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Determining the Effects of Propionate on *Listeria monocytogenes* Lysozyme Susceptibility

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Abstract

Listeria monocytogenes is a gram-positive facultative anaerobe and harmful pathogen transmitted through contaminated food. Listeriosis, the infection associated with *L. monocytogenes*, is rare but potentially fatal, with a twenty to thirty percent mortality rate. For that reason, the lack of safe strategies to prevent infections can be detrimental to public health. Current infection preventative strategies rely on stringent food surveillance and recalls, but we want to determine alternative tactics to further protect the public from *L. monocytogenes*. More specifically, we want to identify environmental factors that can compromise the ability of *L. monocytogenes* to cause infections before the pathogen reaches the intestines. For example, propionate is generally recognized as safe by the FDA and is used as an additive in various food products. Alongside being a food additive, propionate is a gut metabolite found in our intestinal tract. In past studies, our lab has demonstrated that propionate exposure in *L. monocytogenes* can lead to changes in growth and pathogenesis. To determine how propionate exposure affects *L. monocytogenes* survival and fitness in the gastrointestinal tract, my thesis project therefore studies the effects of propionate on *L. monocytogenes* resistance to the lysozyme found in our saliva. If propionate enhances *L. monocytogenes* lysozyme resistance, the use of propionate in food products might contribute to *L. monocytogenes* survival during transmission between food and our gastrointestinal tract. However, if propionate decreases *L. monocytogenes* resistance to lysozyme, it could be beneficial to use propionate as an efficient infection preventative strategy. To better understand the functions of propionate in *L. monocytogenes* lysozyme resistance, I performed several trials of a lysozyme growth experiment and lysozyme survival assay.

Acknowledgements

I would like to acknowledge all the individuals and organizations that have allowed me to pursue this honors thesis project. Without their support and mentorship, this project would not have been possible. This research was done in the Sun Lab and funded by the Honors Program, Biology Department's Robert J. Kearns Summer Scholarship, Berry Summer Thesis Institute, College of Arts and Sciences Dean's Summer Fellowship, and the Premedical Program's Szabo Grant. Furthermore, the mentorship of Dr. Yvonne Sun has meant so much to me throughout my undergraduate studies while working in her lab. Dr. Sun's continuous support and constructive criticism allowed me to flourish as a researcher and lifelong learner. Finally, I would like to thank my classmates, lab mates, friends, and family members for their endless encouragement.

University *of* Dayton

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Introduction

The Oral Microbiome

Many believe that digestion begins in the stomach, but in reality, it commences in the oral cavity during the process of chewing. While chewing, there are numerous antimicrobial mechanisms in our mouth that are constantly at work. Over 700 bacterial species reside in the oral cavity and play a pivotal role in regards to human health and the immune system[1]. These bacterial species can have varying effects on the food or drink particles they potentially encounter, and vice versa. Major oral bacteria phylum containing 96 percent of the oral taxa in the human oral microbiome are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Spirochaetes*, and *Fusobacteria*[2]. Within the various habitats in the oral cavity, the composition of such species is subject to change depending on environmental factors. For example, studies have shown that alcohol consumption can affect the overall composition of the oral microbiome[1]. If a commonly consumed beverage by adults has the ability to notably alter the oral microbiota, there is cause for concern about what other substances can affect the oral microbiome.

The oral microbiome plays a crucial role in fighting off diseases and infections. When the microbiome is thrown off balance, it can lead to diseases that may escalate to affect the entire body. Joshua Lederberg coined the term microbiome "to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body space and have been all but ignored as determinants of health and disease"[2]. This reinforces the evident connection between oral and systemic health. While pathogenic bacteria have the potential to disrupt the oral microbiome, saliva and gingival

crevicular fluid are constantly at work to provide nutrients for maintaining a healthy and balanced microbiota. Furthermore, hypothiocyanite production is catalyzed by lactoperoxidase reinforcing antimicrobial activities such as inhibiting bacterial glycolysis[3]. Proteins form a protective coating that shields tooth surfaces from acid attacks[3]. Simultaneously, our microbiome can be perturbed by diseases in other parts of the body. For example, obesity, metabolic syndrome, multiple sclerosis, among other chronic health conditions are associated with perturbations of the gut microbiome[4]. These diseases are directly associated with a less diverse microbiome since a more diverse microbiome is an indicator of a healthy one. This is because a less diverse microbiome indicates a lack of substantial defenses against infection and disease. In summary, our oral microbiome can cause both oral and systemic diseases when not properly protected from pathogens[5]. Therefore, it is essential to maintain a healthy microbiome, in which the "good" bacteria outnumber the "bad" bacteria.

When it comes to oral health and the chemical processes occurring in our mouths, there are a multitude of chemical interactions at play. With that being said, there are both advantageous and disadvantageous chemical occurrences that impact our oral health as a whole. It's important to note that there are numerous strategies for maintaining excellent oral health, such as a healthy diet with limited sugar and balanced acidity. A caveat that comes with this is that while there are multiple ways to retain oral hygiene, there are also an abundance of factors that might be working against you. Genetics can play a role in your oral microbiome composition and potential dental issues. Oral health has been overlooked by many for years, often due to a lack of knowledge about its relationship with systemic health.

Oral Health vs. Systemic Health

Oral health is often placed on a different plane of existence by the general public in comparison to physical health. When people commit to a lifestyle change, they often start by eating healthier and exercising, while neglecting oral hygiene. As a result, the importance of oral health is taken less seriously. However, the truth remains that oral health is a vital component of an individual's overall well-being. For instance, according to the CDC, oral diseases may lead to issues regarding impairments of speaking, eating, and learning[6]. Quality of life can be significantly impacted by cavities, severe gum disease, and severe tooth loss[6]. This goes to show that many health conditions are linked to oral health in ways that often escape public awareness.

Improper oral health care can result in health problems elsewhere in the body. *Propionibacterium acnes* is a bacterium that is a part of the normal microbiota of the skin, oral cavity, gastrointestinal tract, and genitourinary tract[7]. Moreover, the bacterium operates as an opportunistic pathogen that causes invasive infections, including those related to dental implants in the oral cavity[7]. Implants are a common option that dental patients resort to in order to increase the longevity of the image of their teeth. Dental infections associated with these implants have the possibility to develop into a more serious concern affecting an individual’s overall health. This shows that the state of one’s oral microbiome also impacts their overall health, and an infection in the oral cavity indicates an unbalanced microbiome. Despite the oral cavity harboring one of the most diverse and unique microbiomes, it remains relatively understudied in the field of microbiology today.

The microbiome of the gut is a popular topic of study and receives more attention in comparison to the microbiome of the oral cavity[8]. However, it's essential to note that food that is consumed enters the oral cavity prior to entering the gut. Viewing the second destination of food as more important than the gatekeeper of the human body seems almost inefficient. Studies have found associations between various diseases and changes in the abundances of organisms in the oral cavity[8]. Along with these findings, particular microbiomes within habitats of the oral cavity have direct implications with disease and infection. For example, individuals with poor dental health had tongue microbiomes enriched in pneumonia-associated bacteria[8]. Such research underscores the significant role that oral health plays in one's overall health.

While health problems can originate in the oral cavity, there are instances in which health problems lead to poor oral health. For example, diabetes, a health condition characterized by irregular blood glucose levels, reduces an individual's resistance to infection. Individuals diagnosed with this condition are more susceptible to dental issues and gum disease[9]. This finding further emphasizes the interconnected nature of oral health and general health while the two disciplines are physically separated.

