocyclin A (Balay et al., 2018).

1.2 Literature review:

1.2.1 Biofilms:

Most of the contamination with *L. monocytogenes* for RTE meat products can be attributed to post processing contamination after any intervention (heating or high-pressure processing) step that may have been applied to the RTE food product) (Fagerlund et al., 2021; Lee et al., 2017; Møretrø and Langsrud, 2004; Ricci et al., 2018). This is due in part to the ability of *L.*

monocytogenes to form biofilms and persist in the food processing plants including on the equipment surfaces that come in contact with the RTE food products (Ricci et al., 2018).

Biofilms are described as a bacterial population that has attached to some type of surface, which can include stainless steel, plastic, polystyrene and glass (Stepanović et al., 2004). Biofilm forming bacteria includes a wide range of organisms, including both the gram-positive and gram-negative bacteria (Preda and Săndulescu, 2019). Biofilm themselves typically consists of many different bacterial species that all co-exist within the biofilm (Dincer et al., 2020) and formation of a biofilm usually consists of five steps: the platonic phase (or the free cell phase), followed by adherence, microcolony, macrocology and lastly dispersion (or detachment) phase of the biofilm formation process. At the start of the biofilm formation process, the free/plantonic bacterial cells can start to reversibly attach to a type of surface (Rossi et al., 2017). Then in the second step, a monolayer of the biofilm is formed and the bacterial cells produce an extracellular matrix, which allows the bacterial cells to irreversibly attach to the surface. The extracellular matrix contains polysacarides, nucleic acids, and proteins among other compounds (Rossi et al., 2017). In the third stage, a multilayer appears as the microcolony of the biofilm is developed. Then as the biofilm becomes a mature biofilm, it takes on this characteristic bump or mushroom shape (fourth stage) (Vasudevan, 2014). This fourth step is also where the bacterial cells in the biofilm can start to make small signalling molecules that allow for communication among the bacteria in the biofilm (Rossi et al., 2017; Solano et al., 2014), known as quorum sensing. Lastly, some of the bacterial cells can start to disperse or detach from the mature biofilm and return to step 1 (plantonic lifestyle) of the biofilm formation process (Vasudevan, 2014). If this occurs in a ready-to-eat meat processing facility, the biofilm formation cycle could repeat itself and another

biofilm could establish in another area of the plant, further contributing to the post-processing contamination of the RTE food product.

Due to both the communication among bacteria in the biofilm (quorum sensing) as well as the extracellular polymeric matrix produced by the biofilm, the organisms in the biofilm are shielded from various environmental stresses (Solano et al., 2014). In relation to the food industry, the extracellular polymeric matrix can protect the organisms in the biofilm from dehydration while helping to concentrate the nutrients available in the biofilm (Rossi et al., 2017). The communication among bacterial cells also contribute to the resistance of the organisms in the biofilms to chemicals and antibiotics (Paula et al., 2020; Rossi et al., 2017). All of these advantages of biofilms added together allows the organisms to be resistant to the general cleaning and disinfection practices that are currently used in the food production industry, and allow the biofilms to remain on equipment surfaces and potentially contaminate the food product produced in the facility (Giaouris et al., 2005)

In food processing facilities, biofilms themselves are typically made up of many different bacterial species all living together (i.e. there will not be a biofilm consisting of only *L. monocytogenes* in a ready-to-eat meat facility). On the surfaces of equipment and structures in meat processing facilities, the microbial microbiota mainly consists of gram-negative bacteria, such as *Pseudomonas*, *Acinetobacter*, *Psychrobacter*, *Enterobacteriaceae*, and *Stenotrophomonas* (Fagerlund et al., 2021). However, gram-positive bacteria such as *Carnobacterium*, *Staphylococcus*, and *Leuconostoc* can also be present.

1.2.2 Persistence of *L. monocytogenes* in meat processing plants

In meat processing facilities, *L. monocytogenes* has the ability to establish itself in various places within the facility and persist for extended periods of time. Persistence of a strain is determined when an isolate of a particular strain is repeatedly detected over a duration of at least three months or longer (Cherifi et al., 2018).

To determine if isolates of *L. monocytogenes* are of the same strain when isolated form a meat processing facility at different times, whole genome sequencing and single nucleotide polymorphism (SNP) analysis can be used (Pightling et al., 2018). In recent years, whole genome sequencing (WGS) has helped the food industry when it comes to the typing of foodborne bacteria, such as *L. monocytogenes* during outbreaks (Pightling et al., 2018). This is because whole genome sequencing allows for the high resolution typing of these pathogens and can faster and more accurately identify the origins of contamination (Pightling et al., 2018).

With whole genome sequencing, the answer to whether two or more bacteria are the same strain or if they are from the same contamination source, can be determined by examining the number of single nucleotide polymorphisms (SNPs) (Rahman et al., 2022). Single nucleotide polymorphisms (SNPs) are differences in the DNA sequence in the single nucleotides (A, T, C or G). Although there is no hard cut-offs or thresholds for the number of single nucleotide polymorphisms for two bacteria to be considered the same strain, it is generally accepted that a low number of SNPs (< 20) is enough to consider two bacteria the same strain in outbreak investigations (Rahman et al., 2022).

Alvarez-Molina et al (2021) used the criteria of < 20 SNPs and a sampling frequency of more than three months between isolates to analyze a meat processing plant that was newly opened. In

their research, they sampled the newly open meat processing facility for a total of ten separate times/visits, for a total study duration of 1.5 years(Alvarez-Molina et al., 2021). During the ten visits, they sampled from a variety of places in the facility, including both food contact and non-food contact surfaces, using random sampling, for the presence of *L. monocytogenes*. They sequenced the 18 isolates and analyzed the number of SNPs (Alvarez-Molina et al. 2021). Through comparative genomics, they found very low numbers of single nucleotide polymorphisms (0 and 2 SNPs) were between two pairs of the strains of *L. monocytogenes* isolated from the facility (Alvarez-Molina et al., 2021). This low value of SNPs indicated that the pairs can be considered the same bacterial strain, and because these pairs of *L. monocytogenes* strains were isolated from the same processing room in the meat processing facility, with at least 3 months apart in isolation dates, this demonstrated that these strains are very likely persistent *L. monocytogenes* strains in this meat processing facility.

Lachmann et al. (2021) investigated a German outbreak of listeriosis for a study period of 5 years from 2014-2019 with a total of 39 confirmed cases covering a wide geographic area in Germany. In their study, they collected isolates from both the food samples and the confirmed human cases of listeriosis (Lachmann et al., 2021). Similar to the study by Alvarez-Molina et al. (2021), Lachmann et al (2021) sequenced the isolates they obtained. They found that 11 SNPs (a range of 0 SNPs to 11 SNPs) was the greatest difference between the isolates from the confirmed human cases of listeriosis and the food sample isolates. As the number of SNPs was fewer than 20, this is an indication the isolates are the same strain and that the strain of *L. monocytogenes* has also very likely persisted in the company for at least five years. These studies and others (Harrand et al., 2020; Kolanowski et al., 2023; Palaiodimou et al., 2021), provide evidence for the long-term persistence of individual strains of *L. monocytogenes* in processing plants that

prepare RTE foods. The long-term persistence and continued outbreaks of listeriosis associated with RTE meats supports the need for strategies to control *L. monocytogenes* in the food processing environment and in RTE meats.

1.2.3 Bacteriocins for control of *L. monocytogenes* in RTE foods

There is a need for strategies for control of the presence of *L. monocytogenes* in processing facilities that produce RTE foods to ensure that these foods are safe for consumption by vulnerable populations. Inhibition of the growth of *L. monocytogenes* on RTE meats by bacteriocin-producing lactic acid bacteria or culture supernatants of bacteriocin-producing lactic acid bacteria has been reported in the research literature (Barcenilla et al., 2023; de Azevedo et al., 2020; Fernández et al., 2023; Iacumin et al., 2020; Junges da Costa et al., 2021; Montiel et al., 2019; Vijayakumar and Muriana, 2017). The combination of bacteriocins with other antimicrobials or technologies can have additive effects. For example, when nisin or lactacin AL705 are combined with high-pressure processing, there are additive effects and the combination inhibits the growth of *L. monocytogenes* during storage (Dallagnol et al., 2017; Teixeira et al., 2018). One of the challenges of the use of bacteriocins to control the growth of *L. monocytogenes* is that there is the possibility that it can develop resistance to bacteriocins, which may result in outgrowth during long-term storage of RTE meats (Bodie et al., 2023). A multi-hurdle approach to controlling *L. monocytogenes* in RTE meats maybe the best approach as it may limit the development of resistance during storage. One strategy is to use bacteriocin-producing organisms that produce more than one bacteriocin to limit the development of resistance in RTE foods during storage. The combination of bacteriocins from *Latil. curvatus* and *Latil. sakei* with nisin was able to provide a synergistic impact to control the growth of *L. monocytogenes* in RTE meat (Castellano et al., 2018). Another example would be the use of *C.*

maltaromaticum UAL307 which produces carnocyclin A and two class II bacteriocins. Balay et al (2018) established that *L. monocytogenes* can develop resistance against carnocyclin A and resistance was dependent on the carbohydrates present. However, little is known about the development of resistance to carnocyclin A by *L. monocytogenes* in food products.

1.2.4 Bacteriocin classification, mode of action

In the classification of bacteriocins produced by lactic acid bacteria proposed by Alvarez-Sieiro et al, there are three classes of bacteriocins. Class 1 consists of peptides that are modified during synthesis are and are less than 10 kDa (Alvarez-Sieiro et al., 2016). The modifications give the bacteriocins unique structures and amino acids such as head to tail cyclization, glycosylation or lanthionine which impact their activity. Class 1 bacteriocins also include a leader peptide, which is responsible for the peptide's inactivity until the leader peptide is cleaved, transportation and recognition by enzymes. Class 2 includes bacteriocins that are not modified during synthesis and class 3 refers to bacteriocins that exceed 10 kDa in size, and are unmodified (Alvarez-Sieiro et al., 2016).

The general mode of action for these bacteriocins is that they are able to form pores in the cell membrane of the target bacteria by initiating the permeabilization of the cell membrane (Alvarez-Sieiro et al., 2016). The pores formed will lead to the leakage of cell substrates and dissipation of the membrane proton motive force, which ultimately results in death of the cell (Desiderato et al., 2022; Li et al., 2023). In this process, the bacteriocins may or may not utilize docking molecules, such as the mannose phosphotransferase system or lipid II, to initially interact with the cell membranes of the target bacteria. depending on the specific bacteriocin's mode of action (Alvarez-Sieiro et al., 2016).

Class Ia. lanthipeptides

Within the numerous numbers of lanthipeptides produced by lactic acid bacteria, nisin (produced by *Lactococcus lactis*) is notably the most researched bacteriocin (Kaur et al., 2011). In the case of nisin, it acts by inhibiting the target bacteria by first displacing lipid II from its original location, and then forming a pore in the bacteria by inserting the displaced lipid II into the target cell membrane (Breukink et al., 1999; Guo et al., 2023; Hasper et al., 2006). As lipid II also plays a crucial role in the process of peptidoglycan biosynthesis, this shows that nisin can inhibit the formation of the cell wall in addition to forming pores in the cell membrane in the target bacteria using lipid II as a docking molecule (Lubelski et al., 2008).

Class Ib. head-to-tail cyclized peptides

Class Ib refers to heat to tail cyclized peptides, meaning the bacteriocin is circular in shape as a peptide bond links together the N and C termini of the bacteriocin (Alvarez-Sieiro et al., 2016). This circular structure as mentioned gives these peptides more stability against pH, proteases and a wide range of temperatures (Martin-Visscher et al., 2008b). Head to tail cyclized bacteriocins also possess high net positive charges, which is thought to help when they interact with the target bacteria cell membrane that is negatively charged, and potentially eliminate the need for a receptor molecule in this process (Alvarez-Sieiro et al., 2016). Bacteriocins in this class include Enterocin AS-48, carnocyclin A, garvicin ML, and lactocyclin Q. Among them, two well characterized bacteriocin are carnocyclin A and Enterocin AS-48. Both of these form pores in the target bacteria but have different modes of action (Alvarez-Sieiro et al., 2016).

In the case of Enteriocin AS-48, it was initially demonstrated that the bacteriocin generates non-selective pores (ion channels) in the cell membrane, which leads to the loss of low molecular

weight compounds and ions and eventually cell death (Galvez et al., 1991). However, through X-ray crystallography experiments, it was discovered that enterocin AS-48 can also exists as dimers (pH dependent) (Cebrián et al., 2015). In this mechanism of action, the dimeric form of enterocin AS-48 undergoes transformation when it initially interacts with the cell membrane to its monomeric form, as the membrane is acidic, and the peptide is in its monomeric form at a low pH (Cebrián et al., 2015). Once in its monomeric state, the bacteriocin inserts into the cell membrane by hydrophobic and electrostatic interactions, leading to pore formation and death of the target cell (Cebrián et al., 2015).

For carnocyclin A, the bacteriocin consists of four helices that make up its globular structure and encases a hydrophobic core (Martin-Visscher et al., 2008). For its mechanism of action, it is able to form voltage dependant and anion selective pores in the target bacterial lipid bilayers as it interacts electrostatically, which leads to the leakage of ions, loss of proton motive force and eventually cell death (Gong et al., 2009).

Although it was previously thought that the mechanism of action of circular bacteriocins do not require any receptor molecules, it has been recently suggested that receptor molecules may be involved in the mode of action of bacteriocins like carnocyclin A, where the receptor molecule can help with the docking of the bacteriocin to the target cell (Acedo et al., 2018; Balay, 2019).

Class IIa. pediocin-like bacteriocins

Class IIa includes pediocin-like bacteriocins that work against foodborne pathogens such as *L. monocytogenes* (Alvarez-Sieiro et al., 2016). Within this class, pediocin PA-1 is the most researched bacteriocin. For its mechanism of action, the bacteriocin forms pores as it inserts itself into the target bacterial membrane by binding to the mannose phosphotransferase system

receptors- responsible for the transport of mannose and glucose- which leads to cell death (Diep et al., 2007; Zhu et al., 2022b).

1.2.5 Bacteriocin resistance

In response to bacteriocins, *L. monocytogenes* is able to respond quickly in a variety of ways including modifications to their cell wall and regulation of gene expression related to carbohydrate transport systems such as the mannose PTS system (Citartan et al., 2016).

Bacteriocins have high net positive charges that facilitates their interaction with the target bacteria cell membrane that is negatively charged (Alvarez-Sieiro et al., 2016) and changes to the bacteria cell membrane are used in the development of resistance to bacteriocins. For example, in response to nisin, *Lactococcus lactis* is able to include more D-alanine residues, which are positively charged into its cell membrane (Giaouris et al., 2008). The more positive cell membrane makes it difficult for nisin to bind to the cell wall, contributing to nisin resistance. Mantovani and Russell et al also reported that increasing the positive charge in the bacterial cell wall increased the resistance of *Streptococcus bovis* to nisin (Mantovani and Russell, 2001).

L. monocytogenes can modify its cell membrane and make it more rigid so it is more difficult for nisin to penetrate its membrane and form pores (Ming and Daeschel, 1995). Nisin resistant *L. monocytogenes* incorporates more straight-chain fatty acids and fewer branched chain fatty acids, increasing the rigidity of the cell membrane (Ming and Daeschel, 1995). However, in response to leucocin A, resistant *L. monocytogenes* variants have a more fluid cell membrane as the membrane was modified to contain more unsaturated acyl chains (Vadyvaloo et al., 2002) In response to leucocin A, *L. monocytogenes* can include more L-lysine and D-alanine residues, both positively charged into its cell membrane, to increase the membrane positive charge

(Vadyvaloo et al., 2004). Similar mechanisms of resistance of increasing the fluidity of its membrane were observed in the *L. monocytogenes* resistance to circular bacteriocins such as enterocin AS-48 (Mendoza et al., 1999).

Beyond the regulation of the cell membrane, *L. monocytogenes* can also utilize divalent cations to develop resistance to bacteriocins, such as nisin (Crandall and Montville, 1998). For example, the bacterial cell membrane can become more rigid in the presence of divalent cations, which induces a tighter packing of the fatty acyl chains in response to nisin (Kaur et al., 2011). The interaction between the cell membrane, which is negatively charged, and the divalent cations can hinder bacteriocin binding to the cell membrane of *L. monocytogenes* (Crandall and Montville, 1998).

These impact of these modifications, more positively charged cell membranes, more rigid or fluid membranes and divalent cations, are dependent on the bacteriocin and the target bacteria. However, they can all increase the resistance to bacteriocins by making it more challenging for the bacteriocin to bind to the bacteria membrane, form pores and eventually lead to death of the cell.

The mannose phosphoenolpyruvate-dependent phosphotransferase systems (PTS) has been linked to bacteriocin resistance. In *L. monocytogenes*, seven PTS have been identified for transport of carbohydrates (Stoll and Goebel, 2010). The mannose PTS is responsible for the transport of glucose and fructose, the preferred carbohydrates for growth of *L. monocytogenes* (Premaratne et al., 1991).

The mannose PTS system identified as a docking receptor molecule for various bacteriocins and regulation of gene expression has been observed as a mechanism of resistance to these

bacteriocins (Gravesen et al., 2002; Jeckelmann and Erni, 2020; Kaur et al., 2011; Ramnath et al., 2000). The EIIC and EIID subunits of the mannose PTS serve as receptor molecules for bacteriocins (Kjos et al., 2011; Wu et al., 2020). The mannose PTS system has been demonstrated as a receptor molecule for Class II bacteriocins, including leucocin A (Diep et al., 2007; Ramnath et al., 2000) lactococcin A (Kjos et al., 2011) and pediocin PA-1 (Zhu et al., 2022a) but has not been identified as a receptor to carnocyclin A. The regulation of mannose PTS have been observed in resistance of *L. monocytogenes* to pediocin (Liu et al., 2019). However, the regulation (whether upregulation or downregulation occurs) is dependent on the carbohydrate source present as well as the strain of *L. monocytogenes* (Liu et al., 2023, 2019).