More often than not, hospitals and dental offices operate as separate entities. At one point in time, dentistry wasn't a profession but a trade, and the field was reliant on medicine to build a strong foundation[10]. The tie between oral health and medical health was severed with the development of health insurance[10]. In fact, dental insurance and medical insurance serve two entirely different purposes; medical insurance is intended to cover unpredictable, high-cost expenses, and dental insurance is intended to cover predictable, low-cost preventative care[10]. This method places low-income populations at

a higher risk of being unable to afford necessary, high-cost dental care. The historical separation of dentistry and medicine remains prevalent today, but now, people are gaining awareness of the strong correlation between oral and medical health. More and more people are advocating for the need to reunite the two fields in order to make healthcare more accessible to everyone. On a personal note, oral health and holistic health is and always has been of great value to me. This genuine interest is what first peaked my interest in microbiology, particularly foodborne pathogens that enter our bodies through the oral cavity.

America's Broken Food System–A Nationwide Problem

Before food undergoes digestion in the stomach and intestines, it goes through several points in the journey before entering the human body. These points occur along the food system, which entails that many different types of foods are exposed to a diverse number of environmental factors and conditions. At these locations in the food system, food is highly susceptible to contamination when poorly packaged and handled. While food can be directly sold from farmers to consumers, it's essential to consider the entire food supply chain. America's food system begins at the farm production sector, where farmers worldwide often use pesticides to eliminate and control disease carriers in agriculture. These farmers eventually sell their products to processing and manufacturing sectors, including meat packers, bakeries, and consumer product goods companies that turn raw materials into processed food products of high value[11]. Different foods may pass through various sectors, each with its unique personnel, environment, and protocols. This begs the question: what exactly happens to our food in these different sectors of the food system? Roughly estimated 1 trillion pounds of crop products are converted into 664

billion pounds of beverages and edible food[11]. This shows that the food we digest undergoes a plethora of changes before it reaches our plate.

Our food and what it encounters play a pivotal role in all human health. Farmers often implement chemicals when growing their produce, and the use of these chemicals can have repercussions on the consumers who purchase their products. While limiting chemical use in food production may be a controversial issue, it is an important implication to consider when regarding food safety. Further, the food products can encounter various types of bacteria that detrimentally affect an individual's health and well-being. If not effectively controlled, these bacteria can contaminate the food consumed by the public, leading to foodborne illnesses across the nation.

Investigating *Listeria monocytogenes*

One specific bacterium that our food may come into contact with in the food system is *Listeria monocytogenes*. *L. monocytogenes* is a foodborne pathogen that can persist in multiple food products prevalent within the food system. This gram-positive bacterium is responsible for causing the lethal infection known as listeriosis. One is able to contract the disease by consuming contaminated food or by handling contaminated food and transferring the bacteria from their hands to their mouth. Food items that are particularly susceptible to contamination include produce, dairy products, and pre-packaged foods. Listeriosis is an uncommon but potentially fatal infection with a mortality rate of 20 to thirty percent[12].

Certain groups of individuals, such as the elderly, pregnant women, and the immunocompromised, are at a higher risk of contracting the disease[12]. Protecting these vulnerable populations from anything we consume is a significant concern in

microbiology research. While there are infection preventative strategies currently in place, we want to discover methods to weaken *L. monocytogenes* ability to cause infection before the bacteria reaches the gastrointestinal tract.

L. monocytogenes is unique as a bacterium since it has developed mechanisms to thrive even in extreme and inadequate conditions. To elaborate, *L. monocytogenes* has a tolerance for both acidic and salty conditions, high and low temperatures, and low moisture content[12]. These adaptations allow the bacteria to survive in prepackaged food products and processing plants within the food system. From an evolutionary perspective, the bacteria has been adopting survival mechanisms to persist in different environmental conditions using its own structure and resources. Results of a previous study found that *L. monocytogenes* is able to utilize three enzymes (PgdA, PbpX, and OatA) and two regulators of gene expression (DegU and Rli31) in order to resist lysozyme[13].

Furthermore, I aim to investigate the genetic factors that may contribute to the fitness and pathogenesis of *Listeria* in varying conditions. With that being said, my project centers on enhancing *L. monocytogenes* lysozyme resistance susceptibility. Increasing the bacterium's lysozyme susceptibility can diminish its fitness and pathogenicity in the human body. Lysozyme is an intriguing subject of study as there is still much to discover about its functionality and composition.

Lysozyme Susceptibility of *Listeria*

In the early 1900s, Alexander Fleming gained widespread recognition as a prominent microbiologist. He was widely recognized for his groundbreaking discovery of penicillin. Further, Fleming made the discovery of the first antibiotic in the realm of science through a series of experiments in which he tested the survival of bacteria,

specifically *Staphylococcus aureus*, when exposed to a mysterious mold that he cultivated in a petri dish[14]. After isolation of the mold, Fleming was able to determine the biology of what is now termed, penicillin, as the world's first antibiotic in history.

Prior to discovering penicillin and making history, he made an accidental discovery of the enzyme, lysozyme, in 1922[15]. Moreover, he discovered that lysozyme had an inhibitory effect on the bacteria[15]. This serendipitous discovery revealed an important function of the human immune system and ultimately led to his remarkable discovery of penicillin. However, Fleming noted that lysozyme was only effective against a small number of non-harmful bacteria[14]. He goes on to describe non-harmful bacteria as non-pathogenic bacteria, in which human tears and sputum can dissolve a majority of air-borne microbes[15]. Fleming's initial discovery of lysozyme underscored the enzyme's significance in the human body.

Lysozyme is an enzyme that catalyzes a chemical reaction, the hydrolysis of the β-1,4-linkages between N-acetyl-d-glucosamine (NAG) and N-acetylmuramic acid (NAM) residues in peptidoglycan[16]. NAG and NAM are the main components present in the bacterial cell wall, known as peptidoglycan. Peptidoglycan is a polymer composed of polysaccharide chains cross-linked by short peptides. Exposure of bacteria to lysozyme can result in the degradation of bacterial cell walls, ultimately leading to bacterial cell death. Lysozyme is abundant and naturally produced in bodily secretions, such as tears, saliva, and human breast milk[17]. Moreover, there are varying levels of lysozyme prevalent in the human spleen, thymus, and pancreas. Among various human secretions, human tears contain the highest level of lysozyme present in the body, ranging from 3000 to 5000 ppm[18].

While the entirety of the functionalities and properties of lysozyme are still unknown, it is clear that lysozyme has systemic effects on human health. For instance, sarcoidosis is the growth of collections of inflammatory cells, called granulomas, in the body. An individual with sarcoidosis has an increased concentration at onset of the disease since lysozyme plays a role in granuloma formation[19]. Additionally, amyloidosis, a disorder involving the buildup of amyloid proteins, has a connection to lysozyme. Studies have shown that hereditary renal amyloidosis is associated with a lysozyme gene mutation in Phe57Ile[20]. These results emphasize that lysozyme's function can be unpredictable. In response to the prevalence of lysozyme in the body, a wide range of bacteria has evolved resistance against the degradative action of lysozyme.

The Antimicrobial Activity of Propionate

When exploring factors that can alter *L. monocytogenes* lysozyme resistance, one particular molecule that comes to mind is propionate. The molecule is naturally present in our gut as a fermentation metabolite. Propionate is classified as a short chain fatty acid (SCFA) and has the ability to alter *L. monocytogenes* membrane composition[21]. Further, propionate is generally recognized as safe by the FDA and is used as an additive in various food products[22]. Our lab has previously demonstrated that propionate exposure in *L. monocytogenes* can lead to changes in growth and pathogenesis. For example, the production of listeriolysin O (LLO), one of the main virulence factors of *L. monocytogenes*, was impacted by propionate pretreatment depending on the availability of oxygen[23]. However, the underlying regulatory mechanisms still remain unclear. Propionate exposure significantly influenced L. monocytogenes infection outcomes, regardless of whether the treatment occurred before or during infection[24]. Our

examination of isogenic mutants of *L. monocytogenes* has allowed us to determine if genetics play a role in pathogenesis and infection. We have previously found that CodY plays a role in the regulation of virulence genes, which may suggest that its presence is detrimental to the infection process[25].