As downregulation of the mannose PTS could lead to slower growth of *L. monocytogenes*, upregulation of other PTS systems to offset this downregulation have been documented (Balay, 2019; Dalet et al., 2001; Gravesen et al., 2002). For example, *L. monocytogenes* variants resistant to pediocin PA-1 upregulate the β-glucoside-specific PTS (Gravesen et al., 2002).

In other studies, two modes of resistance for *L. monocytogenes* were discovered where for the more resistant isolates, downregulation of the mannose PTS was reported (Kjos et al., 2011). However, in the less resistant isolates, there was no downregulation of the mannose PTS system, suggesting that resistance to bacteriocins is diverse and dependant on various factors (Kjos et al., 2011). The same phenomenon was observed by Tessema et al who reported that strains of *L. monocytogenes* that were highly resistant to sakacin P under expressed *mptA*, which encodes for the PTS mannose permease EII but in less resistant *L. monocytogenes*, the mptA was overexpressed (Tessema et al., 2011).

With respect to carnocyclin A, previous research demonstrated that *L. monocytogenes* resistance also involves the downregulation of the mannose PTS system, which indicates that the mannose PTS can serve as a receptor molecule for carnocycin A (Balay, 2019). If the mannose PTS system acts as a receptor molecule and the sensitivity of *L. monocytogenes* increases in the presence of glucose or fructose, then downregulation of gene expression can be a mechanism of action for resistance to the carnocyclin A by *L. monocytogenes*. This supports the idea that the formation of pores may not be the only mechanism of action for carnocylin A in *L. monocytogenes* (Gong et al., 2009). However, this proposed mechanism of action for carnocyclin A is still not fully elucidated. Research by Balay (2019) also found that although the presence of different carbohydrates led to the downregulation of the mannose PTS system in bacteriocin resistant *L. monocytogenes* strains, there was also upregulation of gene expression for other sugar transport systems, such as the cellobiose specific PTS system (Balay, 2019).

In addition to cell wall modifications and regulation of the carbohydrate PTS systems, ABC transporters are also involved in the resistance to bacteriocins (Collins et al., 2010; Gandhi and Chikindas, 2007; Kaur et al., 2011). There is also evidence that resistance to bacteriocins leads to more broader stress responses rather than specific stress responses in sugar transport systems. For example, isolates of *L. monocytogenes* that are resistant to carnocyclin A induced changes not only in genes related to carbohydrate PTS systems, but also in cell membrane synthesis, virulence related factors, metabolism and transcriptional regulation (Balay, 2019).

Research on mechanism of resistance of *L. monocytogenes* to other circular bacteriocins is limited and has focused on resistance to enterocin AS-48, a cyclic bacteriocin. The development of resistance to enterocin AS-48 has been linked to changes in cell thickness and a disorganized cytoplasmic membrane (Mendoza et al., 1999). It is probable that the resistance of *L.*

monocytogenes to cyclic bacteriocins is multifactorial and may involve general stress mechanisms of the cell.

1.3 Research hypothesis and objectives

The aim of the current research was to determine the impact of carbohydrates on the development of resistance in *L. monocytogenes* exposed to carnocyclin A in a food product. This will provide information on the potential for growth of *L. monocytogenes* during storage when bacteriocins are used to improve the safety of RTE meat products. This research tested the following overall hypothesis:

Different carbohydrates induce resistance of *L. monocytogenes* to carnocyclin A in a strain dependent manner in cooked ground meat.

To test this hypothesis, the objectives of this research were to:

1. determine the impact of carnocyclin A on the growth of *L. monocytogenes* in cooked ground beef supplemented with different carbohydrates;
2. determine the impact of carbohydrates on expression of genes involved in *L. monocytogenes* resistance to carnocyclin A; and
3. sequence the genomes of strains of carnocyclin resistant *L. monocytogenes* and the wild types strains to determine the impact of carnocyclin A on the genomes of *L. monocytogenes*.

2 Materials and Methods

2.1 Bacterial strains and culture conditions

Three outbreak strains of *Listeria monocytogenes* (*L. monocytogenes* FSL R2-499, *L. monocytogenes* FSL C1-056 and *L monocytogenes* FSL J1-177) were used in this study and are listed in Table 2.1 (Fugett et al., 2006). The three strains of *L. monocytogenes* were selected as previous research demonstrated that *L. monocytogenes* FSL R2-499, *L. monocytogenes* FSL C1-056, and *L monocytogenes* FSL J1-177 were one of the most resistant, intermediately resistant and one of the most sensitive strains, respectively, to carnocyclin A when grown in different carbohydrates (Balay et al., 2018). All strains of *L. monocytogenes* were stored at -80°C in 30% glycerol and were prepared by mixing equal volumes of an overnight culture of *L. monocytogenes* with 60% glycerol in Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Mississauga, ON).

Prior to use in experiments, frozen stocks of *L. monocytogenes* were streaked onto Tryptic Soy Agar (TSA; Becton, Dickinson and Company, Mississauga, ON) and incubated overnight at 37°C. A single colony was picked and inoculated into 10 mL of TSB and grown aerobically at 37°C for 24 h with 200 RPM agitation.

2.2 Carnocyclin A and determination of carnocyclin A activity units:

The carnocyclin A (3.3 mM) used in experiments was partially purified, and stored at -20°C until needed (Balay et al., 2018). To determine the activity units of the partially purified carnocyclin A (hereafter referred to as carnocyclin A), spot-on-lawn antibacterial assays were performed. The carnocycin A was thawed at room temperature for 30 min and diluted in serial two-fold dilutions with 0.1% peptone water (Becton, Dickinson and Company, Mississauga, ON), up to

the 1:4112 dilution. Overnight cultures (100 µL) of each of the 3 strains of *L monocytogenes* were inoculated (0.1%) into TSA and agar was poured into petri dishes and allowed to set. Each dilution of carnocyclin A (10 µL) was spotted onto the TSA and air dried before incubating at 37°C for 24 h.

After incubation, the concentration of carnocylin A that gave a clear zone was recorded for each strain of *L monocytogenes* and the activity units were calculated as the reciprocal of the highest dilution at which the growth of *L. monocytogenes* was inhibited by carnocyclin A (Ahn and Stiles, 1992).

2.3 Growth of *L monocytogenes* in cooked ground beef with different carbohydrates and carnocyclin A

2.3.1 Preliminary experiments to determine amount of carnocyclin A to use in meat

To determine the amount of carnocyclin A for subsequent experiments that allowed the survival of resistant derivatives under long term incubation at 4°C, preliminary experiments with various amounts of carnocyclin A were done. Briefly, ground beef (24% fat) was cooked in a circulating water bath for 45 min set at 71 °C, (Thermo Scientific NESLAB EX 7, Waltham, MA). Then, 50, 75, 100, 125, 150, 200 and 350 µL of thawed carnocyclin A was added to 5 g of cooked ground beef (24% fat) supplemented with 2% dextrose or fructose and mixed massaging by hand in a vacuum package bag (Unipac, Edmonton, AB) for 1 min. Overnight cultures of *L. monocytogenes* FSL R2-499 and *L. monocytogenes* FSL C1-056 (grown in TSB at 37°C for 24 hours at 200 RPM) were diluted to approximately 10^5 colony forming units (CFU)/mL in 0.1% peptone (Becton, Dickinson and Company). The diluted *L. monocytogenes* cultures (50 µL) were inoculated into the cooked ground beef and mixed by hand massaging the package for 1 min. The samples were vacuum packaged (Multivac Inc. Model C200, Kansas City, MO) and stored

at 15°C and 4°C, and sampled after 0 (defined as day of the experiment), 1, 3 and 14 days of storage to determine the growth of *L. monocytogenes* FSL R2-499 and *L. monocytogenes* FSL C1-056. Enumeration of *L. monocytogenes* was done by plating the samples on prepoured plates of TSA and PALCAM (Oxoid, Nepean, ON) with added supplement (Oxoid). Samples were enriched in Listeria Enrichment Broth (Becton, Dickinson and Company) that was incubated at 37 °C for up to 72 h and plated onto PALCAM agar to determine the presence of low numbers of surviving cells.

The aliquot of 50 µL of the carnocyclin A added to 5 grams of cooked ground beef allowed the growth of *L. monocytogenes* FSL C1-056, thus, this amount was used in future experiments.

2.4 Impact of carnocyclin A on the growth of *L monocytogenes* in the presence of different carbohydrates in cooked ground beef

For growth experiments in meat, samples of cooked ground beef (24 % fat) supplemented with three carbohydrates (dextrose, fructose or sucrose) were used. For each treatment, the final formulation consisted of 5 grams of cooked ground beef with 2% carbohydrate (either dextrose, fructose or sucrose), 10^3 CFU/g of either *L. monocytogenes* R2-499, *L. monocytogenes* CL-056 or *L. monocytogenes* J1-177) and 50 µL of carnocyclin A. The final cell density of *L. monocytogenes* was confirmed by plating ground beef samples onto TSA that was incubated at 37 °C for 24 h.

To prepare samples, *L. monocytogenes* FSL R2-499, *L. monocytogenes* FSL C1-056 and *L monocytogenes* FSL J1-177 strains from frozen glycerol stocks were streaked onto TSA and incubated overnight at 37°C. Ground beef (1 kg; 24% fat) was transferred from the -20°C freezer and thawed overnight at 4 °C. Thawed ground beef (100 g) was placed in a vacuum package bag (Unipac, Edmonton, AB) and 2% (w/w) of either dextrose, fructose or sucrose was added to the

meat. The meat and carbohydrates were hand massaged for one min before the bag was vacuum packaged (Multivac Inc. Model C200). After vacuum packaging, the 100 grams of ground beef was flattened to approximately 0.3 cm and cooked for 45 min in a circulating water bath set at 71 °C, (Thermo Scientific NESLAB EX 7, Waltham, MA). Immediately after cooking, the ground beef was placed into ice water for 60 s and stored at 0 °C overnight.

The cooked ground beef with carbohydrates was transferred from the 0 °C cooler to 22 °C and held 10 min prior to use in experiments. Carnocyclin A was thawed at room temperature for 20 min prior to use. The optical density (OD_{630nm}) of each overnight culture of *L. monocytogenes* was recorded to ensure that the cell density had reached approximately 10^9 CFU/mL. Each culture of *L. monocytogenes* was centrifuged at 8000 x *g* for 10 min and the pellets were resuspended in a 0.85% saline. *L. monocytogenes* cultures were serially diluted in 0.1% peptone to 10^5 CFU/mL prior to inoculation of the cooked ground beef.

For the preparation of individual samples, 100 grams of cooked ground beef was divided into two 50 gram samples, which was the total amount needed for all 8 sampling times. For each bag, 50 µL of carnocyclin A was added to 50 g of cooked ground beef (supplemented with either dextrose, fructose or sucrose) and thoroughly mixed in with a sterile swab for 2 min. Controls without carnocyclin A were also prepared for each carbohydrate. Each bag of cooked ground beef was inoculated with 50 µL of the diluted cultures of either *L. monocytogenes* FSL R2-499, FSL C1-056 or FSL J1-177 and thoroughly mixed with a sterile swab for 3 min. This resulted in an inoculation level 10^3 CFU/g. The inoculated cooked ground beef was divided into 8 individual sample bags, with each sample consisting of 5 grams of the cooked ground beef

mixture. Each bag was vacuum packaged (Multivac Inc. Model C200, Kansas City, MO) and incubated at 4 °C until analysis.

The treatments consisted of samples with each of the three strains of *L. monocytogenes*, with and without the addition of the carbohydrates (dextrose, fructose or sucrose) and with or without carnocyclin A. All codes for all treatments are listed in Table 2.2. The experiment was replicated three times.

2.4.1 Sampling

Both the treated and control samples were analyzed after 0 (designated as the day *L. monocytogenes* and carnocyclin A were put into the ground beef), 1, 3, 7, 16, 30, 48, and 80 d of storage at 4 °C. For each of the 5 g samples, packages were aseptically opened, 5 mL of sterile 0.1% peptone water was added, and the sample was mixed for one min using a stomacher (Stomacher Lab-Blender400, A.J. Seward, London UK) before hand mixing for one min. After mixing, 100 µL of the ground beef slurry was serially diluted using sterile 0.1% peptone water, and plated onto prepoured plates of TSA and PALCAM agar, which were incubated at 37 °C for 48 h or 72 h, respectively. Where the cell counts were expected to be below the detection limit, Listeria Enrichment Broth (Oxoid) was pipetted into the sample bag in a 1:9 ratio (Heath Canada) and samples were incubated at 37 °C for up to 72 h and plated onto PALCAM agar to determine the presence of low numbers of surviving cells.

After enumeration of cell counts, individual colonies were isolated from TSA plates and inoculated into TSB with 30% glycerol and stored at -80°C for analysis of resistance to carnocyclin A.

2.5 Identification of isolates of *L monocytogenes* resistant to carnocyclin A

To determine if the three strains of *L monocytogenes* became resistant to carnocyclin A after growth in cooked ground beef with different carbohydrates, a total of 48 isolates obtained after growth of strains in ground beef were tested using a modified protocol from Balay et al (Balay et al., 2018). Briefly, basal media [500 mL of distilled H_2O, 10 g of either dextrose, fructose or sucrose, 5 g Proteose Peptone No. 3 (Becton, Dickinson and Company), 5 g Beef Extract (Becton, Dickinson and Company), 2.5 g Yeast Extract (Becton, Dickinson and Company), 0.5 g Tween® 80, 1 g disodium phosphate monohydrate and 0.5 mL of sterile basal media salt solution (10 g magnesium sulfate, 5 g manganese sulfate dissolved in 100 mL distilled H_2O)], was filtered using a 0.22 µm pore diameter filter (Fisher Scientific, Canada)]. To test resistance, 96 well microtiter plates with a total well volume of 200 µL were used. In each individual well, 150 µL of the basal media with either dextrose, fructose or sucrose was added along with 4.95 µL of carnocyclin A. Overnight cultures of each of the isolates of *L. monocytogenes* recovered from ground beef were centrifuged (8000 x *g*) for 10 min, resuspended in sterile 0.1% peptone (Becton, Dickinson and Company) and diluted to 10^7 CFU/mL, before adding 50 µL of the culture to individual wells for a total well volume of 200 µL. The negative control consisted of 200 µL of the basal media supplemented with each of the three carbohydrates (dextrose, fructose and sucrose). The other controls included of 150 µL of basal media and 50 µL of the parent strains of *L. monocytogenes* FSL R2-499, FSL C1-056 and FSL J1-177 that had not been exposed to carnocyclin A.

After placing a clear optical film (MicroAmp, Applied Biosystems, Thermo Fischer Scientific, Burlington, Ontario, Canada) over the 96 well microtiter plate, it was placed in a spectrophotometer (Varioskan Flash, Thermo Scientific, USA) and incubated for 40 h at 25 °C. The spectrophotometer was set to shake the plate every 1 h for 10 s at 680 RPM and the OD_{560nm}

was measured and recorded every 40 min to generate the growth curves. This experiment was replicated for each culture three times. Isolates that were able to grow in the presence of carnocyclin A and had shorter lag phases than their parent strains were determined to be resistant.

2.6 Quantification and detection of glucose in ground beef

To determine if the amount of glucose in the ground beef used in the growth experiment impacts the resistance of *L. monocytogenes* to carnocyclin A, the amount of glucose present in ground beef was measured. To extract glucose from the ground beef, a protocol from Rood et al (2022) was followed. Briefly, the ground beef was homogenized in 1x phosphate buffered saline (PBS) adjusted to pH 7.4 (1 g meat:2 mL PBS). The homogenized ground beef (0.7 grams) was hydrolyzed with 1 mL 0.1 M HCl at 100 °C for 2 h in a circulating water bath (Polyscience 730 Immersion Circulator, Illinois USA). After hydrolysis, the pH was adjusted using 1 M acetate buffer (pH 5.6) to approximately a pH of 5.

Glucose was quantified using the Glucose Colorimetric Detection Kit (Thermo Fisher Scientific, Canada), which has an assay sensitivity of 0.413 mg/dL glucose. A slight modification was made to the procedure where the hydrolyzed meat samples were diluted 1:20 in the Assay Buffer prior to use with the Glucose Colorimetric Detection Kit. Measurements were done with a spectrophotometer (Varioskan Flash, Thermo Scientific, USA) at OD_{560nm}.

Using the standard curve generated by glucose standards, the following formula was used to determine the glucose present:

$$y=0.0982x$$

Where y is the absorbance measured by the spectrophotometer and x is mg glucose/dL.

The measured absorbance of five samples of cooked ground beef was used to obtain the mg glucose/dL.

2.7 Impact of glucose in ground beef on gene expression

To determine if the level of glucose in ground beef affected the expression of genes involved in resistance to carnocyclin A (Table 2.3), *L. monocytogenes* J1-177 was inoculated into 10 mL of basal media (as above) supplemented with either the amount of glucose in the ground beef or no glucose was added and the media was incubated for 24 h at 37°C with 200 RPM agitation. Reverse transcription q-PCR (described below) was done to compare gene expression with and without glucose.

To determine the impact of carnocyclin A and carbohydrates on gene expression in 4 strains of *L. monocytogenes* that were resistant to carnocyclin A and the 2 parent strains, colonies of *L. monocytogenes* from TSA were inoculated into 10 mL of basal media and grown with 98 µl of carnocyclin A added to the basal media for the resistant strains, and with 15 µl of carnocyclin A for the parent strains. Cultures were grown aerobically at 26°C until an OD_{600} of 0.5 to 0.6 nm was reached.

Isolation of RNA was done following the RNA extraction from rumen Tissue or Fluid protocol of Dr. Guan's Lab (2015) with some modifications. Briefly, 2 mL of the *L. monocytogenes* cultures were centrifuged at 8000 x *g* for 10 min in 2 mL bead tubes (Precellys Bertin Technologies, France). The sample was homogenized at 8 °C using Precellys 24 homogenizer

(Bertin Technologies). The modifications included adding the high salt solution before the isopropyl alcohol. Rather than vortexing samples after the addition of chloroform (Thermo Fisher Scientific, USA) or the addition of high salt solution plus IPA, the sample tubes were inverted for ten to twenty times. Centrifugation using the IEC MICROCL 17R Centrifuge (Thermo Electron Corporation, Waltham, United States) after the ethanol wash step was performed at 12600 x *g* instead of 12000 x g for 5 min at 4 °C. The RNA was dissolved in either 30 or 50 µl molecular grade water and stored at -80 °C prior to use in experiments.

Isolation of DNA was carried out following a modified Promega wizard genomic DNA purification kit protocol (ThermoFisher Scientific 2021). The modifications include using 5 mL of the overnight *L. monocytogenes* culture instead of 1 mL and washing the cells with 1x phosphate buffered saline (PBS) prior to pelleting the cells. In addition, the protein precipitation steps (steps 10 to 12) were performed three times, where 200 µl of the protein precipitation solution was added the first two times and no protein precipitation solution was added the third time, followed by adding 750 µl of room temperature isopropanol rather than 600 µl. The DNA was rehydrated by adding 100 µl of the DNA rehydration solution and incubating at 65°C for 1 h (mixed every 15 minutes by gently flicking the tube) and placed overnight in a 4°C refrigerator. The DNA was stored at -20 °C prior to use in experiments.

The quantity and quality (A260/A280 and A260/A230) of isolated RNA and DNA were measured with a NanoDrop One Spectrophotometer (Thermo Fisher Scientific, USA). For RNA measurements, 1 µl of the RNA solution was used and for DNA measurements, 1.5 µl of the DNA solution was used.

To confirm that the primers designed were specific to *L. monocytogenes* C1-056 prior to qPCR, both PCR and colony PCR were carried out on DNA that was reverse transcribe from RNA and colonies grown on TSA, respectively. The working solution of the primer (100 µM) was prepared following the manufacturer's instructions. The PCR reaction mix was made by mixing 10 µL DreamTaq Hot Start Green PCR master mix (Thermo Fisher Scientific, USA) with 6 µL primer (10 µM forward and reverse), 2 µL Nuclease-free water and 2 µL of DNA in a PCR tube or a colony from TSA. The tubes were placed in the PCR machine and the PCR program with the PCR parameters (94.0 °C for 2 min, 30 cycles of PCR (95.0°C for 30 s, 60.0 °C for 30 s, 72.0 °C for 1 min) was performed.

To view the PCR products, a 2.0% agarose gel with 26 wells was made and each well was loaded with 10 µL of the PCR product. A 1 Kb DNA ladder (Thermo Fisher Scientific, USA) loaded into the first well. Gel electrophoresis was carried out at 130 V for 1 h before the gel was imaged (Alpha Imager HP, Alpha Innotech, USA)

Prior to reverse transcription, the isolated RNA samples were treated with DNase using the RQ1 RNase-Free DNase kit following the manufacture's protocol (Promega, USA). The RNA was reverse transcribed to cDNA using the QuantiTect Reverse Transcription kit following the quick start protocol (Qiagen, Germany) and stored at -20 °C prior to use in experiments.

Each qPCR reaction mixture consisted of 12.5 of QuantiFast Master Mix (2x) (Thermo Fisher Scientific, USA), 2.5 µL of 10 µM forward primer and reverse primer (Table 2.3), 1 µL undiluted cDNA and 6.5 µL of nuclease free water (Thermo Fisher Scientific, USA). For each biological replicate, two technical replicates were used for the real-time PCR reaction. The reaction mixture was loaded into a 96 well PCR reaction plate (Thermo Fisher Scientific, USA)

and the procedure for operating the 7500 fast QuantStudio Real-Time PCR System (Applied Biosystems, USA) was followed. In addition to the samples, negative controls with both RNA (after DNase treatment but prior to reverse transcription) or nuclease free water (instead of cDNA) were included. The plate was vortexed briefly and centrifuged (1000 x *g* for 10 s) before placing into the qPCR machine. The conditions for the real-time PCR reactions were: hold stage at 95.0 °C for 20 s, 40 cycles of PCR (95.0°C for 3 s, 60.0 °C for 1 min) and the melt curve stage (95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s) to confirm that the amplification was specific.

The comparative threshold method was used for the relative quantification of template RNA. For comparative (CT) calculations, normalization to the control conditions was done according to Pfaffl (2001). The formula used is as follows:

$\Delta CT = CT\ control - CT\ sample$

Normalization to the reference gene (16S rRNA gene) was done using the following formula:

$\Delta\Delta CT = \Delta CT\ target\ gene - \Delta CT\ reference\ gene$

The target genes are listed in Table 2.3, and the reference gene was 16S rRNA.

The $\log_2$ normalized relative gene expression was calculated using the formula below and visualized using a bar graph.

$\log_2 2^{-\Delta\Delta CT}$

2.8 DNA sequencing and SNP analysis

To determine the impact of carnocyclin A and carbohydrates on the genomes of the strains of *L. monocytogenes*, the resistant strains of *L. monocytogenes* isolated during this study were sequenced using Nanopore sequencing technology. DNA libraries were prepared with genomic DNA by the Nanopore Native Barcoding Kit 24 V14 (SQK-NBD114.24) and sequenced on the MinION platform using the R.10.4.1 flow cell. Reads underwent base calling in super-high accuracy mode by Guppy version 6.5.7 (Oxford Nanopore Technologies). The genome assembly was obtained by Flye, followed by annotation with Prokka, and polished by Medaka using wf-bacterial-genomics v0.3.1 provided by EPI2ME Labs (Oxford Nanopore Sequencing). The parent strains had been sequenced previously (Balay, 2019).

SNP analysis was performed to determine the number of SNPs between the parent (carnocyclin A sensitive) strains of *L. monocytogenes* and the resistant strains of *L. monocytogenes*. The variants were called by utilizing SNP-sites v2.51 from a pseudo whole-genome alignment produced by Snippy v4.6.0 (https://github.com/tseemann/snippy). For the analysis, only the SNPs were called (no deletions and insertions) and only the differences in ATGC were called (no Ns or other annotations in the genome alignment). Subsequently, the variant sites were transformed into a pairwise SNP matrix using SNP-dists v0.8.2 (https://github.com/tseemann/snp-dists).

2.9 Statistical Analysis:

All experiments were replicated three times. Data was analyzed using SAS (University Edition) where Analysis of Variance (ANOVA) was done with Tukey's Studentized Range test to determine differences among means. The significance value used was $P<0.05$**.**

Table 2.1 Strains of *L monocytogenes* used in this study (Fugett et al., 2006).

Strain designation	Lineage	Serotype	Origin
FSL R2-499	II	1/2a	Human isolate associated with US outbreak '00
FSL C1-056	II	1/2a	Isolated from human sporadic case '98
FSL J1-177	I	1/2b	Isolated from human sporadic case '97

Table 2.2 Codes for treatments and controls used to determine the impact of carnocyclin A on the growth of *L monocytogenes* in the presence of different carbohydrates in cooked ground beef.

Code	Strain of *L monocytogenes*	Carbohydrate	Presence of carnocyclin A
RFA	FSL R2-499	Fructose	carnocyclin A
RDA	FSL R2-499	Dextrose	carnocyclin A
RSA	FSL R2-499	Sucrose	carnocyclin A
RA	FSL R2-499	No carbohydrate	carnocyclin A
RF	FSL R2-499	Fructose	No carnocyclin A
RD	FSL R2-499	Dextrose	No carnocyclin A
RS	FSL R2-499	Sucrose	No carnocyclin A
R	FSL R2-499	No carbohydrate	No carnocyclin A
CFA	FSL C1-056	Fructose	carnocyclin A
CDA	FSL C1-056	Dextrose	carnocyclin A
CSA	FSL C1-056	Sucrose	carnocyclin A
CA	FSL C1-056	No carbohydrate	carnocyclin A
CF	FSL C1-056	Fructose	No carnocyclin A
CD	FSL C1-056	Dextrose	No carnocyclin A
CS	FSL C1-056	Sucrose	No carnocyclin A
C	FSL C1-056	No carbohydrate	No carnocyclin A
JFA	FSL J1-177	Fructose	carnocyclin A
JDA	FSL J1-177	Dextrose	carnocyclin A
JSA	FSL J1-177	Sucrose	carnocyclin A
JA	FSL J1-177	No carbohydrate	carnocyclin A
JF	FSL J1-177	Fructose	No carnocyclin A
JD	FSL J1-177	Dextrose	No carnocyclin A
JS	FSL J1-177	Sucrose	No carnocyclin A
J	FSL J1-177	No carbohydrate	No carnocyclin A
carnocyclin A	uninoculated	No carbohydrate	carnocyclin A
Cooked ground beef	uninoculated	No carbohydrate	No carnocyclin A

Table 2.3 Primers of *L monocytogenes* and amplicon sizes for qPCR reactions

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Amplicon size	Reference
16S rRNA (Housekeeping gene)	TTAGCTAGTT GGTAGGGT	AATCCGGACA ACGCTTGC	550	(Tasara and Stephan, 2007)
PTS mannose transporter subunit IIA	AAGGTGGCAC TCCGTTTAAT	CGACTGAGGA ACGCTTCTAAT	109	This study
PTS mannose transporter subunit IIB	CGAATCGGTTG AAGACGCTTA	GGCTGTTCCTT CTCTTTGCT	97	This study
PTS fructose transporter subunit IIA	TGGTGGAACT CCGTGTAATG	TCTCCAAGGCT AAGTGGAATG	108	This study
PTS beta-glucoside transporter subunit IIABC	TCCAGGTCTTG TCGGAGATA	GGCGTTGCTTC TACCATTTG	120	This study
Sucrose phosphorylase	GTCTGAGAACT TCCGAGTGATG	TGTACGGCGC TTCGTTATC	98	This study

3 Results

3.1 Activity of carnocyclin A

To determine the activity of carnocyclin A against *L. monocytogenes* R2-499, *L. monocytogenes* C1-056, and *L. monocytogenes* J1-177, spot-on-lawn antibacterial assays were performed. Carnocyclin A had the lowest activity against *L. monocytogenes* R2-499 at 3,200 activity units (AU)/mL, followed by 8,533 AU/mL for *L. monocytogenes* C1-056. Carnocyclin A had the highest activity against *L. monocytogenes* J1-177, at 10,667 AU/mL.

3.2 Impact of carnocyclin A on the growth of *L monocytogenes* in the presence of different carbohydrates in cooked ground beef

The growth curves for each strain of *L. monocytogenes* in cooked ground beef supplemented with either 2% fructose, dextrose or sucrose and in the presence or absence of 50 µL of carnocyclin A are shown in Figures 3.1, 3.2 and 3.3 for each of the three strains. For the control samples of carnocyclin A and cooked ground beef, counts on TSA and PALCAM agar counts remained below the detection limit (1 log CFU/g meat) for all of the sampling days (days 0 to 80) throughout the experiment.

At all sampling times, the presence of carnocyclin A significantly decreased the cell counts of *L. monocytogenes* R2-499 (Figure 3.1) compared to the strain grown without carnocyclin A. When *L. monocytogenes* R2-499 was exposed to carnocyclin A, the number cells decreased initially by 1 log compared to samples without addition of carnocyclin A and by 3 days of storage counts were approximately 2 log CFU/g lower than the samples without carnocyclin A at 0 days of storage. In the presence of carnocyclin A, the growth of *L. monocytogenes* R2-499 was inhibited until 7 d of storage, regardless of the presence of the different carbohydrates. After 16 d of storage, in the presence of carnocyclin A, the counts for the control with no carbohydrate

added were significantly higher ($P<0.05$) than the counts for the sample with added sucrose. After 30 d of storage, in the presence of carnocyclin A, the counts were significantly lower ($P<0.05$) in samples supplemented with dextrose as compared to the control with no carbohydrate added.

Carnocyclin A significantly decreased the growth of *L. monocytogenes* CL-056 in cooked ground beef throughout storage (Figure 3.2) compared to growth in samples without carnocyclin A. When *L. monocytogenes* CL-056 was exposed to carnocyclin A, the number cells decreased initially by 1 log compared to samples without addition of carnocyclin A and by 7 days of storage counts were approximately 3 log CFU/g lower than the samples without carnocyclin A at 0 days of storage. The growth of *L. monocytogenes* CL-056 was inhibited by carnocyclin A until after 7 d of storage. There were no significant differences in cell counts among cooked ground beef samples with carnocyclin A that were supplemented with fructose, dextrose and sucrose.

Carnocyclin A significantly decreased the growth of *L. monocytogenes* J1-177 in cooked ground beef at all storage times (Figure 3.3). When *L. monocytogenes* J1-177 was exposed to carnocyclin A, the number cells decreased initially by 1 log compared to samples without addition of carnocyclin A and by 7 days of storage counts were approximately 4 log CFU/g lower than the counts for samples without carnocyclin A at 0 days of storage. Throughout storage, there was high variability among the three replicates in the survival of *L. monocytogenes* J1-177 in the presence of carnocyclin A. At each sampling day after 0 d of storage, counts for some replicates were below the limit of detection by enrichment, while enumeration was possible with other samples. The data in Figure 3.3 for samples grown in the presence of carnocyclin A are either single data points from one replicate or means of two or three replicates. Table 3.1

shows the number of samples at each sampling time that were below the limit of detection by enrichment. The variability in the ability to enumerate samples inoculated with *L. monocytogenes* J1-177 makes it difficult to assess the impact of carbohydrates on the growth in the presence of carnocyclin A.

There was a significant difference ($P<0.05$) among the cell counts of *L. monocytogenes* R2-499, *L. monocytogenes* CL-056 and *L. monocytogenes* J1-177 strains after 3 days of storage, where carnocyclin A had a limited impact on the growth of *L. monocytogenes* R2-499, followed by *L. monocytogenes* CL-056 and the greatest impact on the growth of *L. monocytogenes* J1-177.

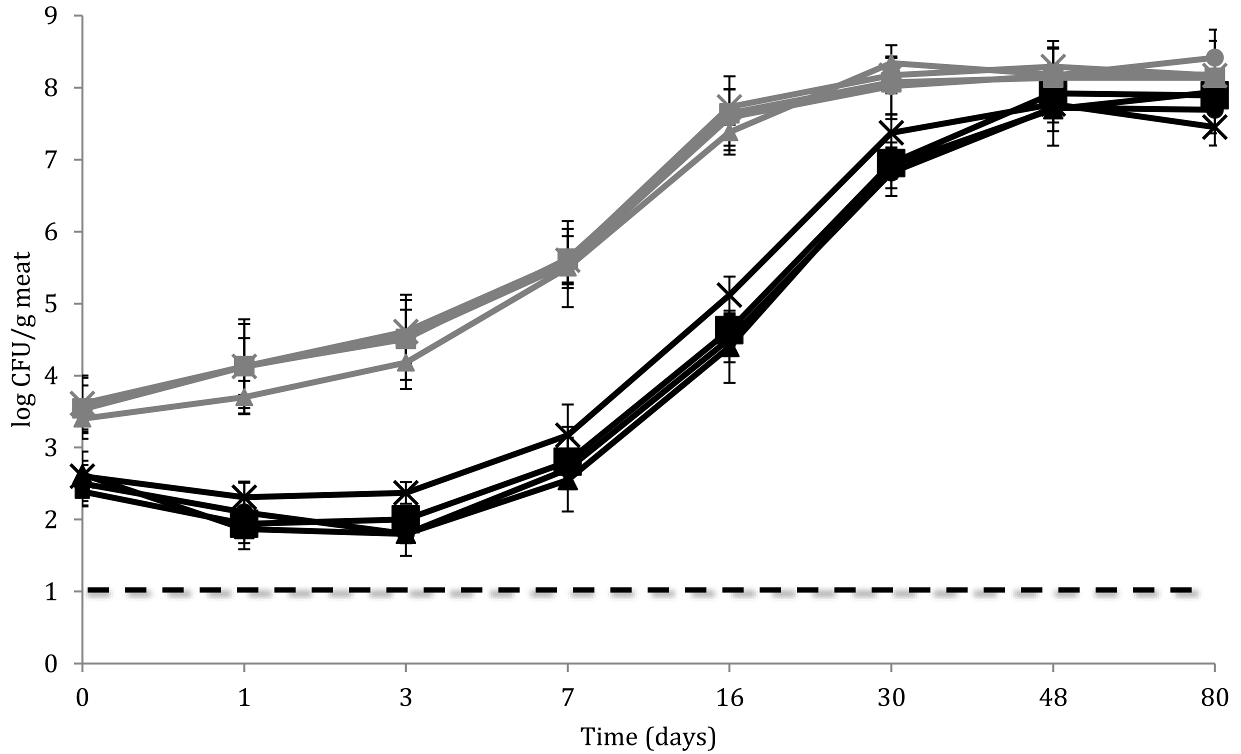

Figure 3.1 Mean cell counts of *L. monocytogenes* R2-499 in the presence of different carbohydrates in cooked ground beef with and without the addition of carnocyclin A stored at 4 °C. Counts were determined on tryptic soy agar. Grey lines and symbols are cell counts for *L. monocytogenes* R2-499 grown in cooked ground beef without carnocyclin A and supplemented with fructose (■), dextrose (●), sucrose (▲), or no carbohydrate added (X). Black lines and symbols are cell counts for *L. monocytogenes* R2-499 in cooked ground beef with carnocyclin A supplemented with fructose (■), dextrose (●), sucrose (▲), or no carbohydrate added (X). The data are the means of counts for three replicated experiments and the error bars are the standard deviations. Significance among treatments at individual sampling times are indicated by an *. 1 log CFU/ g meat is the limit of quantification (dashed line) and data points at zero are below the limit of detection by enrichment.