To determine how propionate exposure affects *L. monocytogenes* survival and fitness in the gastrointestinal tract, my thesis project therefore studies the effects of propionate on *L. monocytogenes* resistance to lysozyme, which is found in our saliva. If propionate enhances *L. monocytogenes* lysozyme resistance, the use of propionate in food products might contribute to *L. monocytogenes* survival during transmission between food and our gastrointestinal tract. However, if propionate decreases *L. monocytogenes* resistance to lysozyme, it could be beneficial to use propionate as an efficient infection preventative strategy. To better understand the functions of propionate in *L. monocytogenes* lysozyme resistance, I conducted a series of experiments examining several factors including oxygen variability, genes, and varying concentrations of lysozyme.

Methods

Strains

Listeria monocytogenes wild type (WT) strain 10403s and two isogenic mutants of *L. monocytogenes* were utilized in experiments. These mutant strains include the *ΔcodY* and Δ*sigB*, which have clean deletions in the *codY* and *sigB* genes, respectively. On a weekly basis, these strains were streaked onto fresh LB (Fisher BP 14262) or brain heart infusion (BHI, BD 211059) plates.

Growth Conditions

For each experiment, *Listeria* was grown overnight in BHI media, which was prepared by filter sterilization to ensure consistency across batches. Subsequently, overnight liquid cultures were incubated in either aerobically or anaerobically environments at the temperature 37℃. Under aerobic conditions, the culture tubes were agitated at 250 rpm. On the other hand, the anaerobic cultures were placed in an anaerobic chamber (Type A, Coy Laboratory) with a nitrogenous atmosphere, 2-3% hydrogen gas, a dehumidifier, and a palladium catalyst to remove residual oxygen.

Propionate Supplementation

Propionate was used in its salt form, sodium propionate, to maintain a stable pH of the BHI media. Sodium propionate stock solutions at 1 M were prepared through filter sterilization, aliquoted, and stored in the freezer prior to experimentation. The treated bacterial cultures received 25 mM propionate before overnight incubation.

Lysozyme

Lysozyme, sourced from chicken egg white in powdered form, was typically dissolved in a liquid solution for our project. Lysozyme stock solutions, at 100 mg/5 mL, were also prepared through filter sterilization, aliquoted, and stored in the freezer prior to experimentation. We tested various concentrations of lysozyme in the following experiments.

Lysozyme Growth Assay

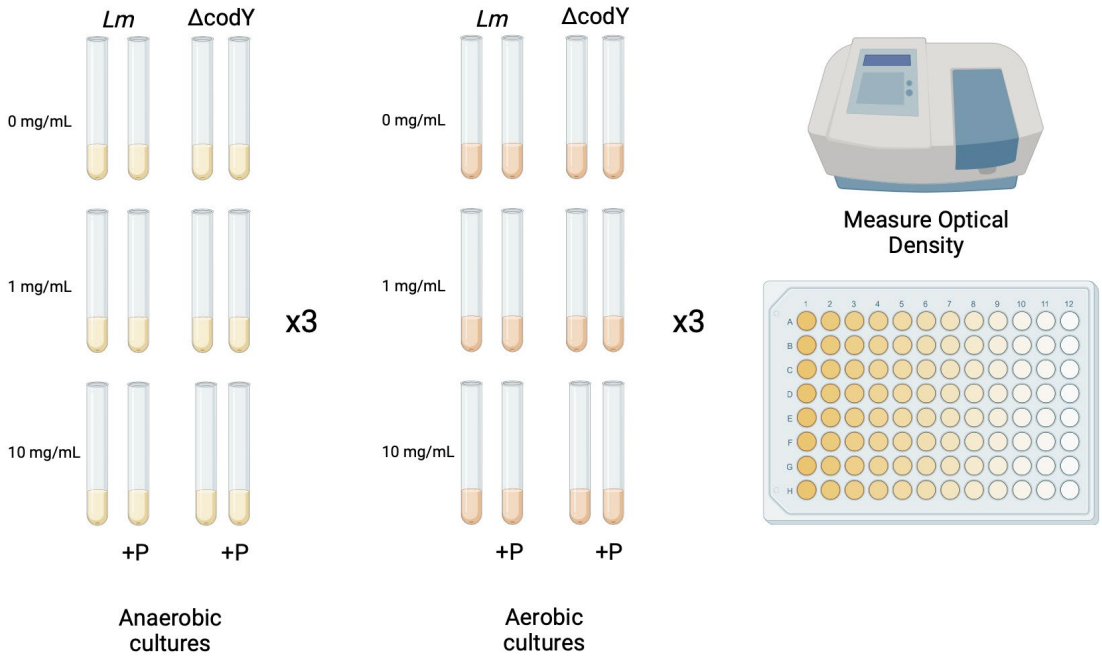

Figure 1. A schematic of Lysozyme Growth Assay Protocol. Image created in BioRender.com.

In the first set of experiments, we assessed the growth of *Listeria monocytogenes* under various conditions, including the absence or presence of oxygen, 0 mM or 25 mM propionate, and 0 mg/mL, 1 mg/mL, or 10 mg/mL lysozyme. While performing this

assay to study the growth of *L. monocytogene*s in response to stress, I collaborated with my colleague, Elizabeth Herr. In order to sufficiently measure such growth across the different experimental conditions, we filter sterilized 150 mL BHI separately from 100 mL BHI with 10 mg/mL lysozyme. Using these two solutions, we created three BHI growth media with 0 mg/mL, 1 mg/mL, or 10 mg/mL lysozyme in three conical tubes, each inoculated with one wild type colony. We repeated this step with the *ΔcodY* mutant strain with another three conical tubes. Following this, we prepared and labeled 72 plastic culture tubes and transferred 50 uL propionate into the designated tubes. Using an electronic pipette, we transferred 1 mL from each of the inoculated conical tubes into 12 of the designated culture tubes. Once all proper volumes were transferred, we stored the aerobic cultures in the incubator and the anaerobic cultures in the anaerobic chamber for 16 to 18 hours. Following 16 to 18 h of incubation time, we collected optical density data and performed t-tests to determine statistical significant differences between the tested conditions. After thorough analysis of these data, I repeated this experimental procedure with the Δ*sigB* deletion mutant. The data was analyzed by various comparisons and described under “Results.”