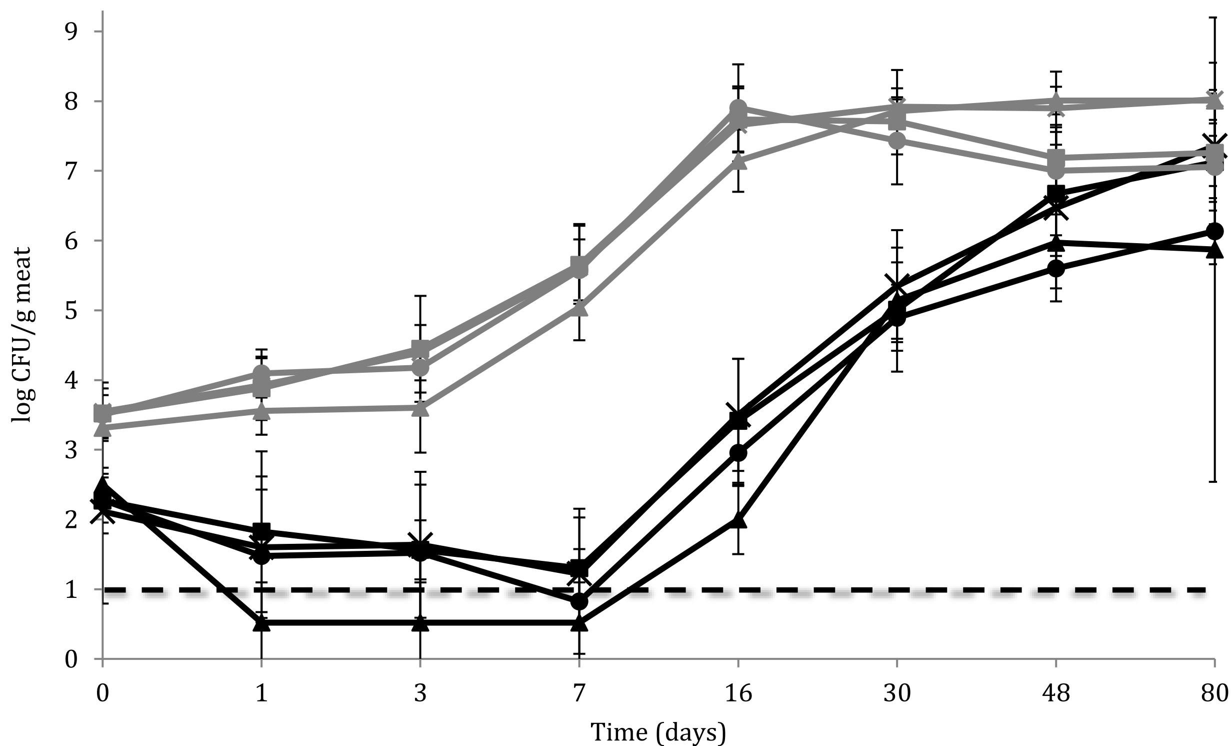

Figure 3.2 Mean cell counts of *L. monocytogenes* C1-056 in the presence of different carbohydrates in cooked ground beef with and without the addition of carnocyclin A stored at 4 °C. Counts were determined on tryptic soy agar. Grey lines and symbols are cell counts for *L. monocytogenes* C1-056 grown in cooked ground beef without carnocyclin A and supplemented with fructose (■), dextrose (●), sucrose (▲), or no carbohydrate added (X). Black lines and symbols are cell counts for *L. monocytogenes* C1-056 in cooked ground beef with carnocyclin A supplemented with fructose (■), dextrose (●), sucrose (▲), or no carbohydrate added (X). The data are the means of counts for three replicated experiments and the error bars are the standard deviations. Significance among treatments at individual sampling times are indicated by an *. 1 log CFU/ g meat is the limit of quantification (dashed line) and data points at zero are below the limit of detection by enrichment.

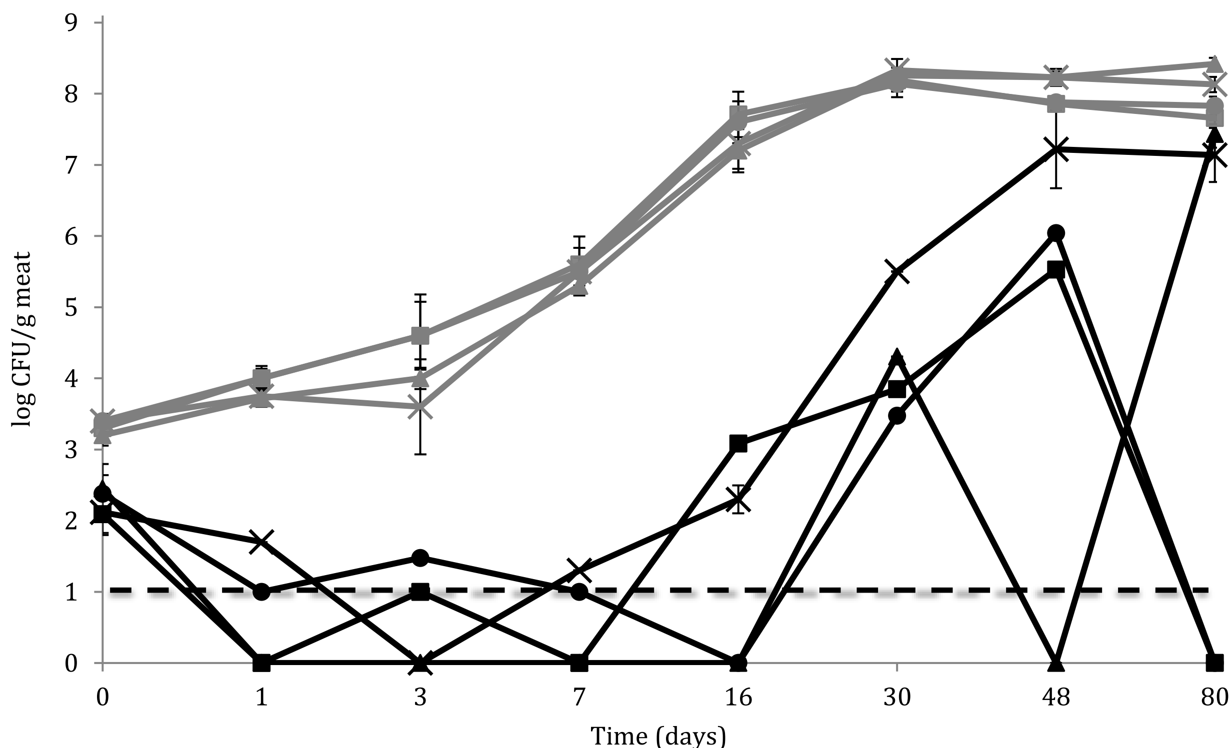

Figure 3.3 Mean cell counts of *L. monocytogenes* J1-177 in the presence of different carbohydrates in cooked ground beef with and without the addition of carnocyclin A stored at 4 °C. Counts were determined on tryptic soy agar. Grey lines and symbols are cell counts for *L. monocytogenes* J1-177 grown in cooked ground beef without carnocyclin A and supplemented with fructose (■), dextrose (●), sucrose (▲), or no carbohydrate added (X). Black lines and symbols are cell counts for *L. monocytogenes* J1-177 in cooked ground beef with carnocyclin A supplemented with fructose (■), dextrose (●), sucrose (▲), or no carbohydrate added (**X**). The data are the means of counts for three replicated experiments and the error bars are the standard deviations. If no error bars are present, the data represents cell counts from one replicate or means of two replicates. Table 3.1 shows the number of samples at each sampling time that were below the limit of detection by enrichment. Significance among treatments at individual sampling times are indicated by an *. 1 log CFU/ g meat is the limit of quantification (dashed line) and data points at zero are below the limit of detection by enrichment.

Table 3.1 Number of replicates at each sampling time where counts of *L. monocytogenes* were below the limit of detection by enrichment.

Sampling time (d)	Number of replicates where samples were below limit of detection by enrichment			
	Carbohydrate			
	None	Dextrose	Fructose	Sucrose
1	2	1	3	3
3	3	1	2	3
7	2	2	3	3
16	0	3	1	3
30	1	1	1	1
48	0	2	1	3
80	0	3	3	0

3.3 Identification of resistant strains of *L monocytogenes* to carnocycin A

To determine if the three strains of *L monocytogenes* became resistant to carnocyclin A during growth in ground beef supplemented with different carbohydrates, a total of 48 isolates collected from TSA plates from cooked ground beef samples stored for 16, 30, 48 or 80 d. Isolates were grown in basal media, with either fructose, dextrose or sucrose, in the presence or absence of

carnocyclin A and their growth curves were analyzed. For *L. monocytogenes* J1-77, 3 resistant isolates were obtained, one isolate each from samples of cooked ground beef with each of the three carbohydrates. For *L. monocytogenes* C1-06, one isolate from cooked ground beef supplemented with sucrose was resistant to carnocyclin A and none of the strains of *L. monocytogenes* R2-499 collected from stored cooked ground beef were resistant to carnocyclin A.

To determine if isolates from the cooked ground beef had similar growth patterns, isolates from the cooked ground beef that were resistant or sensitive to carnocyclin A and their parent strains were grown for 40 h in basal media supplemented with carbohydrates.

Isolates of *L. monocytogenes* J1-117 retrieved from cooked ground beef and grown in basal media with carnocyclin A and either fructose or dextrose reached maximum population after 30 h of growth (Figure 3.4). The isolates of *L. monocytogenes* J1-177 and *L. monocytogenes* C1-056 that were isolated from ground beef supplemented with sucrose and grown in basal media with sucrose reached maximum population after18 h of growth (Figure 3.4). In contrast, the parent strains of *L. monocytogenes* J1-177 and C1-056 and an isolate of *L. monocytogenes* FSL R2-499 grown in sucrose had similar growth patterns and didn't reach maximum population until after 35 h of growth (Figure 3.4). The parent strains of *L. monocytogenes* J1-177 grown in basal media supplemented with fructose or dextrose were not able to grow in the presence of carnocyclin A (Figure 3.4). The isolate of *L. monocytogenes* R2-499 obtained from the cooked ground beef was labelled as sensitive to carnocyclin A (CSAs). The isolates of *L. monocytogenes* J1-177 and C1-056 that were able to grow in the presence of carnocyclin A were labelled as resistant.

Isolates of *L. monocytogenes* J1-177 (JSA) and C1-056 (CSA) grown in basal media without carnocyclin A supplemented sucrose with reached maximum population between 15 to 22 h of growth (Figure 3.5). In basal media supplemented with sucrose the parent strain of C1-056 reached maximum population at approximately 22 h of growth whereas the parent strain of J1-177 didn't reach maximum population until after 30 h of growth. The parent strain and isolate of *L. monocytogenes* R4-99 reached a maximum population after 18 h when grown in sucrose but the isolate had a much lower cell density than the parent strain. Isolates of *L. monocytogenes* J1-177 grown in either fructose or dextrose didn't reach a maximum population until approximately 25 h of growth (Figure 3.5) whereas their parent strains grew slowly and never reached a maximum population after 40 h of growth (Figure 3.5).

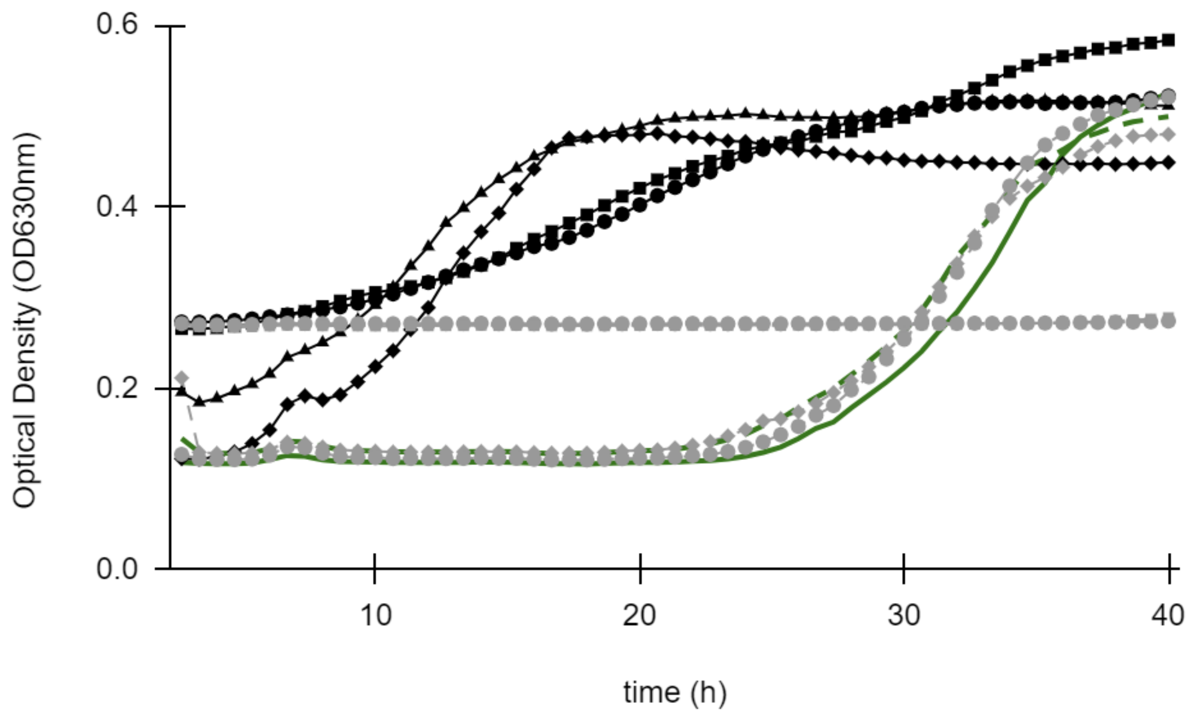

Figure 3.4 Growth of resistant strains of *L. monocytogenes* J1-177 (grown with fructose ●, dextrose ■, sucrose ◆), and *L. monocytogenes* C1-056 (grown with sucrose ▲), carnocyclin A sensitive *L monocytogenes* FSL R2-499 (grown with sucrose —) isolated from cooked ground beef and their parent strains (grey symbols or dashed green line) in basal media with carnocyclin A and either fructose, dextrose or sucrose. The OD_{630nm} was measured every 40 min for 40 h during incubation at 25°C (n=3).

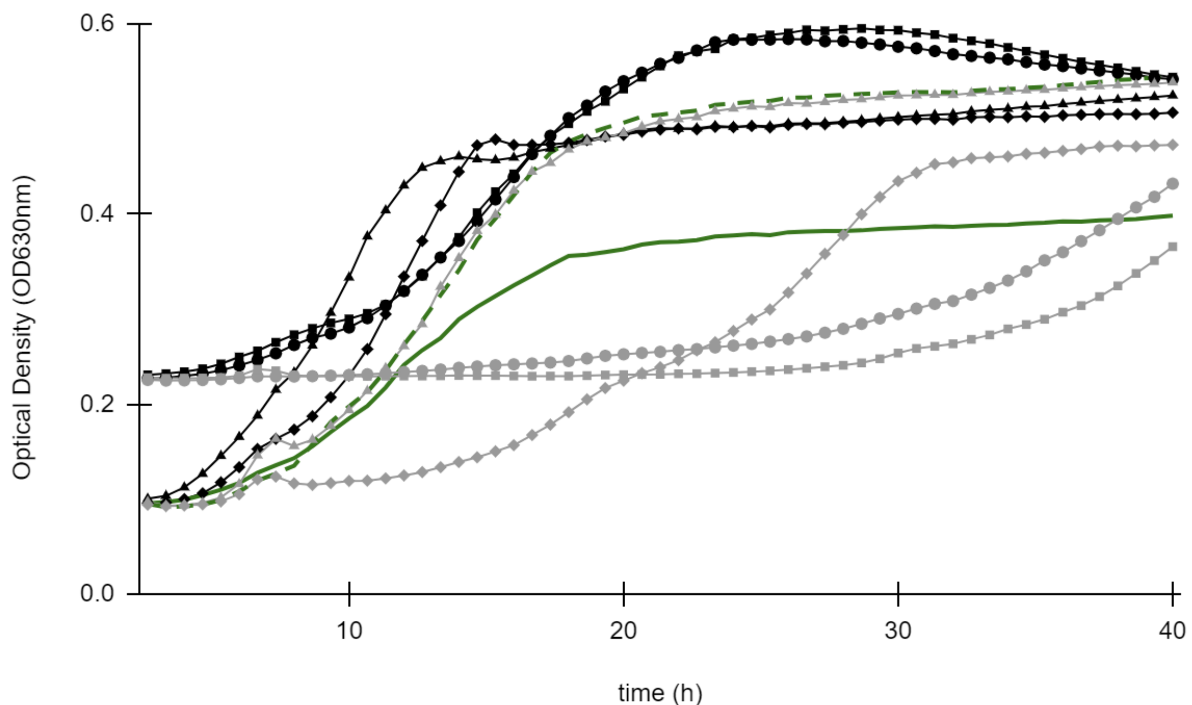

Figure 3.5 Growth of resistant strains of *L. monocytogenes* J1-177 (grown with fructose ●, dextrose ■, sucrose ◆), and *L. monocytogenes* C1-056 (grown with sucrose ▲), carnocyclin A sensitive *L monocytogenes* FSL R2-499 (grown with sucrose —) isolated from cooked ground beef and their parent strains (grey symbols or dashed green line) in basal media without carnocyclin A and either fructose, dextrose or sucrose. The OD_{630nm} was measured every 40 min for 40 h during incubation at 25°C (n=3).

In the presence of carnocyclin A, resistant isolates (JFAr, JDAr JSAr and CSAr) had a shorter lag phase compared to the parent strains of *L. monocytogenes* FSL C1-056 and FSL J1-177 (Table 3.2). Resistant isolate CSAr had the shortest lag phase, followed by JSAr and then JFAr and JDAr. In the absence of carnocyclin A, the lag phase of the parent strains varied depending on the carbohydrate the strains had been grown in. Strains grown in the presence of sucrose had a much shorter lag phase than parent strains growth in fructose of dextrose. This difference was not as apparent in the resistant isolates where the length of the lag phase for each of the strains was similar (Table 3.2).

Table 3.2 Lag phase (h) of parent and resistant strains of *L. monocytogenes* isolated from cooked ground beef grown in basal media with or without carnocyclin A. Lag phase was estimated from growth curves in Figures 3.4 and 3.5.

Strain of *L, monocytogenes*	Carbohydrate	Lag phase (h)	
		Parent strain	Resistant isolate
Grown with carnocyclin A			
J1-177	Fructose	22	9
J1-177	Dextrose	22	9
J1-177	Sucrose	22	5
C1-056	Sucrose	22.5	4
Growth without carnocyclin A			
J1-177	Fructose	15	6
J1-177	Dextrose	25	5
J1-177	Sucrose	9	5
C1-056	Sucrose	7	3

3.4 Quantification and detection of glucose in ground beef

The glucose present in the cooked ground beef used in the growth experiment was quantified using the Glucose Colorimetric Detection Kit (Thermo Fisher Scientific, USA). The amount of glucose present in the cooked ground beef was 0.651g/L and this was added to the basal media to determine if there was an impact on gene expression.

3.5 Quantity and quality of isolated RNA and DNA

Cultures of *L. monocytogenes* were harvested in the exponential growth phase for RNA isolation. All samples had A260/A280 ratios in the range of 1.69 to 2.25 and A260/A230 ratios between 0.52 and 1.83 (Table 3.3).

Table 3.3 Mean and standard deviation for the quantity and quality of RNA isolated from the resistant strains grown in basal media with carbohydrate added to an OD_{600} to 0.5 to 0.6 nm (n=3).

Isolate and carbohydrate (sample code)	Quantity (ng/µL)	A_{260}/A_{280}	A_{260}/A_{230}
J1-177 Fructose (JFAr)	36.7 ± 0.84	1.79 ± 0.16	0.97± 0.64
J1-177 Dextrose (JDAr)	24.35 ± 9.26	1.98 ± 0.19	1.0 ± 0.55
J1-177 Sucrose (JSAr)	42.53 ± 15.68	1.86 ± 0.03	0.90 ± 0.22
C1-056 Sucrose (CSAr)	48.5 ± 19.51	1.84 ± 0.13	1.11 ± 0.59

Cultures of *L. monocytogenes* grown overnight were harvested in the stationary phase for DNA isolation. All samples had A260/A280 ratios greater or equal to 1.80 and A260/A230 ratios between 1.84 and 2.17 (Table 3.4).

Table 3.4 Quantity and quality of DNA isolated from strains grown in in TSB with carbohydrate added to an OD_{600} to 0.5 to 0.6 nm (n=3).

Isolate and carbohydrate (sample code)	Quantity (ng/µL)	A_{260}/A_{280}	A_{260}/A_{230}
J1-177 Fructose (JFAr)	176.4	1.83	1.90
C1-056 Sucrose (CSAr)	106.8	1.83	2.17
J1-177 Dextrose (JDAr)	133.5	1.80	1.95
J1-177 Sucrose (JSAr)	151.7	1.82	2.10
R2-499 Sucrose (RSAs)	154.1	1.80	1.84

3.6 Primer testing and colony PCR

PCR was performed to confirm that the primers designed were specific to the strains of *L. monocytogenes* prior to qPCR. The PCR products (Figure 3.6) were approximately 100 bp, similar to the amplicon size of the primers tested (Table 2.3).

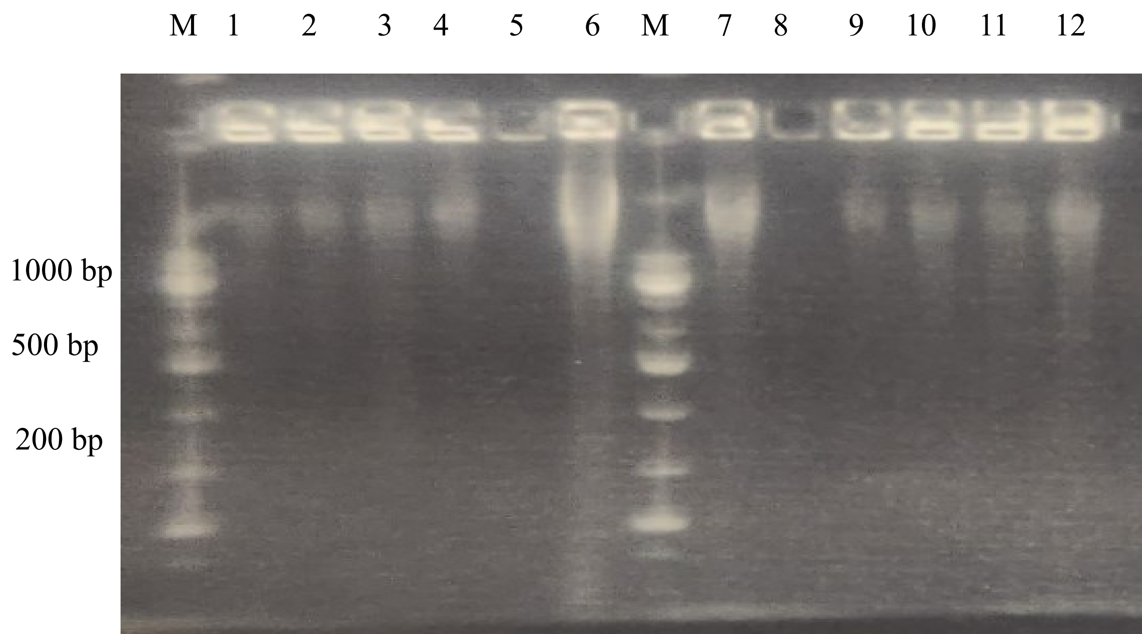

Figure 3.6. Detection of PCR products for primers for the 16S rRNA (lane 1, 7), PTS mannose transporter subunit IIA (lane 2, 8), PTS mannose transporter subunit IIB (lane 3, 9), PTS fructose transporter subunit IIA (lane 4, 10) sucrose phosphorylase (lane 5, 11), and PTS β-glucoside transporter subunit IIABC (lane 6, 12) amplified from *L. monocytogenes* C1-056 with DNA that was either reverse transcribed from isolated RNA (Lanes 1 to 6) or obtained from a colony (7 to 12). A 1 Kb DNA ladder loaded into the wells labelled M. Gel electrophoresis was done at 130 V for 1 h on a 2.0% agarose gel before the gel was imaged.

3.7 Reverse transcription -qPCR and gene expression

3.7.1 Gene expression of resistant isolates of *L. monocytogenes*

Reverse transcription -qPCR was performed to determine the gene expression of resistant isolates of *L. monocytogenes* (JFAr, JDAr, JSAr and CSAr) for genes of interest (Table 2.3). The genes of interest were the PTS mannose transporter subunit IIA, PTS mannose transporter subunit IIB, PTS fructose transporter subunit IIA, sucrose phosphorylase and the PTS β-glucoside transporter subunit IIABC. These were selected based on the work of D. Balay (2019) who reported that these

genes were either up or down regulated when strains were grown in the presence of different carbohydrates.

For the resistant isolate JFAr, there was no significant difference in the gene expression levels among all five of the genes tested (Figure 3.7). For JDAr, the PTS mannose transporter subunit IIA and subunit IIB gene expression was downregulated (Figure 3.8). There was significant upregulation in the PTS mannose transporter subunit IIB and the sucrose phosphorylase gene in the JSAr isolate (Figure 3.9). For the CSAr isolate, there was significant differences in gene expression of the PTS mannose transporter subunit IIA and the PTS β-glucoside transporter subunit IIABC where upregulation of the genes was detected (Figure 3.10).

3.7.2 Impact of glucose in ground beef on gene expression

Reverse transcription -qPCR was also done to determine if the level of glucose in ground beef impacted the expression of genes involved in resistance to carnocyclin A. There was no significant difference in the gene expression level of the five genes that were tested (PTS mannose transporter subunit IIA, PTS mannose transporter subunit IIB, PTS fructose transporter subunit IIA, sucrose phosphorylase and the PTS β-glucoside transporter subunit IIABC) when 0.651g/L of glucose was added to basal media.

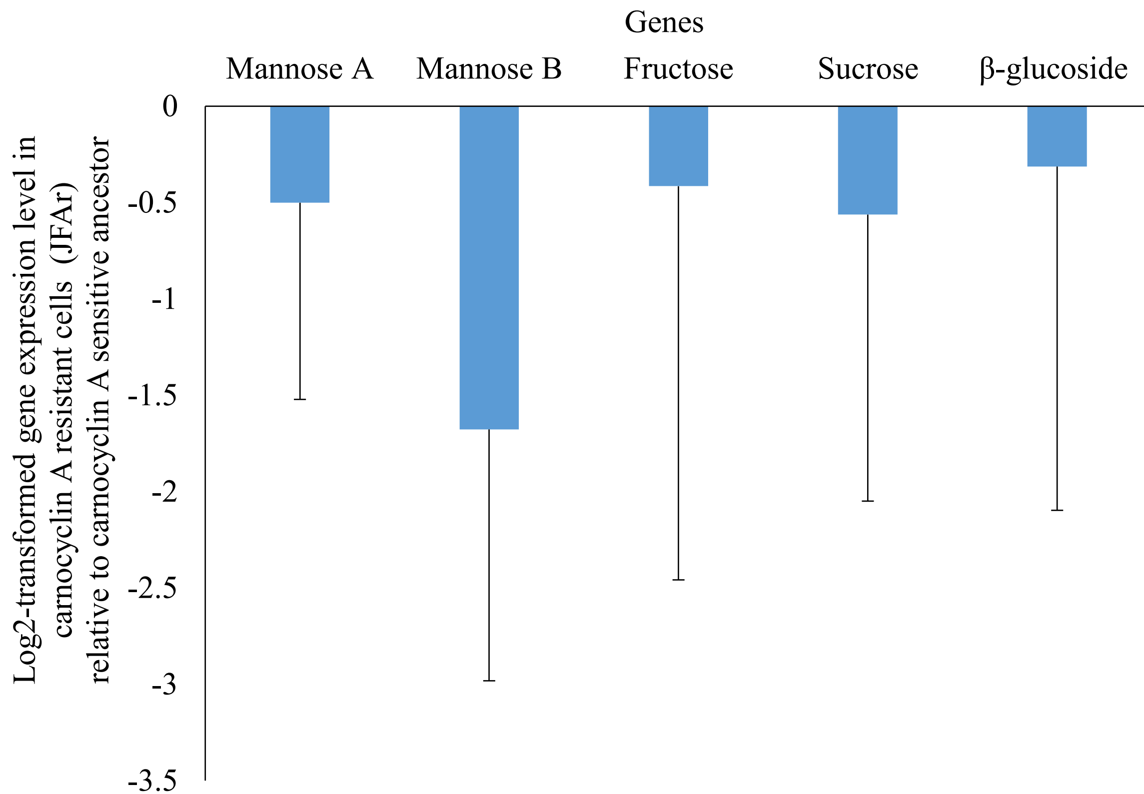

Figure 3.7 Relative fold expression of the PTS mannose A subunits (mannose A and B), PTS fructose transporter subunit IIA (Fructose), sucrose phosphorylase (Sucrose), and β-glucoside transporter subunit IIABC (β-glucoside) genes in carnocyclin A resistant strains of *L monocytogenes* J1-117 (JFA) grown in fructose relative to the carnocyclin A sensitive parent strain. The bars represent the mean gene expression levels and the error bars represent standard deviation for three replicated experiments. *L monocytogenes*.

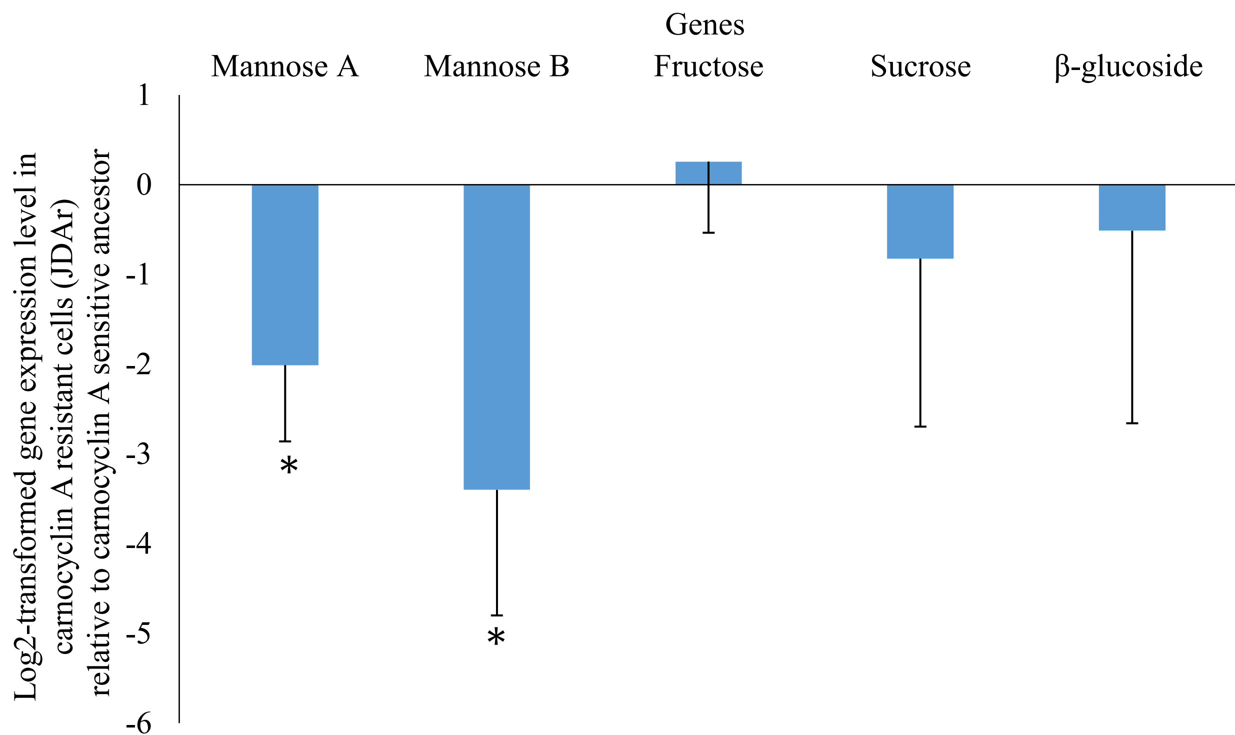

Figure 3.8 Relative fold expression of the PTS mannose A subunits (mannose A and B), PTS fructose transporter subunit IIA (Fructose), sucrose phosphorylase (Sucrose), and β-glucoside transporter subunit IIABC (β-glucoside) genes in carnocyclin A resistant strains of *L monocytogenes* J1-177 (JDAr) grown in dextrose relative to the carnocyclin A sensitive parent strain. The bars represent the mean gene expression levels and the error bars represent standard deviation for three replicated experiments. *L monocytogenes*. Significance is represented by * and was determined using an unpaired t-test with $P<0.05$.

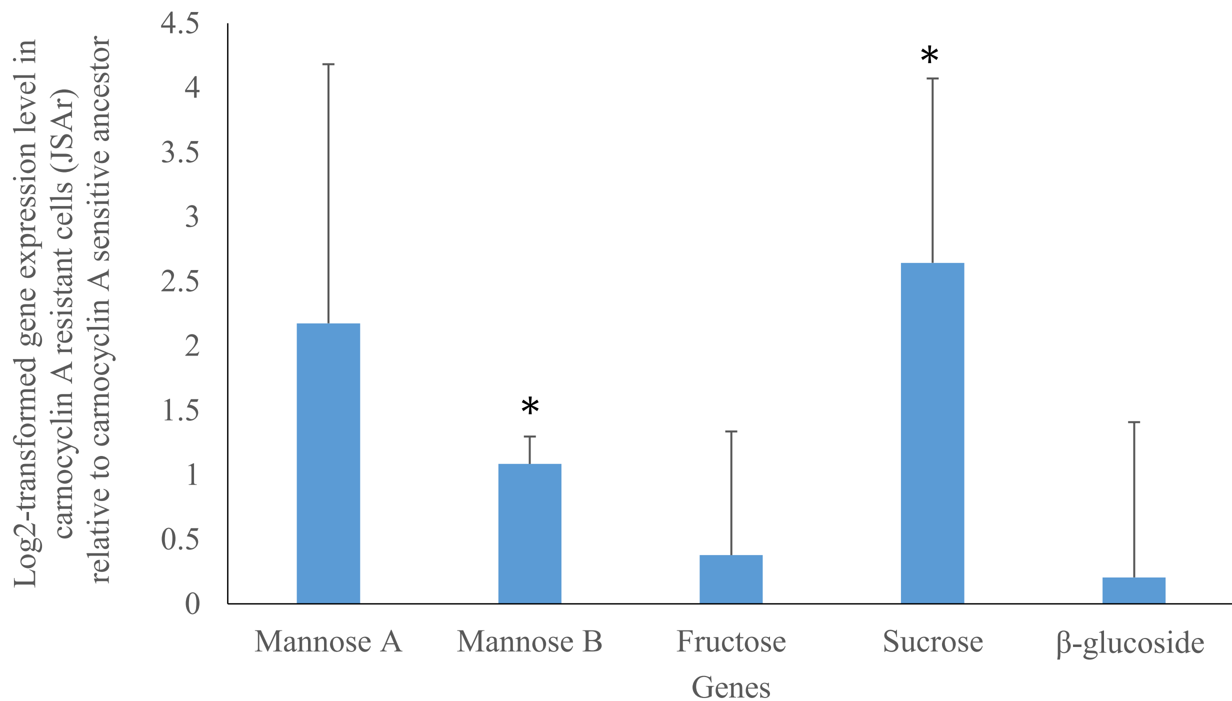

Figure 3.9 Relative fold expression of the PTS mannose A subunits (mannose A and B), PTS fructose transporter subunit IIA (Fructose), sucrose phosphorylase (Sucrose), and β-glucoside transporter subunit IIABC (β-glucoside) genes in carnocyclin A resistant strains of *L monocytogenes* J1-177 grown in sucrose (JSAr) relative to the carnocyclin A parent strain (JS). The bars represent the mean gene expression levels and the error bars represent standard deviation for three replicated experiments. *L monocytogenes*. Significance is represented by * and was determined using an unpaired t-test with $P<0.05$.