Lysozyme Survival Assay

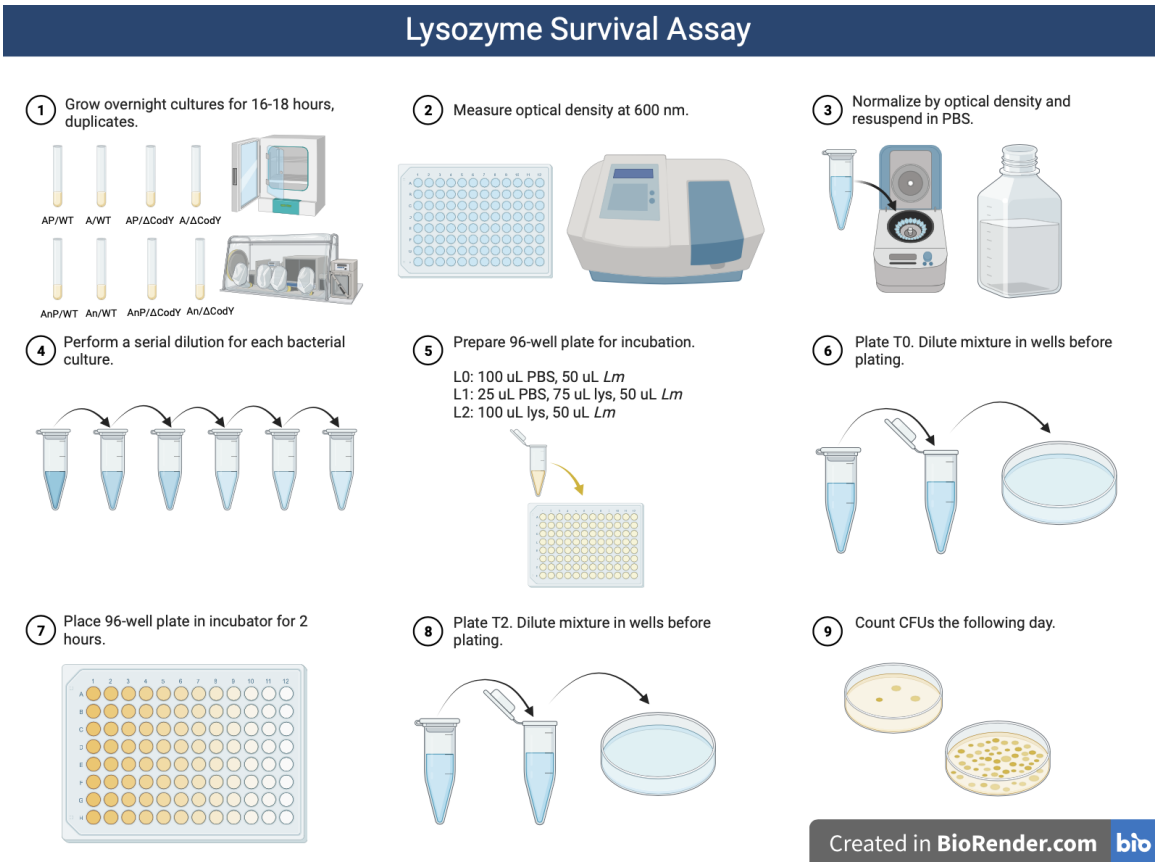

Figure 2. A schematic of Lysozyme Survival Assay Protocol. Image created in BioRender.com

In the second set of experiments, we assessed the survival of *Listeria monocytogenes* after lysozyme exposure under various conditions, including the absence or presence of oxygen, 0 mM or 25 mM propionate, and 0 mg/mL, 10 mg/mL, or 20 mg/mL lysozyme. Overnight cultures were incubated at 37°C either aerobically or anaerobically. Overnight is also defined at 16 to 18 hours in this protocol. Following the incubation period, optical density data was collected at an absorbance of 600 nm. Next, 1 mL of each culture was spun down in the microcentrifuge, and the supernatant was properly disposed of via vacuum flask. I normalized each culture by their respective absorbance value and resuspending the spun down bacteria in phosphate-buffered saline (PBS) to OD of 1. Once normalized by optical density, I performed a 1:1000 serial dilution. A 96-well plate was prepared for the second half of the experiment, consisting of survival testing. The wells labeled with 0 mg/mL lysozyme had 50 uL of bacteria and 100 uL PBS. The wells labeled with 10 mg/mL lysozyme had 50 uL of bacteria, 7.5 uL lysozyme, and 92.5 uL PBS. Lastly, the wells labeled with 20 mg/mL lysozyme had 50 uL bacteria, 75 uL lysozyme, and 25 uL PBS. Before plating T0, the bacteria from the wells were diluted to a factor of 1:100. To note, several trials were performed to determine the correct dilution factor required for measurable colonies. After T0 was plated, the 96-well plate is placed into the incubator at 37°C for 2 hours. Following this incubation period, T2 was plated with a dilution factor of 1:100, and colonies were counted 24 to 36 h post plating. The data was analyzed by various comparisons and described under “Results.”

Results

To gain a better understanding of how propionate affects *L. monocytogenes* growth after a prolonged period of time, Elizabeth Herr and I collaborated on a series of growth experiments. We measured the growth of *L. monocytogenes* by collecting optical density data from our overnight cultures. With the addition of propionate, we found that bacterial growth decreased under aerobic conditions (Fig. 3). Moreover, the combination of 1 mg/mL lysozyme and propionate also hindered *L. monocytogenes* growth in our overnight cultures (Fig. 3). Under anaerobic conditions, the combination of 1 mg/mL lysozyme and 25 mM propionate significantly decreased the growth of *L. monocytogenes* (Fig. 4). Subsequently, the addition of propionate alone and lysozyme alone resulted in a significant reduction of bacterial growth, however, the growth of propionate was significantly greater than lysozyme-only cultures (Fig. 3). Additionally, the growth of cultures with only lysozyme was significantly less than cultures with a combination of lysozyme and propionate (Fig. 4).

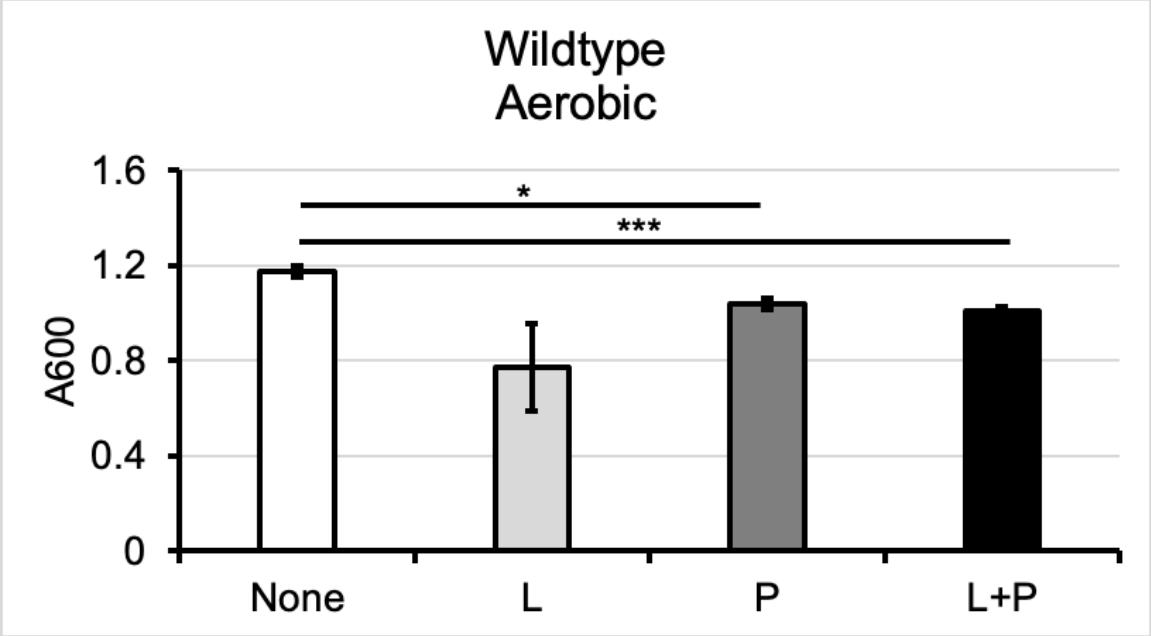

Figure 3. Mean optical density values of aerobic wild type cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