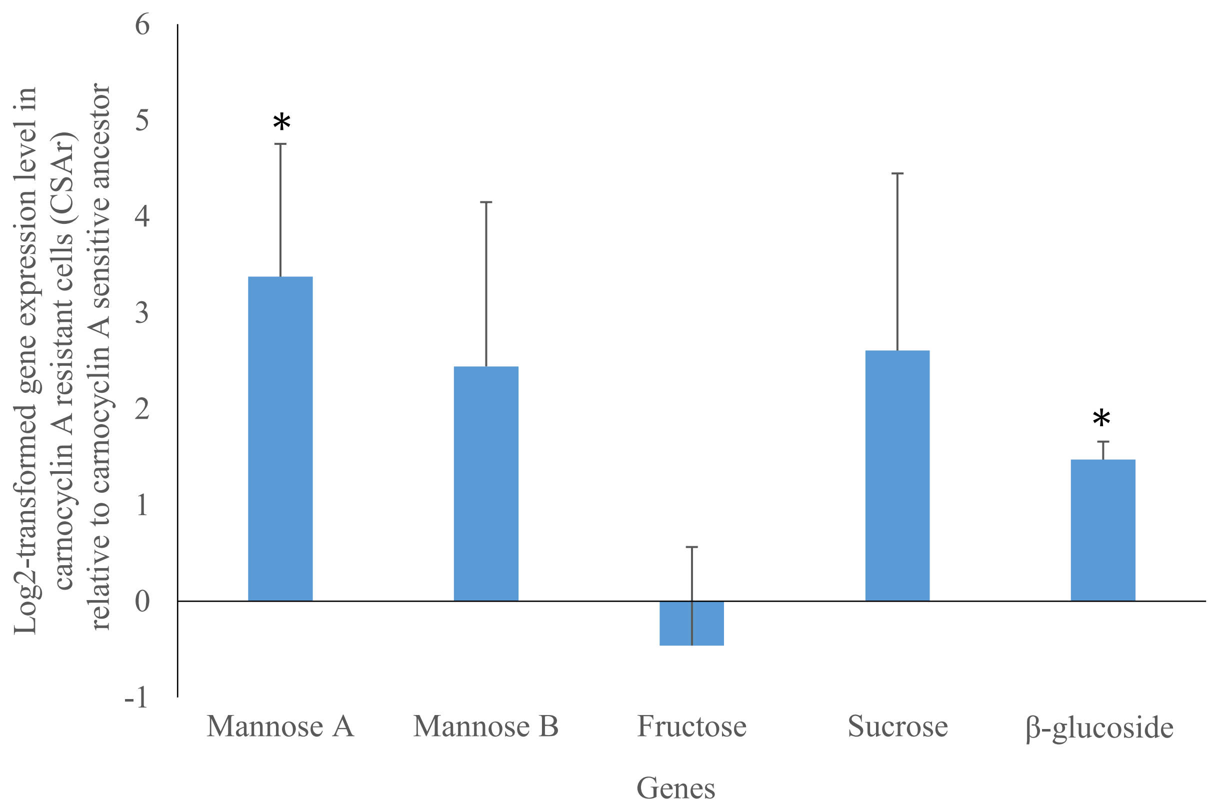

Figure 3.10 Relative fold expression of the PTS mannose A subunits (mannose A and B), PTS fructose transporter subunit IIA (Fructose), sucrose phosphorylase (Sucrose), and β-glucoside transporter subunit IIABC (β-glucoside) genes in carnocyclin A resistant strain of *L monocytogenes* (CSAr) relative to the carnocyclin A sensitive parent strain. The bars represent the mean gene expression levels and the error bars represent standard deviation for three replicated experiments. *L monocytogenes*. Significance is represented by * and was determined using an unpaired t-test with $P<0.05$.

3.8 Differences in single nucleotide polymorphisms

To determine the number of SNPs between the parent (carnocyclin A sensitive) strains of *L. monocytogenes* and the resistant strains of *L. monocytogenes* the genomes were either sequenced or their sequences were obtained from the laboratory database. The sensitive *L. monocytogenes* R2-499 isolated from meat with sucrose (RSA) had 1 SNP difference from the parent strain. The resistant strains of *L. monocytogenes* C1-056 (CSAr) and *L. monocytogenes* J1-177 JDAr, JFAr and JSAr had 41, 88,608, 1 and 1 SNPs, respectively.

For the sensitive *L. monocytogenes* isolated from meat with sucrose (RSA), there was 1 SNP in the UDP-N-acetylenolpyruvoylglucosamine reductase (EC 1.1.1.158) (synonymous variant), and 1 deletion resulting in a frameshift variant in the PTS system, mannose-specific IID component. For the resistant C1-056 (CSAr), there were no SNPs related to sugar transport systems in the 41 SNPs. But there were SNPs in Internalin A (synonymous variant), Multidrug resistance ABC transporter ATP-binding and permease protein, and drug resistance transporter, Bcr/CflA family. For the resistant J1-177 (JFAr), there was 1 SNP in a hypothetical protein (SAV1845) and 2 frameshift variants in Cobyrinic acid A, C-diamide synthase and NtrC family Transcriptional regulator, ATPase domain. For the resistant J1-177 (JSAr), there was also 1 SNP in a hypothetical protein (SAV1845) and 2 frameshift variants in Cobyrinic acid A,C-diamide synthase and NtrC family Transcriptional regulator, ATPase domain.

For the resistant *L. monocytogenes* J1-177 (JDAr), which had 88,608 SNPs, there were SNPs relating to some of the sugar transport systems such as the cellobiose phosphotransferase system YdjC-like protein, oligopeptide ABC transporter, periplasmic oligopeptide-binding protein

OppA, oligopeptide transport system permease protein OppC, putative regulator of the mannose operon, mannose specific IIA, IIB, IIC and IID component PTS system, cellobiose-specific IIC component, putative sucrose phosphorylase, PTS system, mannitol-specific IIB and IIC component, PTS system, fructose-specific IIB and IIC components/PTS system, and PTS system, glucose-specific IIA component.

There were also SNPs in cell wall-binding protein, Lipid A export ATP-binding/permease protein MsbA, Listeria antigen LmaA, Listeria protein LmaB, LmaC and LmaD (associated with virulence), drug resistance transporter (Bcr/CflA family), multidrug resistance ABC transporter ATP-binding and permease protein, HPr kinase/phosphorylase and RNA polymerase sigma-54 factor RpoN, sigma factor RpoD and sigma factor SigB.

Table 3.5 Comparison of SNPs between carnocyclin A sensitive parent strains and the resistant strains of *L. monocytogenes* isolated after storage in cooked ground beef. The variants were called by utilizing SNP-sites v2.51 from a pseudo whole-genome alignment produced by Snippy v4.6.0.

Strain comparison	Number of SNPs
JDAr vs J1-177	88,608
CSAr vs C1-056	41
RSA vs R2-499	1
JFAr vs J1-177	1
JSA vs J1-177	1

4 Discussion

Carnocyclin A was least active against *L. monocytogenes* FSL R2-499 (the strain is most resistant), followed by *L. monocytogenes* FSL C1-056, and then *L. monocytogenes* FSL J1-177 (most sensitive to carnocyclin A). This is similar to what Balay et al (2018) reported previously with the bacteriocin leucocin A, where *L. monocytogenes* FSL R2-499 was the most resistant strain, followed by *L. monocytogenes* FSL C1-056 and being the least resistant (most sensitive) strain. This indicates that there is variability among strains of *L. monocytogenes* in their sensitivity to carnocyclin A (Balay et al., 2018). This phenomenon has also been reported for other bacteriocins (Ennahar and Deschamps, 2000; Schöbitz et al., 2003).

The presence of carnocyclin A in cooked ground beef lengthened the lag phase of *L. monocytogenes* in a strain dependent manner and depended on the carbohydrate that was present. This demonstrates that the resistance of *L. monocytogenes* to carnocyclin A is dependent on both the strain as well as the carbohydrate source present. Previous research by Balay et al demonstrated that resistance of *L. monocytogenes* to carnocyclin A (in broth media) is dependent on the strain and the carbohydrate present, and that the impact of the specific carbohydrate was found to be slightly different as the result showed sucrose increased the resistance of *L. monocytogenes* R2-499 to carnocyclin A (Balay et al., 2018).

Overall, the strains of *L. monocytogenes* were more resistant to carnocyclin A when there was no carbohydrate present than when carbohydrates (fructose, dextrose and sucrose) were present. This would seem to support the idea that if sugar transport systems are used as docking molecules for carnocyclin A, then the presence of different carbohydrates would impact the resistance of *L. monocytogenes* to carnocyclin A differently. For example, if carnocyclin A utilized the Mannose PTS system (which transports fructose and glucose) as a docking molecule,

then the presence of fructose or glucose (dextrose) could increase the sensitivity of *L. monocytogenes* to carnocyclin A. However, there is a lack of data regarding specific docking molecules for carnocyclin A in *L. monocytogenes*. Others have reported variation in growth rates of *L. monocytogenes* in the presence of carnocyclin A with different carbohydrates in broth systems (Balay et al., 2018).A similar observation was made for the impact of carbohydrates on the growth of *L. monocytogenes* that is resistant to pediocin and sakacin P. Pediocin resistant *L. monocytogenes* grows significantly slower in glucose, mannose and cellobiose (Liu et al., 2023) and sakacin resistant *L. monocytogenes* grows slower in the presence of glucose and mannose (Tessema et al., 2011).

Although carnocyclin A was able to significantly reduce and inhibit the growth of all three strains of *L. monocytogenes* after refrigerated storage in cooked ground beef, all three strains were able to grow with prolonged storage times with the exception of *L. monocytogenes* J1-177 supplemented with sucrose after 48 d of storage, or in cooked ground beef supplemented with dextrose and fructose after 80 d of storage. This may suggest that if carnocyclin A is used to inhibit strains of *L. monocytogenes* in RTE meats that have a long storage life, the growth during storage should be taken into consideration to utilize the bacteriocin in conjunction with other antimicrobials or technologies (hurdle concept) to prevent the re-growth of *L. monocytogenes* in the food product (Castellano et al., 2018). By combining carnocyclin A with another technology or antimicrobial, this could potentially lessen the concern of *L. monocytogenes* becoming resistant to carnocyclin A during the prolonged storage time of ready to eat meat products.

Isolates of *L. monocytogenes* recovered from the cooked ground beef did become resistant to carnocyclin A as indicated by the shorter lag phase when compared to the parent strains of *L.*

monocytogenes. Balay et al (2018) reported in that strains of *L. monocytogenes* that were resistant to carnocyclin A maintained their resistant phenotype. The carnocyclin A resistant strains isolated in the current study also maintained their resistant phenotype.

For the transport of carbohydrates in *L. monocytogenes*, seven PTS (phosphoenolpyruvate-dependent phosphotransferase systems) have been identified (Stoll and Goebel, 2010). PTS is made up of sugar specific enzyme II complexes and 2 phosphotransferase proteins, and the enzyme II complexes consists of 4 protein subunits (subunits IIA, IIB, IIC and IID) (Jeckelmann and Erni, 2020). where IIC and IID are the integral membrane complexes. As the sugar is transported by subunits IIC and IIDA through the integral membrane, subunits IIA and IIB are responsible for transferring the phosphoryl groups of the sugar. The mannose PTS is one of the seven sugar transport systems in *L. monocytogenes*, responsible for the transport of glucose and fructose, which are the preferred carbohydrates of *L. monocytogenes* and has been implicated as a docking receptor molecule for bacteriocins (Jeckelmann and Erni, 2020; Premaratne et al., 1991).

In this study, for the gene expression of isolates of *L. monocytogenes* J1-177 that were resistant to carnocyclin A, there was downregulation in the Mannose PTS in the presence of dextrose for the resistant strain that had been isolated from cooked ground beef with dextrose. However, for the resistant isolates of *L. monocytogenes* J1-177 and *L. monocytogenes* C1-056 isolated from meat supplemented with sucrose, there was significant upregulation in both Mannose PTS subunit B and sucrose phosphorylase and in the Mannose PTS subunit A and β-glucosidase, respectively. The downregulation of the mannose PTS in the JDAr isolate, and upregulation of the Mannose PTS and sucrose phosphorylase in the JSAr and CSAr isolates aligned with the

growth curves of the resistant isolates in basal media. The downregulation of the mannose PTS lead to a slower growth compared to a fast growth rate in the CSAr and JSAr isolates. The difference observed in growth between the resistant isolates of *L. monocytogenes* can be due to strain individuality and the carbohydrate present, which influences the down or up regulation of genes related to PTS carbohydrate transport systems. Similar results were reported for differences in growth rates when sakacin P resistant strains of *L. monocytogenes* were grown in mannose (Tessema et al., 2011).

For regulation of genes related to PTS carbohydrate transport systems, past research has demonstrated the downregulation of the Mannose PTS in resistant variants like pediocin (Gravesen et al., 2002; Opsata et al., 2010). When the gene expression level of Mannose PTS A gene in pediocin resistant *Listeria* strains was examined by Liu et al (2019), they found both downregulation and upregulation depending on the pediocin resistant variant, which is similar to the results in this study. In a follow up study Liu et al (2023) that included various carbohydrates to investigate the pediocin resistance of *L. ivanovii*, the presence of glucose and mannose resulted in upregulation of Mannose PTS genes while fructose and cellobiose resulted in downregulation of the same genes. In addition, Tessema et al (2011) reported similar findings where sakacin P resistant *L. monocytogenes* strains repressed the expression of *mptA* (encodes for PTS mannose permease EII) in the strain with higher resistance while overexpressed the *mptA* in strain with lower resistance (Dalet et al., 2001; Tessema et al., 2011). Research by Balay et al (2018) also observed that resistant strains of *L. monocytogenes*, in the presence of various carbohydrates, lead to the downregulation of the Mannose PTS system but upregulated the expression of genes for other sugar transport systems such as the cellobiose specific PTS system.

Based on these findings, it is likely that when different carbohydrates are present *L. monocytogenes* is able to change gene expression (downregulation or up regulation) related to PTS sugar transport systems, depending on the strain of *L. monocytogenes*. When *L. monocytogenes* J1-177 was grown in dextrose with carnocyclin A (JDAr), downregulation of the Mannose PTS system was observed. This could indicate that the possibility of Mannose PTS being involved as a receptor docking molecule for carnocyclin A, as downregulation of the sugar transport system lead to resistance of the *L. monocytogenes* J1-177 strain in this case. However, when *L. monocytogenes* J1-177 or C1-056 was grown in sucrose, upregulation of the Mannose PTS system and sucrose phosphorylase was detected. This indicates that the mechanism of resistance to carnocyclin A is dependent on the carbohydrate and strain but may not be directly related to the Mannose PTS system.

These results together support the hypothesis that the availability of different carbohydrates impacts the growth of resistant isolates of *L. monocytogenes*. In the presence of carnocyclin A, different carbohydrates can impact the preferred carbohydrate system in *L. monocytogenes*. This is similar to what Ibarguren et al (2022) observed in a recent study, where different strains and carbohydrates lead to different growth patterns of resistant variants of *L. monocytogenes*. The results also support the idea that *L. monocytogenes* resistance to carnocyclin A may not be correlated with various sugar transport systems such as the Mannose PTS system.

Comparison of the genomes of the carnocyclin A resistant and sensitive isolates of *L. monocytogenes* with their parent strains, can help identify the changes in the genome in response to the development of resistance to carnocyclin A. Analysis of SNPs can determine if the development of *L. monocytogenes* resistance to carnocyclin A is more of a specific response, for

example, restricted to genes related to PTS carbohydrate transport systems or a more general response, such as changes in various parts of the genome.

In the carnocyclin resistant strain of *L. monocytogenes* that was isolated from meat with dextrose, SNPs were related to carbohydrate transport systems in *L. monocytogenes*. The SNPs observed with the JDAr isolate indicates that *L. monocytogenes* could utilize other sugar PTS systems to become resistant to carnocyclin A. This aligns with van Belkum et al (2023) findings, where lactococcin A and garvicin Q resistant mutants of *L. lactis* also had mutations in the Mannose PTS system, specifically on the IIC and IID subunits. The SNPs in RNA polymerase sigma-54 factor RpoN in the resistant isolate JDAr also supports the idea that the Mannose PTS is involved in resistance as the sigma-54 factor regulates the *mptACD* operon (Dalet et al., 2001). More research would be needed to investigate the gene expression levels of other carbohydrate transport systems in *L. monocytogenes* that are resistant carnocyclin A to support this finding.

In the current study, other SNPs found were related to cell wall binding proteins as well as Lipid A export ATP-binding/permease proteins, which support the idea that changes in the cell wall can also contribute to resistance of *L. monocytogenes* to carnocyclin A. One study on *L. ivanovii* resistance to pediocin reported 12 SNPs in the resistant variants when compared to the parent strain (Liu et al., 2020). This included SNPs that were related to genes encoding internalin B, which are surface proteins that are involved in virulence (Liu et al., 2020). It is possible that changes in the cell surface proteins impact the adhesion of the bacteriocins, and this could be a strategy for development of resistance. The HPr kinase/phosphorylase in *L. monocytogenes* may play a role in bacteriocin resistance (Opsata et al., 2010). As HPr kinase is responsible for phosphorylating HPr, which is involved in sugar transport, it is possible that this is in response to

the downregulation of Mannose PTS system observed in the current study. In response to downregulation of the mannose PTS, *L. monocytogenes* is able to regulate its uptake of carbohydrates with other transport systems (Aké et al., 2011; Hung and Miller, 2009).

The number of SNPs present in the resistant isolates CSAr (41 SNPs) and JSAr (88,608 SNPs) suggest that the development of resistance against carnocyclin A leads to hypermutation of *L. monocytogenes* that is dependent on both the strain and carbohydrate present. In *L. monocytogenes*, the evolutionary rate of change is estimated to be 0.18 to 0.35 SNPs/year (Knudsen et al., 2017). The high number of SNPs present in the resistant isolates obtained from cooked meat suggests there is a more general stress response to the presence of bacteriocins as opposed to specific responses focused only on the PTS carbohydrate systems. The SNPs found in sigma factor SigB also supports the idea of a general stress response as the signa factor SigB regulates the *L. monocytogenes* general stress response and is used by *L. monocytogenes* to adapt to various environmental stresses (Chaturongakul et al., 2008; Dorey et al., 2019). The development of a hypermutation phenotype develops in response to antibiotic stress in *Escherichia coli* (Crane et al., 2021) and in response to changes in a DNA mismatch repair protein in *L. monocytogenes* (Durack et al., 2015). Liu et al (2020) demonstrated that exposure to pediocin causes a high number of mutations in *L. ivanovii*. The results from the SNP analysis in the current study are preliminary and need further confirmation prior to conclusions about the genomic responses of *L. monocytogenes* to carnocyclin A.

These results altogether support the conclusions that there are many different mechanisms of resistance that bacteria utilize such as regulation of cell wall components, virulence factors as

well as other carbohydrate systems. The mechanisms that *L. monocytogenes* activates in response to carnocyclin A is both carbohydrate and strain dependent.

Overall, resistance of *L. monocytogenes* to carnocyclin A is impacted by strain variability and that there are various methods for development of resistance to bacteriocins in *L. monocytogenes*. More research must be done on the regulation of gene expression in the development of resistance to carnocyclin A in strains of *L. monocytogenes*. This study only focused on the expression of 5 genes related to carbohydrate transport systems, but SNPs were found on various parts of the genome that require future research. Future studies should also investigate more carbohydrates and strains of *L. monocytogenes* in the development of resistance to carbohydrates to determine if there were other possible mechanisms of resistance that were not found in this study. In addition, future direction for this study would be to sequence the parent strains of *L. monocytogenes* R2-499 and *L. monocytogenes* C1-056 using Nanopore sequencing technology and repeat the SNP analysis with appropriate programs to eliminate potential differences in SNPs analysis due to variability in whole genome sequences sequenced by different technologies. Future research should also focus on the cross resistance of carnocyclin A with other bacteriocins in ready-to-eat food products. By investigating the cross resistance, this will reduce the risk of the development of resistance by *L. monocytogenes* and further inform the application of bacteriocins in the food industry.

Bibliography

Acedo, J.Z., Chiorean, S., Vederas, J.C., van Belkum, M.J., 2018. The expanding structural variety among bacteriocins from Gram-positive bacteria. FEMS Microbiol Rev 42, 805–828. https://doi.org/10.1093/FEMSRE/FUY033

Ahn, C., Stiles, M.E., 1992. Mobilization and expression of bacteriocin plasmids from *Carnobacterium piscicola* isolated from meat. Journal of Applied Bacteriology 73, 217–228. https://doi.org/10.1111/J.1365-2672.1992.TB02981.X

Aké, F.M.D., Joyet, P., Deutscher, J., Milohanic, E., 2011. Mutational analysis of glucose transport regulation and glucose-mediated virulence gene repression in *Listeria monocytogenes*. Mol Microbiol 81, 274–293. https://doi.org/10.1111/J.1365-2958.2011.07692.X

Alvarez-Molina, A., Cobo-Díaz, J.F., López, M., Prieto, M., de Toro, M., Alvarez-Ordóñez, A., 2021a. Unraveling the emergence and population diversity of *Listeria monocytogenes* in a newly built meat facility through whole genome sequencing. Int J Food Microbiol 340, 109043. https://doi.org/10.1016/J.IJFOODMICRO.2021.109043

Alvarez-Molina, A., Cobo-Díaz, J.F., López, M., Prieto, M., de Toro, M., Alvarez-Ordóñez, A., 2021b. Unraveling the emergence and population diversity of *Listeria monocytogenes* in a newly built meat facility through whole genome sequencing. Int J Food Microbiol 340, 109043. https://doi.org/10.1016/J.IJFOODMICRO.2021.109043

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., Kuipers, O.P., 2016. Bacteriocins of lactic acid bacteria: extending the family. Appl Microbiol Biotechnol 100, 2939. https://doi.org/10.1007/S00253-016-7343-9

Balay, D., 2019. The efficacy of bacteriocins to control *Listeria monocytogenes*. PhD Thesis. University of Alberta. https://era-library-ualberta-ca.login.ezproxy.library.ualberta.ca/items/ba901091-4bf8-494e-a0b1-8ec6f52905cd

Balay, D.R., Gänzle, M.G., McMullen, L.M., 2018. The effect of carbohydrates and bacteriocins on the growth kinetics and resistance of *Listeria monocytogenes*. Front Microbiol 9, 307215. https://doi.org/10.3389/FMICB.2018.00347/BIBTEX

Barcenilla, C., Puente, A., Cobo-Díaz, J.F., Alexa, E.-A., Garcia-Gutierrez, E., O'Connor, P.M., Cotter, P.D., González-Raurich, M., López, M., Prieto, M., Álvarez-Ordóñez, A., 2023. Selection of lactic acid bacteria as biopreservation agents and optimization of their mode of application for the control of *Listeria monocytogenes* in ready-to-eat cooked meat products. Int J Food Microbiol 403, 110341. https://doi.org/10.1016/j.ijfoodmicro.2023.110341

Bodie, A.R., O'Bryan, C.A., Olson, E.G., Ricke, S.C., 2023. Natural antimicrobials for *Listeria monocytogenes* in ready-to-eat meats: current challenges and future prospects. Microorganisms 2023, Vol. 11, Page 1301 11, 1301. https://doi.org/10.3390/MICROORGANISMS11051301

Breukink, E., Wiedemann, I., Van Kraaij, C., Kuipers, O.P., Sahl, H.G., De Kruijff, B., 1999. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. Science 286, 2361–2364. https://doi.org/10.1126/SCIENCE.286.5448.2361

Castellano, P., Peña, N., Ibarreche, M.P., Carduza, F., Soteras, T., Vignolo, G., 2018. Antilisterial efficacy of *Lactobacillus* bacteriocins and organic acids on frankfurters. Impact on sensory characteristics. J Food Sci Technol 55, 689–697. https://doi.org/10.1007/S13197-017-2979-8/METRICS

CDC, 2023. *Listeria* (Listeriosis) [WWW Document]. URL https://www.cdc.gov/listeria/outbreaks/peaches-11-23/index.html (accessed 1.16.24).

Cebrián, R., Martínez-Bueno, M., Valdivia, E., Albert, A., Maqueda, M., Sánchez-Barrena, M.J., 2015. The bacteriocin AS-48 requires dimer dissociation followed by hydrophobic interactions with the membrane for antibacterial activity. J Struct Biol 190, 162–172. https://doi.org/10.1016/J.JSB.2015.03.006

Chaturongakul, S., Raengpradub, S., Wiedmann, M., Boor, K.J., 2008. Modulation of stress and virulence in *Listeria monocytogenes*. Trends Microbiol 16, 388–396. https://doi.org/10.1016/J.TIM.2008.05.006

Cherifi, T., Carrillo, C., Lambert, D., Miniaï, I., Quessy, S., Larivière-Gauthier, G., Blais, B., Fravalo, P., 2018. Genomic characterization of *Listeria monocytogenes* isolates reveals that their persistence in a pig slaughterhouse is linked to the presence of benzalkonium chloride resistance genes. BMC Microbiol 18, 1–13. https://doi.org/10.1186/S12866-018-1363-9/FIGURES/6

Citartan, M., Raabe, C.A., Hoe, C.H., Rozhdestvensky, T.S., Tang, T.H., 2016. Bacterial sRNAs: regulation in stress. Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria 1, 108–114. https://doi.org/10.1002/9781119004813.CH9

Collins, B., Joyce, S., Hill, C., Cotter, P.D., Ross, R.P., 2010. *TelA* contributes to the innate resistance of *Listeria monocytogenes* to nisin and other cell wall-acting antibiotics. Antimicrob Agents Chemother 54, 4658–4663. https://doi.org/10.1128/AAC.00290-10/ASSET/9F8256BD-AF1C-490F-9323-CE7A1D1D3553/ASSETS/GRAPHIC/ZAC9991093800003.JPEG

Crandall, A.D., Montville, T.J., 1998. Nisin resistance in *Listeria monocytogenes* ATCC 700302 is a complex phenotype. Appl Environ Microbiol 64, 231–237. https://doi.org/10.1128/AEM.64.1.231-237.1998/ASSET/2DED82BA-9F9D-4D61-80FF-0AF17750C5A0/ASSETS/GRAPHIC/AM0181053005.JPEG

Crane, J.K., Alvarado, C.L., Sutton, M.D., 2021. Role of the SOS response in the generation of antibiotic resistance in vivo. Antimicrob Agents Chemother 65. https://doi.org/10.1128/AAC.00013-21/SUPPL_FILE/AAC.00013-21-S0001.PDF

Dalet, K., Cenatiempo, Y., Cossart, P., Glaser, P., Amend, A., Baquero-Mochales, F., Berche, P., Bloecker, H., Brandt, P., Buchrieser, C., Chakraborty, T., Charbit, A., Couvé, E., De Daruvar, A., Dehoux, P., Domann, E., Dominguez-Bernal, G., Durant, L., Entian, K.D., Frangeul, L., Fsihi, H., Garcia del Portillo, F., Garrido, P., Goebel, W., Gomez-Lopez, N., Hain, T., Hauf, J., Jackson, D., Kreft, J., Kunst, F., Mata-Vicente, J., Ng, E., Nordsiek, G., Perez-Diaz, J.C., Remmel, B., Rose, M., Rusniok, C., Schlueter, T., Vazquez-Boland, J.A., Voss, H., Wehland, J., Héchard, Y., 2001. A sigma(54)-dependent PTS permease of the mannose family is responsible for sensitivity of *Listeria monocytogenes* to mesentericin Y105. Microbiology (Reading) 147, 3263–3269. https://doi.org/10.1099/00221287-147-12-3263

Dallagnol, A.M., Barrio, Y., Cap, M., Szerman, N., Castellano, P., Vaudagna, S.R., Vignolo, G., 2017. *Listeria* inactivation by the combination of high hydrostatic pressure and Lactocin AL705 on cured-cooked pork loin slices. Food Bioproc Tech 10, 1824–1833. https://doi.org/10.1007/S11947-017-1956-6/METRICS

de Azevedo, P.O.S., Mendonça, C.M.N., Seibert, L., Domínguez, J.M., Converti, A., Gierus, M., Oliveira, R.P.S., 2020. Bacteriocin-like inhibitory substance of *Pediococcus pentosaceus* as a biopreservative for *Listeria* sp. control in ready-to-eat pork ham. Brazilian Journal of Microbiology 51, 949–956. https://doi.org/10.1007/S42770-020-00245-W/TABLES/2

Desiderato, C.K., Hasenauer, K.M., Reich, S.J., Goldbeck, O., Holivololona, L., Ovchinnikov, K. V., Reiter, A., Oldiges, M., Diep, D.B., Eikmanns, B.J., Riedel, C.U., 2022. Garvicin Q: characterization of biosynthesis and mode of action. Microb Cell Fact 21. https://doi.org/10.1186/S12934-022-01952-9

Diep, D.B., Skaugen, M., Salehian, Z., Holo, H., Nes, I.F., 2007. Common mechanisms of target cell recognition and immunity for class II bacteriocins. Proc Natl Acad Sci USA 104, 2384–2389. https://doi.org/10.1073/pnas.0608775104

Dincer, S., Uslu, F.M., Delik, A., Dincer, S., Uslu, F.M., Delik, A., 2020. Antibiotic Resistance in Biofilm. Bacterial Biofilms. https://doi.org/10.5772/INTECHOPEN.92388

Dorey, A., Marinho, C., Piveteau, P., O'Byrne, C., 2019. Role and regulation of the stress activated sigma factor sigma B (σB) in the saprophytic and host-associated life stages of *Listeria monocytogenes*. Adv Appl Microbiol 106, 1–48. https://doi.org/10.1016/BS.AAMBS.2018.11.001

Durack, J., Burke, T.P., Portnoy, D.A., 2015. A *prl* Mutation in SecY Suppresses Secretion and Virulence Defects of *Listeria monocytogenes secA2* Mutants. J Bacteriol 197, 932. https://doi.org/10.1128/JB.02284-14

Ennahar, S., Deschamps, N., 2000. Anti-*Listeria* effect of enterocin A, produced by cheese-isolated *Enterococcus faecium* EFM01, relative to other bacteriocins from lactic acid bacteria. J Appl Microbiol 88, 449–457. https://doi.org/10.1046/J.1365-2672.2000.00985.X

Fagerlund, A., Langsrud, S., Møretrø, T., 2021. Microbial diversity and ecology of biofilms in food industry environments associated with *Listeria monocytogenes* persistence. Curr Opin Food Sci 37, 171–178. https://doi.org/10.1016/J.COFS.2020.10.015

FAO, WHO, 2022. *Listeria monocytogenes* in ready-to-eat (RTE) food: attribution, characterization and monitoring: meeting report [WWW Document]. URL https://www.who.int/publications/i/item/9789240034969 (accessed 1.29.24).

Farber, J.M., Peterkin, P.I., 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 55, 476–511. https://doi.org/10.1128/MMBR.55.3.476-511.1991

Fernández, M., Hospital, X.F., Caballero, N., Jiménez, B., Sánchez-Martín, V., Morales, P., Haza, A.I., Hierro, E., 2023. Potential of selected bacteriocinogenic lactic acid bacteria to control *Listeria monocytogenes* in nitrite-reduced fermented sausages. Food Control 150, 109724. https://doi.org/10.1016/j.foodcont.2023.109724

Fugett, E., Fortes, E., Nnoka, C., Wiedmann, M., 2006. International Life Sciences Institute North America *Listeria monocytogenes* strain collection: development of standard *Listeria*

monocytogenes strain sets for research and validation studies. J Food Prot 69, 2929–2938. https://doi.org/10.4315/0362-028X-69.12.2929

Galvez, A., Maqueda, M., Martinez-Bueno, M., Valdivia, E., 1991. Permeation of bacterial cells, permeation of cytoplasmic and artificial membrane vesicles, and channel formation on lipid bilayers by peptide antibiotic AS-48. J Bacteriol 173, 886–892. https://doi.org/10.1128/JB.173.2.886-892.1991

Gandhi, M., Chikindas, M.L., 2007. *Listeria*: A foodborne pathogen that knows how to survive. Int J Food Microbiol 113, 1–15. https://doi.org/10.1016/J.IJFOODMICRO.2006.07.008

Giaouris, E., Briandet, R., Meyrand, M., Courtin, P., Chapot-Chartier, M.P., 2008. Variations in the degree of D-alanylation of teichoic acids in *Lactococcus lactis* alter resistance to cationic antimicrobials but have no effect on bacterial surface hydrophobicity and charge. Appl Environ Microbiol 74, 4764–4767. https://doi.org/10.1128/AEM.00078-08/ASSET/5C451B60-B39E-47D1-9B00-12BEF8EACF2D/ASSETS/GRAPHIC/ZAM0150890550004.JPEG

Giaouris, E., Chorianopoulos, N., Nychas, G.J.E., 2005. Effect of temperature, pH, and water activity on biofilm formation by *Salmonella enterica* enteritidis PT4 on stainless steel surfaces as indicated by the bead vortexing method and conductance measurements. J Food Prot 68, 2149–2154. https://doi.org/10.4315/0362-028X-68.10.2149

Glass, K.A., Granberg, D.A., Smith, A.L., McNamara, A.M., Hardin, M., Mattias, J., Ladwig, K., Johnson, E.A., 2002. Inhibition of *Listeria monocytogenes* by sodium diacetate and

sodium lactate on wieners and cooked bratwurst. J Food Prot 65, 116–123. https://doi.org/10.4315/0362-028X-65.1.116

Golden, M.C., McDonnell, L.M., Sheehan, V., Sindelar, J.J., Glass, K.A., 2014. Inhibition of *Listeria monocytogenes* in deli-dtyle turkey breast formulated with cultured celery powder and/or cultured sugar–vinegar blend during storage at 4°c. J Food Prot 77, 1787–1793. https://doi.org/10.4315/0362-028X.JFP-14-059

Gong, X., Martin-Visscher, L.A., Nahirney, D., Vederas, J.C., Duszyk, M., 2009. The circular bacteriocin, carnocyclin A, forms anion-selective channels in lipid bilayers. BBA - Biomembranes 1788, 1797–1803. https://doi.org/10.1016/j.bbamem.2009.05.008

Gravesen, A., Ramnath, M., Rechinger, K.B., Andersen, N., Jänsch, L., Héchard, Y., Hastings, J.W., Knøchel, S., 2002. High-level resistance to class IIa bacteriocins is associated with one general mechanism in *Listeria monocytogenes*. Microbiology (Reading) 148, 2361–2369. https://doi.org/10.1099/00221287-148-8-2361

Guan's lab- edited by YH, 2015. RNA extraction from rumen Tissue or Fluid protocol .