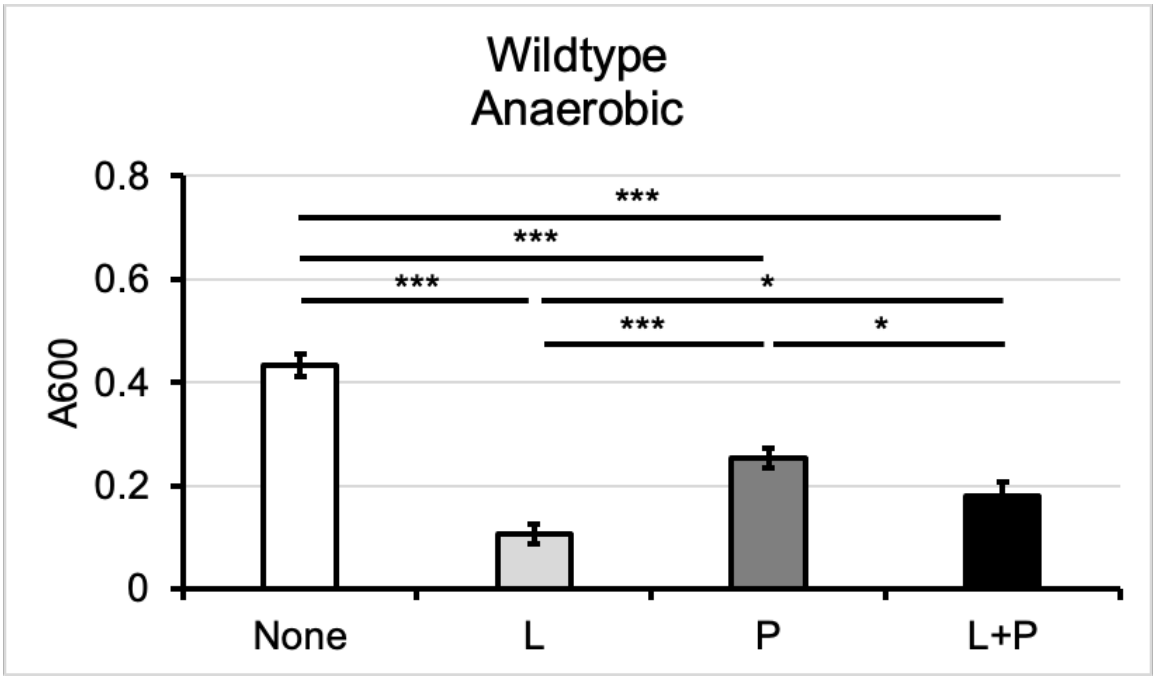

Figure 4. Mean optical density values of anaerobic wild type cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

To further investigate the effects of propionate on *L. monocytogenes* growth, we incorporated two isogenic mutants, *ΔsigB* and *ΔcodY* . Under aerobic conditions, we found significantly less bacterial growth comparing the control with the lysozyme propionate combination in the two mutants (Fig. 5 & 6). There was a significant decrease in the amount of bacteria in the lysozyme and propionate cultures compared to propionate-only cultures in both mutants (Fig. 5 & 6). Further, the addition of propionate alone and lysozyme alone also resulted in a significant decrease of bacterial growth in the *ΔcodY* mutant (Fig. 5). To conclude analysis of the aerobic mutant cultures, there was significantly less bacterial growth in lysozyme and propionate cultures than lysozyme-only cultures in the *ΔcodY* mutant (Fig. 5).

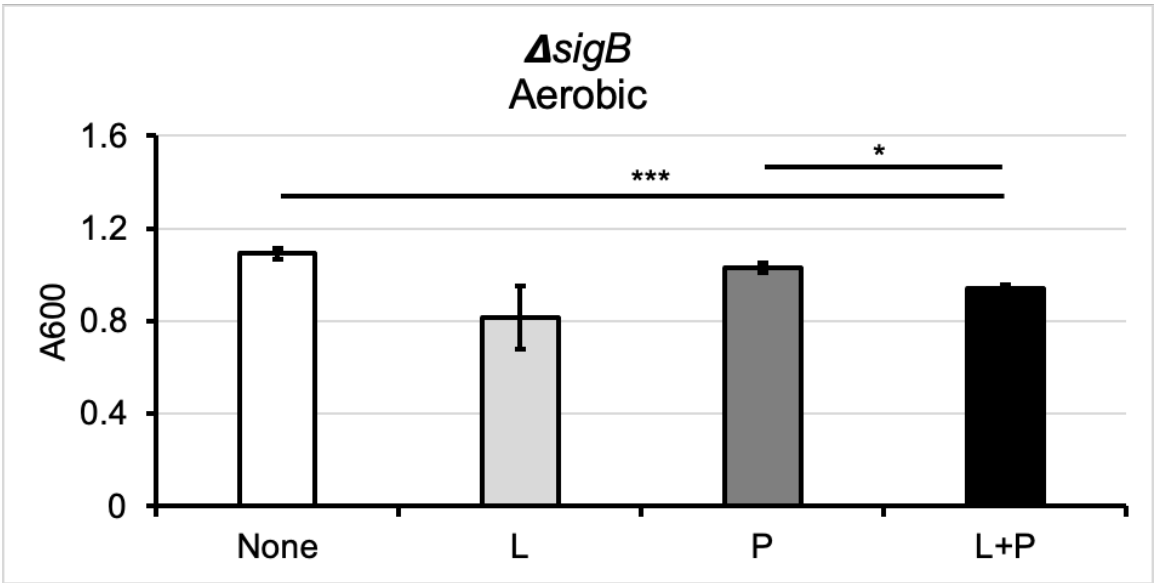

Figure 5. Mean optical density values of aerobic *ΔsigB* cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

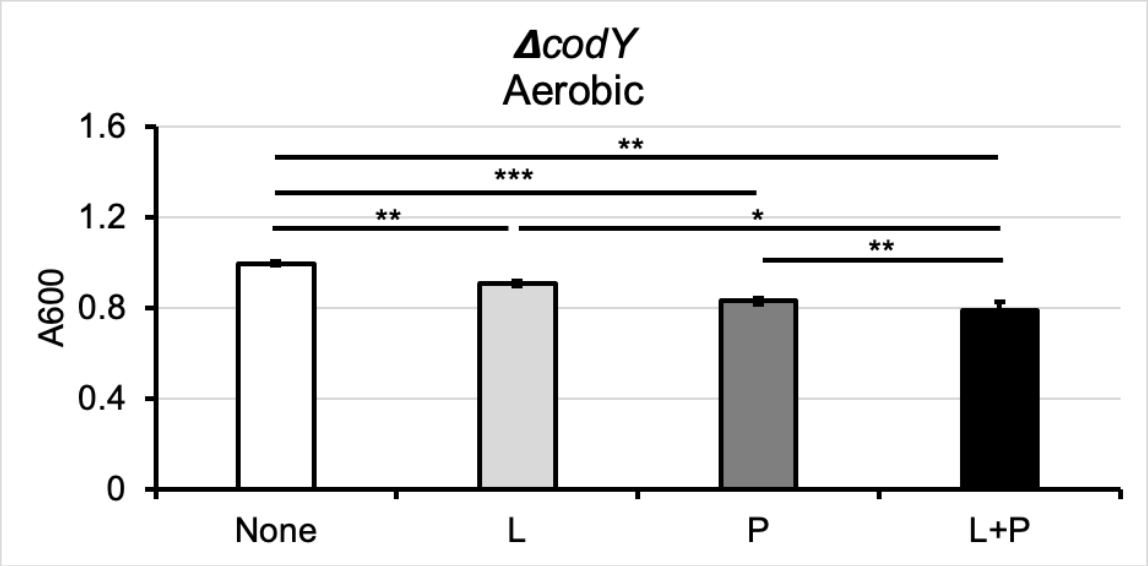

Figure 6. Mean optical density values of aerobic *ΔcodY* cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

Under anaerobic conditions, both the mutants exhibited a significant decrease comparing the control to lysozyme-only cultures (Fig. 7 & 8). For the *ΔsigB* mutant, propionate-only cultures and the combination showed significantly less bacterial growth when compared to the control (Fig. 7). Regarding the *ΔcodY* mutant, there was significantly less bacteria in the combination cultures than the propionate-only cultures (Fig. 8).