Guo, L., Wambui, J., Wang, C., Muchaamba, F., Fernandez-Cantos, M.V., Broos, J., Tasara, T., Kuipers, O.P., Stephan, R., 2023. Cesin, a short natural variant of nisin, displays potent antimicrobial activity against major pathogens despite lacking two C-terminal macrocycles. Microbiol Spectr 11. https://doi.org/10.1128/SPECTRUM.05319-22

Harrand, A.S., Jagadeesan, B., Baert, L., Wiedmann, M., Orsi, R.H., 2020. Evolution of *Listeria monocytogenes* in a food processing plant involves limited single-nucleotide substitutions

but considerable diversification by gain and loss of prophages. Appl Environ Microbiol 86. https://doi.org/10.1128/AEM.02493-19/SUPPL_FILE/AEM.02493-19-S0001.PDF

Hasper, H.E., Kramer, N.E., Smith, J.L., Hillman, J.D., Zachariah, C., Kuipers, O.P., De Kruijff, B., Breukink, E., 2006. An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. Science 313, 1636–1637. https://doi.org/10.1126/SCIENCE.1129818

Health Canada, 2023. Yearly food-borne illness estimates for Canada - Canada.ca [WWW Document]. URL https://www.canada.ca/en/public-health/services/food-borne-illness-canada/yearly-food-borne-illness-estimates-canada.html (accessed 1.10.24).

Health Canada, n.d. Policy on *Listeria monocytogenes* in ready-to-eat foods (2023) - Canada.ca [WWW Document]. 2023. URL https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/listeria-monocytogenes-ready-eat-foods.html (accessed 1.10.24).

Hung, V.K., Miller, K.W., 2009. Regulation of mannose phosphotransferase system permease and virulence gene expression in *Listeria monocytogenes* by the EIItMan transporter. Appl Environ Microbiol 75, 6671–6678. https://doi.org/10.1128/AEM.01104-09/ASSET/92FD63DC-1129-4BA0-AE47-FD3D02B99DA3/ASSETS/GRAPHIC/ZAM0210903930001.JPEG

Iacumin, L., Cappellari, G., Colautti, A., Comi, G., 2020. *Listeria monocytogenes* survey in cubed cooked ham packaged in modified atmosphere and bioprotective effect of selected

lactic acid bacteria. Microorganisms 2020, Vol. 8, Page 898 8, 898. https://doi.org/10.3390/MICROORGANISMS8060898

Ibarguren, C., Guitián, M.V., Lenz, R.M., Cecilia, S.M., Audisio, M.C., 2022. Response of sensitive and resistant *Listeria monocytogenes* strains against bacteriocins produced by different *Enterococcus* spp. strains. Int J Food Microbiol 382, 109928. https://doi.org/10.1016/J.IJFOODMICRO.2022.109928

Jeckelmann, J.M., Erni, B., 2020. The mannose phosphotransferase system (Man-PTS) - Mannose transporter and receptor for bacteriocins and bacteriophages. Biochim Biophys Acta Biomembr 1862. https://doi.org/10.1016/J.BBAMEM.2020.183412

Junges da Costa, R., Pereira da Silva, A., Nobre da Fonseca, R., de Oliveira Hübner, S., Nalério, E.S., de Lima Marques, J., Soares Vitola, H.R., Padilha da Silva, W., Duval, E.H., Fiorentini, Â.M., 2021. Characterization of *Enterococcus faecium* EO1 isolated from mutton and activity of bacteriocin-like substances in the control of *Listeria monocytogenes* in fresh mutton sausage. LWT 141, 110954. https://doi.org/10.1016/J.LWT.2021.110954

Kaur, G., Malik, R.K., Mishra, S.K., Singh, T.P., Bhardwaj, A., Singroha, G., Vij, S., Kumar, N., 2011. Nisin and class IIa bacteriocin resistance among *Listeria* and other foodborne pathogens and spoilage bacteria. https://home-liebertpub-com.login.ezproxy.library.ualberta.ca/mdr 17, 197–205. https://doi.org/10.1089/MDR.2010.0054

Kjos, M., Nes, I.F., Diep, D.B., 2011. Mechanisms of resistance to bacteriocins targeting the mannose phosphotransferase system. Appl Environ Microbiol 77, 3335–3342.

https://doi.org/10.1128/AEM.02602-10/ASSET/6149F4DA-E441-4520-992E-C346CC432002/ASSETS/GRAPHIC/ZAM9991020620006.JPEG

Knudsen, G.M., Nielsen, J.B., Marvig, R.L., Ng, Y., Worning, P., Westh, H., Gram, L., 2017. Genome-wide-analyses of *Listeria monocytogenes* from food-processing plants reveal clonal diversity and date the emergence of persisting sequence types. Environ Microbiol Rep 9, 428–440. https://doi.org/10.1111/1758-2229.12552

Kolanowski, W., Takeuchi-Storm, N., Hansen, L.T., Nielsen, N.L., Andersen, J.K., 2023. Presence and persistence of *Listeria monocytogenes* in the danish ready-to-eat food production environment. Hygiene 2023, Vol. 3, Pages 18-32 3, 18–32. https://doi.org/10.3390/HYGIENE3010004

Lachmann, R., Halbedel, S., Adler, M., Becker, N., Allerberger, F., Holzer, A., Boone, I., Falkenhorst, G., Kleta, S., Al Dahouk, S., Stark, K., Luber, P., Flieger, A., Wilking, H., 2021. Nationwide outbreak of invasive listeriosis associated with consumption of meat products in health care facilities, Germany, 2014e2019. Clinical Microbiology and Infection 27, 1035.e1-1035.e5. https://doi.org/10.1016/j.cmi.2020.09.020

Lee, B.H., Hébraud, M., Bernardi, T., 2017. Increased adhesion of *Listeria monocytogenes* strains to abiotic surfaces under cold stress. Front Microbiol 8, 287050. https://doi.org/10.3389/FMICB.2017.02221/BIBTEX

Li, H., Sun, X., Liao, X., Gänzle, M., 2020. Control of pathogenic and spoilage bacteria in meat and meat products by high pressure: Challenges and future perspectives. Compr Rev Food Sci Food Saf 19, 3476–3500. https://doi.org/10.1111/1541-4337.12617

Li, R., Duan, J., Zhou, Y., Wang, J., 2023. Structural basis of the mechanisms of action and immunity of lactococcin A, a class IId bacteriocin. Appl Environ Microbiol 89. https://doi.org/10.1128/AEM.00066-23/SUPPL_FILE/AEM.00066-23-S0001.PDF

Liu, S., Ding, B., Wang, N., Zhang, P., Xiao, P., 2023. Investigation of the correlation between carbohydrate metabolization and pediocin sensitivity. Biologia (Bratisl) 79, 251–261. https://doi.org/10.1007/S11756-023-01525-0/FIGURES/5

Liu, S., Liu, Y., Takala, T.M., Zhang, P., Wang, S., 2020. Phenotypic comparison and DNA sequencing analysis of a wild-type and a pediocin-resistant mutant of *Listeria ivanovii*. Res Microbiol 171, 115–121. https://doi.org/10.1016/J.RESMIC.2020.02.004

Liu, S., Liu, Y., Zhang, P., 2019. Properties of *Listeria monocytogenes* on acquisition of pediocin resistance. Ann Microbiol 69, 123–130. https://doi.org/10.1007/S13213-018-1411-6/FIGURES/6

Lubelski, J., Rink, R., Khusainov, R., Moll, G.N., Kuipers, O.P., 2008. Biosynthesis, immunity, regulation, mode of action and engineering of the model lantibiotic nisin. Cellular and Molecular Life Sciences 65, 455–476. https://doi.org/10.1007/S00018-007-7171-2/METRICS

Mantovani, H.C., Russell, J.B., 2001. Nisin resistance of *Streptococcus bovis*. Appl Environ Microbiol 67, 808–813. https://doi.org/10.1128/AEM.67.2.808-813.2001/ASSET/9D0F9976-C60B-4E5F-B7D5-388CAFAB1F97/ASSETS/GRAPHIC/AM0211166008.JPEG

Martin-Visscher, L.A., Van Belkum, M.J., Garneau-Tsodikova, S., Whittal, R.M., Zheng, J., McMullen, L.M., Vederas, J.C., 2008a. Isolation and characterization of carnocyclin A, a novel circular bacteriocin produced by *Carnobacterium maltaromaticum* UAL307. Appl Environ Microbiol 74, 4756. https://doi.org/10.1128/AEM.00817-08

Martin-Visscher, L.A., Van Belkum, M.J., Garneau-Tsodikova, S., Whittal, R.M., Zheng, J., McMullen, L.M., Vederas, J.C., 2008b. Isolation and characterization of carnocyclin A, a novel circular bacteriocin produced by *Carnobacterium maltaromaticum* UAL307. Appl Environ Microbiol 74, 4756–4763. https://doi.org/10.1128/AEM.00817-08

Mbandi, E., Shelef, L.A., 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* in meat by combinations of sodium lactate and diacetate. J Food Prot 64, 640–644. https://doi.org/10.4315/0362-028X-64.5.640

McDonnell, L.M., Glass, K.A., Sindelar, J.J., 2013. Identifying ingredients that delay outgrowth of *Listeria monocytogenes* in natural, organic, and clean-label ready-to-eat meat and poultry products. J Food Prot 76, 1366–1376. https://doi.org/10.4315/0362-028X.JFP-12-501

Mendoza, F., Maqueda, M., Gálvez, A., Martínez-Bueno, M., Valdivia, E., 1999. Antilisterial activity of peptide AS-48 and study of changes induced in the cell envelope properties of an AS-48-adapted strain of *Listeria monocytogenes*. Appl Environ Microbiol 65, 618–625. https://doi.org/10.1128/AEM.65.2.618-625.1999/ASSET/2E56F0EB-798F-4516-B13F-32860F954E83/ASSETS/GRAPHIC/AM0290669007.JPEG

Ming, X., Daeschel, M.A., 1995. Correlation of cellular phospholipid content with nisin resistance of *Listeria monocytogenes* Scott A. J Food Prot 58, 416–420. https://doi.org/10.4315/0362-028X-58.4.416

Montiel, R., Quesille-Villalobos, A., Alessandria, V., Medina, M., Cocolin, L.S., Rantsiou, K., 2019. Antilisterial effect and influence on *Listeria monocytogenes* gene expression of enterocin or *Enterococcus faecalis* in sliced dry-cured ham stored at 7°C. J Food Prot 82, 1598–1606. https://doi.org/10.4315/0362-028X.JFP-19-024

Møretrø, T., Langsrud, S., 2004. *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments. Biofilms 1, 107–121. https://doi.org/10.1017/S1479050504001322

Opsata, M., Nes, I.F., Holo, H., 2010. Class IIa bacteriocin resistance in *Enterococcus faecalis* V583: The mannose PTS operon mediates global transcriptional responses. BMC Microbiol 10, 1–13. https://doi.org/10.1186/1471-2180-10-224/FIGURES/4

Palaiodimou, L., Fanning, S., Fox, E.M., 2021. Genomic insights into persistence of *Listeria* species in the food processing environment. J Appl Microbiol 131, 2082–2094. https://doi.org/10.1111/JAM.15089

Paula, A.J., Hwang, G., Koo, H., 2020. Dynamics of bacterial population growth in biofilms resemble spatial and structural aspects of urbanization. Nature Communications 2020 11:1 11, 1–14. https://doi.org/10.1038/S41467-020-15165-4

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res 29, e45–e45. https://doi.org/10.1093/NAR/29.9.E45

Pightling, A.W., Pettengill, J.B., Luo, Y., Baugher, J.D., Rand, H., Strain, E., 2018. Interpreting whole-genome sequence analyses of foodborne bacteria for regulatory applications and outbreak investigations. Front Microbiol 9, 367953. https://doi.org/10.3389/FMICB.2018.01482/BIBTEX

Preda, V.G., Săndulescu, O., 2019. Communication is the key: biofilms, quorum sensing, formation and prevention. Discoveries 7, e100. https://doi.org/10.15190/D.2019.13

Premaratne, R.J., Lin, W.J., Johnson, E.A., 1991. Development of an improved chemically defined minimal medium for *Listeria monocytogenes*. Appl Environ Microbiol 57, 3046. https://doi.org/10.1128/AEM.57.10.3046-3048.1991

Rahman, M.M., Lim, S., Park, Y.C., 2022. Development of Single Nucleotide Polymorphism (SNP)-based triplex PCR marker for serotype-specific *Escherichia coli* detection. Pathogens 11, 115. https://doi.org/10.3390/PATHOGENS11020115/S1

Ramnath, M., Beukes, M., Tamura, K., Hastings, J.W., 2000. Absence of a putative mannose-specific phosphotransferase system enzyme IIAB component in a leucocin A-resistant strain of *Listeria monocytogenes*, as shown by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Appl Environ Microbiol 66, 3098–3101. https://doi.org/10.1128/AEM.66.7.3098-3101.2000

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P.S., Girones, R., Herman, L., Koutsoumanis, K., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Takkinen, J., Wagner, M., Arcella, D., Da Silva Felicio, M.T., Georgiadis,

M., Messens, W., Lindqvist, R., 2018. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA Journal 16, e05134. https://doi.org/10.2903/J.EFSA.2018.5134

Rood, L., Bowman, J.P., Ross, T., Corkrey, R., Pagnon, J., Yang, S.W.T., Kocharunchitt, C., 2022. The effects of glucose on microbial spoilage of vacuum-packed lamb. Meat Sci 188, 108781. https://doi.org/10.1016/J.MEATSCI.2022.108781

Rossi, D.A., Melo, R.T., Mendonça, E.P., Monteiro, G.P., Rossi, D.A., Melo, R.T., Mendonça, E.P., Monteiro, G.P., 2017. Biofilms of Salmonella and Campylobacter in the Poultry Industry. Poult Sci. https://doi.org/10.5772/65254

Schöbitz, R., Suazo, V., Costa, M., Ciampi, L., 2003. Effects of a bacteriocin-like inhibitory substance from *Carnobacterium piscicola* against human and salmon isolates of *Listeria monocytogenes*. Int J Food Microbiol 84, 237–244. https://doi.org/10.1016/S0168-1605(02)00406-3

Sebranek, J.G., Jackson-Davis, A.L., Myers, K.L., Lavieri, N.A., 2012. Beyond celery and starter culture: Advances in natural/organic curing processes in the United States. Meat Sci 92, 267–273. https://doi.org/10.1016/J.MEATSCI.2012.03.002

Solano, C., Echeverz, M., Lasa, I., 2014. Biofilm dispersion and quorum sensing. Curr Opin Microbiol 18, 96–104. https://doi.org/10.1016/J.MIB.2014.02.008

Stepanović, S., Ćirković, I., Ranin, L., Švabić-Vlahović, M., 2004. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett Appl Microbiol 38, 428–432. https://doi.org/10.1111/J.1472-765X.2004.01513.X

Stoll, R., Goebel, W., 2010. The major PEP-phosphotransferase systems (PTSs) for glucose, mannose and cellobiose of *Listeria monocytogenes*, and their significance for extra- and intracellular growth. Microbiology (N Y) 156, 1069–1083. https://doi.org/10.1099/mic.0.034934-0

Tasara, T., Stephan, R., 2007. Evaluation of housekeeping genes in *Listeria monocytogenes* as potential internal control references for normalizing mRNA expression levels in stress adaptation models using real-time PCR. FEMS Microbiol Lett 269, 265–272. https://doi.org/10.1111/J.1574-6968.2007.00633.X

Teixeira, J.S., Repková, L., Gänzle, M.G., McMullen, L.M., 2018. Effect of pressure, reconstituted RTE meat microbiota, and antimicrobials on survival and post-pressure growth of *Listeria monocytogenes* on Ham. Front Microbiol 9, 347030. https://doi.org/10.3389/FMICB.2018.01979/BIBTEX

Tessema, G.T., Møretrø, T., Snipen, L., Axelsson, L., Naterstad, K., 2011. Global transcriptional analysis of spontaneous sakacin P-resistant mutant strains of *Listeria monocytogenes* during growth on different sugars. PLoS One 6, e16192. https://doi.org/10.1371/JOURNAL.PONE.0016192

Vadyvaloo, V., Hastings, J.W., Van der Merwe, M.J., Rautenbach, M., 2002. Membranes of class IIa bacteriocin-resistant *Listeria monocytogenes* cells contain increased levels of desaturated and short-acyl-chain phosphatidylglycerols. Appl Environ Microbiol 68, 5223–5230. https://doi.org/10.1128/aem.68.11.5223-5230.2002

Vadyvaloo, V., Snoep, J.L., Hastings, J.W., Rautenbach, M., 2004. Physiological implications of class IIa bacteriocin resistance in *Listeria monocytogenes* strains. Microbiology (N Y) 150, 335–340. https://doi.org/10.1099/mic.0.26731-0

van Belkum, M.J., Aleksandrzak-Piekarczyk, T., Lamer, T., Vederas, J.C., 2023. *Lactococcus lactis* mutants resistant to lactococcin A and garvicin Q reveal missense mutations in the sugar transport domain of the mannose phosphotransferase system. Microbiol Spectr. https://doi.org/10.1128/SPECTRUM.03130-23

Vasudevan, R., 2014. Biofilms: microbial cities of scientific significance. J Microbiol Exp Volume 1. https://doi.org/10.15406/JMEN.2014.01.00014

Vijayakumar, P.P., Muriana, P.M., 2017. Inhibition of *Listeria monocytogenes* on ready-to-eat meats using bacteriocin mixtures based on mode-of-action. Foods 2017, Vol. 6, Page 22 6, 22. https://doi.org/10.3390/FOODS6030022

Wu, X., Ju, X., Du, L., Wang, L., He, R., Chen, Z., 2020. The Man-PTS subunit IIC is responsible for the sensitivity of *Listeria monocytogenes* to durancin GL. Food Sci Nutr 8, 150–161. https://doi.org/10.1002/FSN3.1285

Zhu, L., Zeng, J., Wang, C., Wang, J., 2022a. Structural basis of pore formation in the mannose phosphotransferase system by pediocin PA-1. Appl Environ Microbiol 88. https://doi.org/10.1128/AEM.01992-21/SUPPL_FILE/AEM.01992-21-S0001.PDF

Zhu, L., Zeng, J., Wang, J., 2022b. Structural basis of the immunity mechanisms of pediocin-like bacteriocins. Appl Environ Microbiol 88. https://doi.org/10.1128/AEM.00481-22/SUPPL_FILE/AEM.00481-22-S0001.PDF