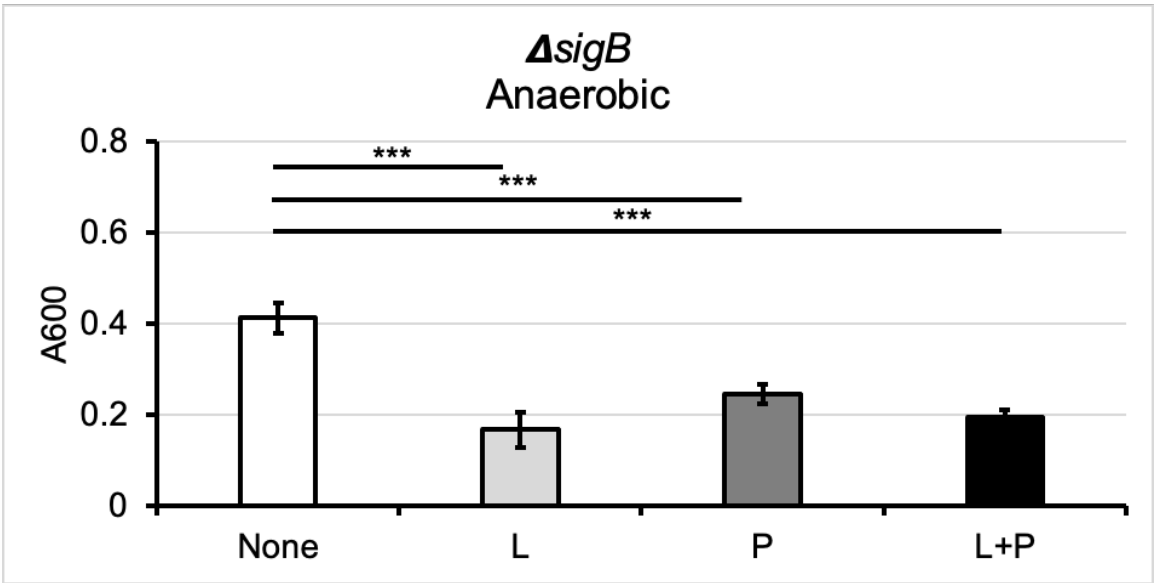

Figure 7. Mean optical density values of anaerobic *ΔsigB* cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

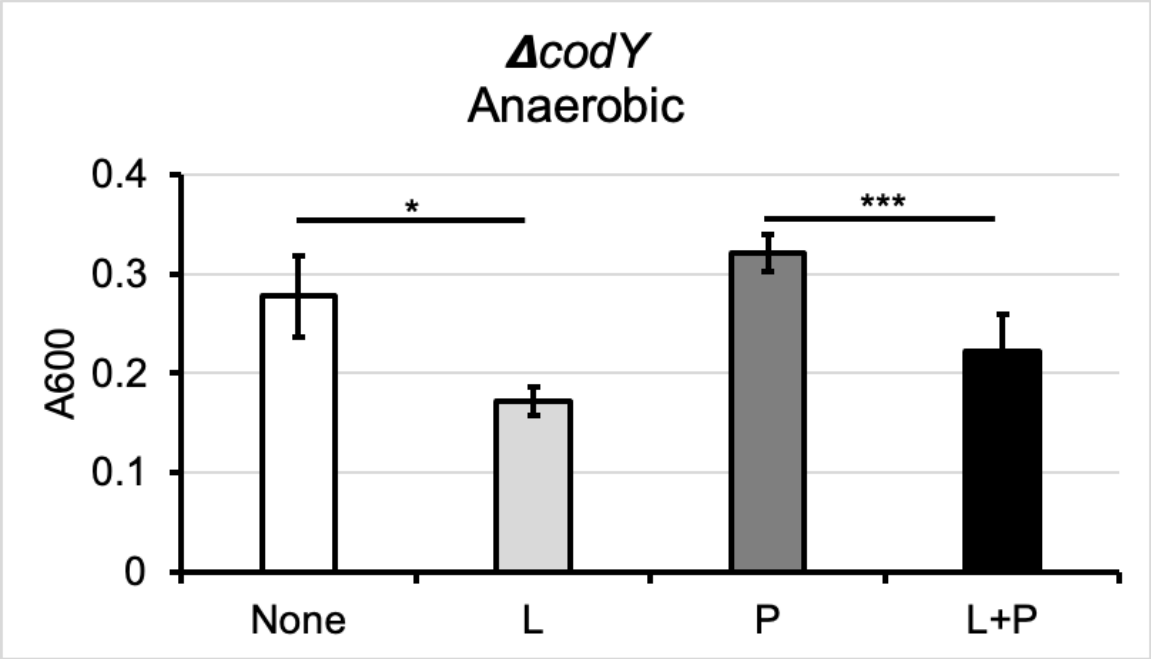

Figure 8. Mean optical density values of anaerobic *ΔcodY* cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

For a better visualization of the role that genetic factors play in *L. monocytogenes* growth, data analysis was performed between the wild type (WT) strain and the isogenic mutants. Under aerobic conditions, each condition (shown in the x-axis) demonstrated a significant decrease in bacterial growth in the *ΔcodY* mutant compared to wild type (Fig. 9). For the control, a significant increase is shown between the WT and the *ΔsigB* mutant (Fig. 9). Further, the lysozyme and propionate cultures demonstrated a significant decrease of growth between WT and the *ΔsigB* mutant (Fig. 9). Under anaerobic conditions, the data exhibits a significant increase in growth between WT and the *ΔcodY* mutant in both propionate only cultures and the combination of propionate and lysozyme (Fig. 10).

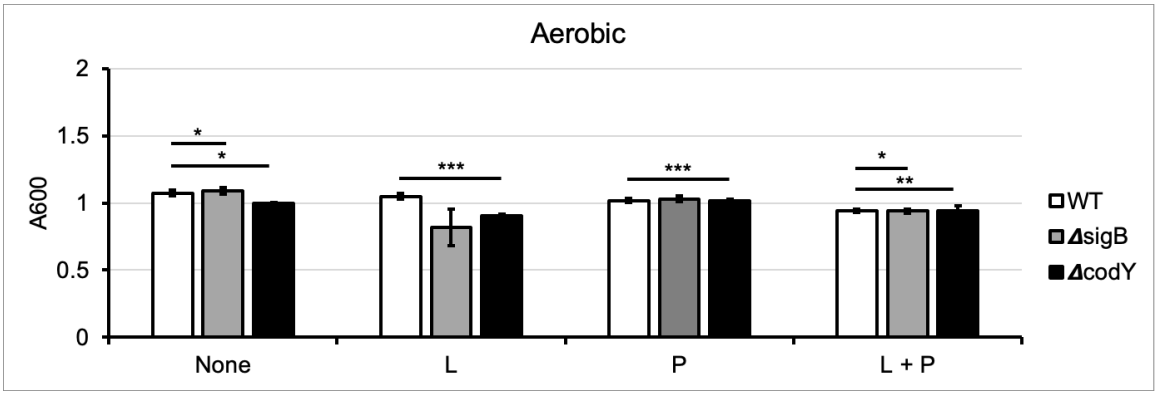

Figure 9. Mean optical density values of aerobic WT, *ΔsigB, ΔcodY* cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

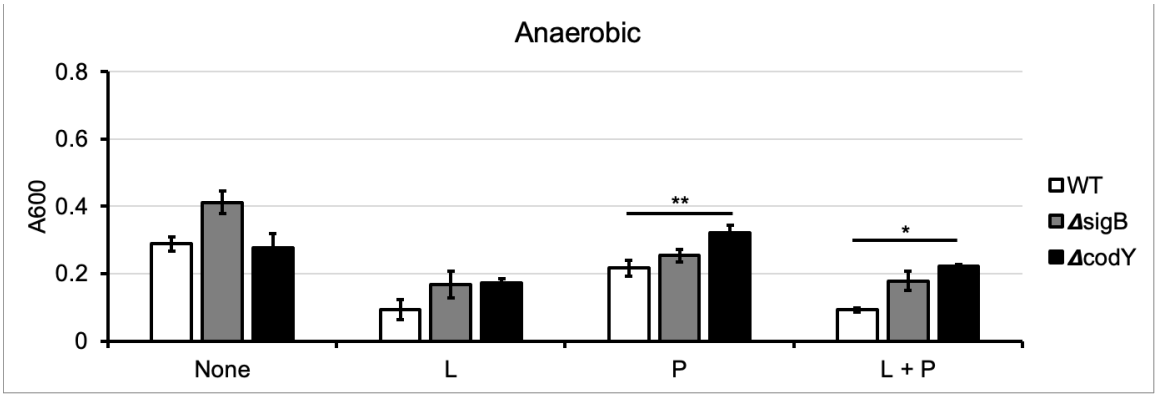

Figure 10. Mean optical density values of anaerobic WT, *ΔsigB, ΔcodY* cultures measured as absorbance at a wavelength of 600 nm. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 9 replicates from 3 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. None = control, L = 1 mg/mL lysozyme, P = 25 mM propionate, L+P = 1 mg/mL lysozyme and 25 mM propionate.

Survival assay data illustrated the proportion of bacteria that survived after lysozyme exposure for 2 hours. Among the anaerobic wild type cultures, there was a significant decrease in survival from the control (no lysozyme) to 20 mg/mL lysozyme when treated with propionate (Fig. 11). However, for the mutant strain, the *ΔcodY* mutant showed a significant increase in survival between aerobic and anaerobic bacteria without lysozyme exposure (Fig. 12). With the addition of 10 mg/mL lysozyme, there was a significant increase in survival between aerobic and anaerobic bacteria (Fig. 12). Additionally, the addition of 20 mg/mL lysozyme caused a significant decrease in survival in aerobic bacteria exposed to 25 mM propionate (Fig. 12). Similarly, there was a significant decrease in survival for bacteria that experienced anaerobic propionate exposure following the addition of 10 mg/mL lysozyme (Fig. 12). Lysozyme exposure of 10 mg/mL and 20 mg/mL resulted in a significant decrease of survival in anaerobic bacteria of the mutant strain (Fig. 12).

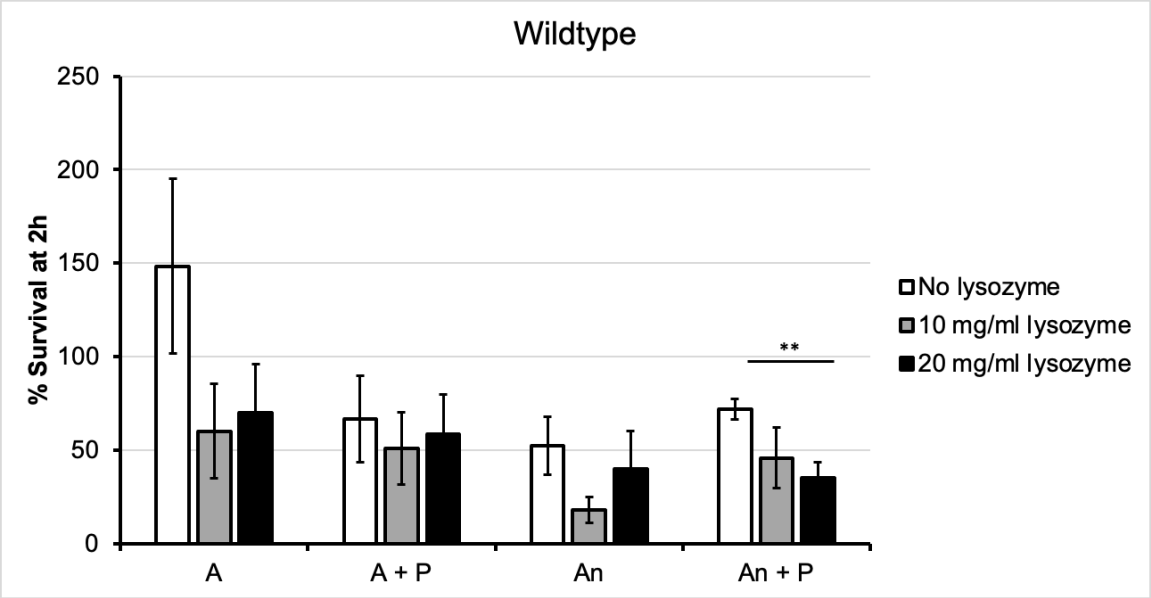

Figure 11. Lysozyme survival percentages of wild type bacteria after 2 hours of lysozyme exposure. The x-axis represents aerobic and anaerobic bacteria either with or without 25 mM propionate. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 10 replicates from 5 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. A = aerobic bacterial, A+P = aerobic bacteria and 25 mM propionate, An = anaerobic bacteria, An+P = anaerobic bacteria and 25 mM propionate.

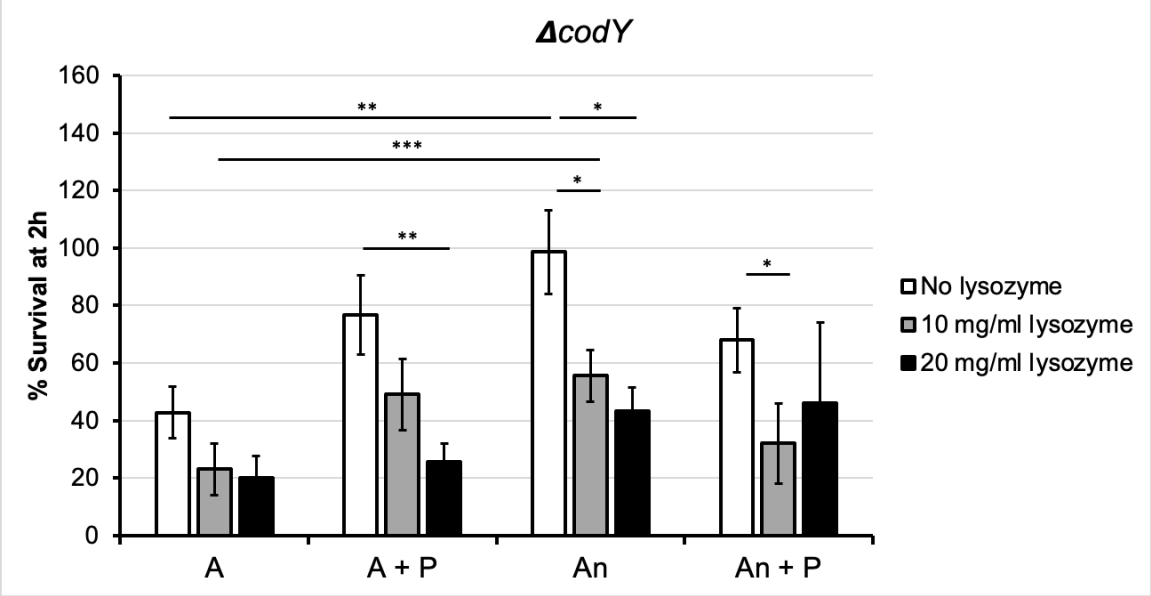

Figure 12. Lysozyme survival percentages of *ΔcodY* bacteria after 2 hours of lysozyme exposure. The x-axis represents aerobic and anaerobic bacteria either with or without 25 mM propionate. The cultures were incubated at 37°C in filter-sterilized BHI. Averages of 10 replicates from 5 independent experiments were plotted with error bars representing standard errors of the means. Asterisks indicate significant difference in pair-wise comparison based on student's t-test with "*" representing $0.01<p<0.05$ and "***" representing $p<0.001$. A = aerobic bacterial, A+P = aerobic bacteria and 25 mM propionate, An = anaerobic bacteria, An+P = anaerobic bacteria and 25 mM propionate.

Conclusion

Lysozyme serves as a first line of defense against the transmission of foodborne pathogens by degrading peptidoglycan, a major component in gram-positive cell walls. Propionate, a commonly used food additive and gut metabolite, was considered for its potential impact on the efficacy of lysozyme as a defense mechanism. The results of multiple experimental trials are summarized in the table below. Survival experiments with the *ΔsigB* mutant have yet to be conducted.

Does propionate affect *L. monocytogenes* growth and/or survival in lysozyme?				
	Growth (1 mg/mL lysozyme)		Survival (10 mg/mL lysozyme)	
	Aerobic	Anaerobic	Aerobic	Anaerobic
WT	No	Yes, propionate increases growth	No	No
ΔcodY mutant	Yes, propionate decreases growth	No	No	No
ΔsigB mutant	No	No	No Data	No Data

These results hold relevance for two different sites within the gastrointestinal tract. First, the oral cavity is a primarily aerobic environment encountered by pathogens such as *L. monocytogenes*. Second, the intestinal tract, where listeriosis infection festers, is an anaerobic environment. In addressing our main research question, "does propionate affect *L. monocytogenes* lysozyme susceptibility," we observed that propionate significantly increased bacterial growth in lysozyme for anaerobic wildtype bacteria. This suggest that propionate may have a role in *L. monocytogenes* ability to persist in the human gut. We also found that this phenotype is absent when the *codY* gene is not present, suggesting a possible role for the *codY* gene in the response of *L. monocytogenes* to anaerobic propionate exposure. Despite the enhancing effects of propionate on anaerobic growth in lysozyme, we do not see significant effects of propionate on *L. monocytogenes* anaerobic lysozyme survival. As to why this might be the case, we rationalized that because the bacteria in my growth experiments were exposed to lysozyme for a prolonged period of time (16-18 h) at a lower lysozyme concentration as opposed to 2 hours of incubation in survival experiments with a higher lysozyme concentration. Additionally, in the survival experiments, colonies were counted 24 to 36 h post-plating, allowing the bacteria time to recover from prior lysozyme exposure. The implications of these results mean that the amount of lysozyme found in human saliva is not sufficient enough to prevent the spread of *L. monocytogenes*. In the anaerobic environment of the intestinal tract, propionate appears to enhance the growth of the pathogenic bacterium. Essentially, propionate potentially enhances *L. monocytogenes* persistence under anaerobic conditions and further research is required to determine the mechanistic details and potential applications.

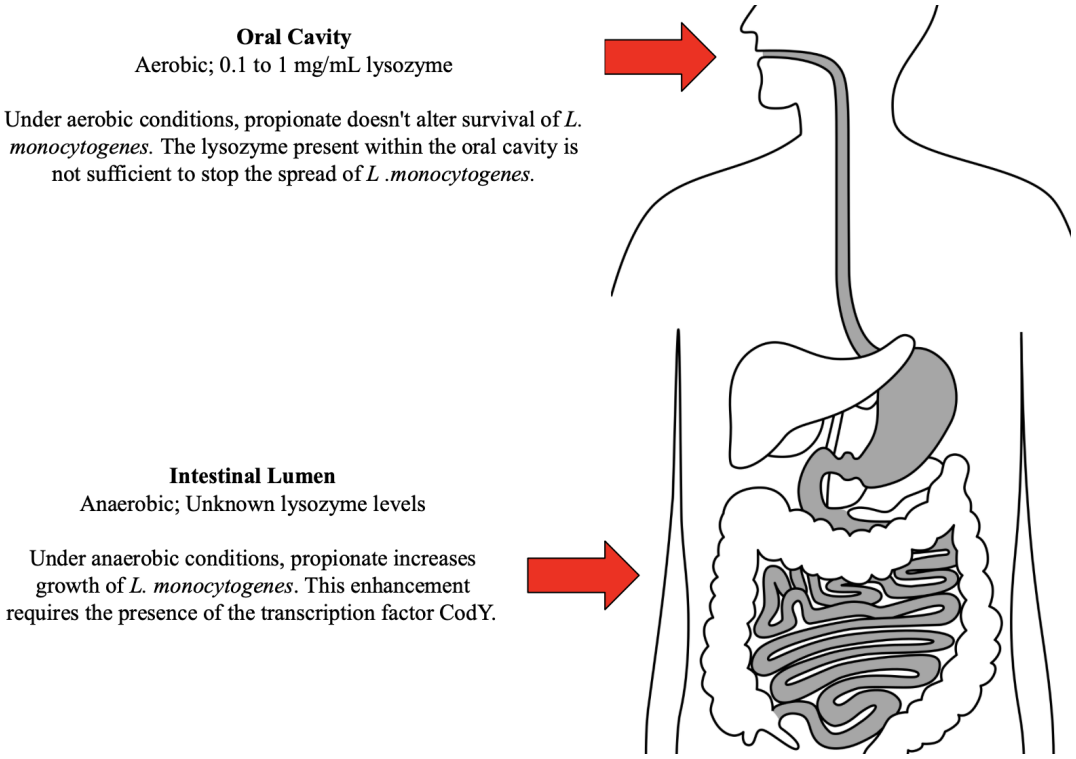

Figure 13. A schematic of implications of our research results.

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Personal Reflection

When I enrolled at the University of Dayton as a pre-dentistry major, I had not anticipated devoting a majority of my studies to independent research and lab work. Looking back, I am profoundly grateful for the opportunity that introduced me to the research process and thesis work as a Berry Summer Thesis Institute Scholar during the summer of 2022. The subsequent year, being a Dean's Summer Fellow, allowed me to fully immerse myself in my work and gain an appreciation for the intricate and impactful aspects of research. While working on my project, I have learned a great deal about myself in terms of the limits of my knowledge, what I can overcome, and what ignites my passion. As I currently pursue admission to dental school, I aim to explore research avenues in the dental profession and continue pushing the boundaries of my academic aspirations.

The reality is that one should never form preconceived expectations about research and lab without experiencing it firsthand. Scientists often discover unexpected findings and may encounter trials that don't yield the anticipated results, and that is perfectly fine. Sometimes the data doesn't answer your initial questions but instead lead to new inquiries. All my initial expectations were swiftly replaced by the actual experience of working in a microbiology lab. I experienced disheartening, humbling, and rewarding experiments and projects that only furthered my curiosity about the subject matter.