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Understanding the Effects of Propionic Acid on Listeria monocytogenes Growth, Survival, and Virulence

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Abstract

Listeria monocytogenes is a foodborne pathogen that causes illnesses with a high fatality rate immunocompromised and elderly individuals[1]. It colonizes the human intestine, which contains low levels of oxygen but high levels of fermentation acids. This work investigates how that environment affects various aspects of *Listeria* pathogenesis. Based on these results, we can ascertain whether propionate supplementation can be explored as an effective method of protecting individuals from *Listeria* infection.

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Introduction

Human health is shaped by our microbiomes – a collection of bacteria, viruses, archaea, and eukaryotic organisms that take residence within our bodies. For example, metabolism of dietary phosphatidylcholine by gut microbes has been correlated with a significantly increased risk of cardiovascular disease[1]. Disturbance of gut microbiota is correlated with neurodegenerative disorders like Alzheimer's disease[2]. Additionally, alterations of the gut microbiota by antibiotics have been shown to increase patient susceptibility to infections by *Clostridium difficile*[3]. However, despite the known correlations of the human microbiome in human health, determining which of its elements lead to these health impacts is a challenging task. Our gut microbiota, for example, differs from person-to-person and changes based on diet and other environmental and host factors. As a result, many studies on the composition of the gut microbiome have taken a bioinformatics approach, using computer algorithms and statistics to analyze and interpret large amounts of biological data to decipher complex correlations between the human gut microbiome and health outcomes. To complement these correlative analyses, our lab takes a reductionist approach, in which we examine individual facets of the microbiome and extrapolate our findings to its broader nature. This approach allows us to offer insight into specific mechanisms of how the human gut microbiota impacts human health.

One aspect of the human gut microbiome is the production of short-chain fatty acids (SCFAs). SCFAs are fatty acids with six or fewer carbons, and they are a product of human gut bacteria fermentation of carbohydrates. The three most common SCFAs are acetate, propionate, and butyrate. Previous research has demonstrated positive correlations between the presence of SCFAs and good health. For example, butyrate was shown to inhibit

cancerous colonocyte proliferation and stimulate cancer cell death in mice[4]. SCFAs have also been shown to have a role in preventing dietary-induced obesity and beneficially impacting glucose metabolism[5]. In addition to understanding these connections between SCFAs and human health, we also know that SCFA levels in the human gut decrease with age[6]. It remains unclear how this decrease in SCFA concentration impacts the overall health of the older population.

However, we do know elderly people are more susceptible to infection by *Listeria monocytogenes*, often exhibiting a fatality rate as high as 30%[7]. One study showed that, between June 2004 and December 2008, 67% of reported *Listeria* cases in Hong Kong were in people above the age of 51[8]**.** The disproportionately high infection and fatality rate is largely due to the weakening of the immune system as we age[9]. Because fatal *Listeria* infections result from *Listeria* breaking the intestinal wall and spreading to other organs like brains and hearts, prevention of fatal infections relies on blocking *Listeria* from breaking the intestinal wall, particularly in the elderly population with a weakened immune defense.

During *Listeria* infections, the pathogen encounters and must adapt to the chemical content of the intestine in order to escape and cause disease. Since that chemical content changes as we age, there is a possibility that those changes – including the decrease in SCFA concentration referenced above – render the elderly more susceptible to *Listeria* infections. If this possibility is true, enriching SCFA levels in the elderly population will help protect them from fatal *Listeria* infections. One of the three most common SCFAs in the human gut is propionate. Propionate is produced under anaerobic conditions within our intestinal lumen, where *Listeria* encounters propionate during its intestinal phase of

infection. However, no research has been done to examine *Listeria* regulation and pathogenesis in response to propionate under anaerobic conditions.

Our lab studies *Listeria* in its interactions with this environment. We hypothesize that propionate serves a protective role against *Listeria* infections. Proving our hypothesis would allow further research into ways to protect the elderly population from *Listeria* infection by safely modifying the intestinal SCFA content to increase the presence of propionate. This could serve as a mechanism of strengthening the elderly's intestinal chemical defense against *Listeria*.

To test our hypothesis, we examined the effects of propionate supplementation on anaerobically-grown *Listeria* in order to approximate the responses from *Listeria* after exposure to environmental conditions in the intestinal lumen before invading our intestines. We tested this with a variety of methods, including the measurements of bacterial growth and fitness, infection ability, level of cytosolic bacteria after phagosomal escape, and toxin and metabolite production. Finally, we isolated *Listeria* from garden vegetables to demonstrate the relevance of the data we obtained using our laboratory strain. Results showed that propionate is a determinant in *Listeria* physiology and pathogenesis, and the effects of propionate on *Listeria* exhibit a strong dependency on the availability of oxygen.

Materials and Methods

Bacterial strains and culture conditions

Two *Listeria monocytogenes* strains were used in these studies: (1) 10403S wild-type and (2) Δ*hly*, a mutant strain which does not produce listeriolysin O due to a deletion of the *hly* gene. *Listeria* were grown overnight from single colonies <1 week old in filter-

sterilized brain heart infusion (BHI) media (Lot 4176589). Bacteria were grown in a 37^{0}C incubator while shaking at 250 rpm. Bacteria were also allowed to grow statically in a temperature-controlled incubator in an anaerobic chamber (Coy Laboratory, Type A). The chamber maintains a nitrogenous atmosphere with 2.5% hydrogen. *Listeria* were grown for 16-18 hours, unless otherwise noted. After growth, optical density (OD) was measured in a 96-well plate at 600 nm with a volume of 200 μL per well using a 96-well plate reader (Biotek Synergy4).

Bacteria were grown with or without supplementation of sodium propionate (Alfa Aesar A11148) at 0, 5, 15, and 25 mM concentrations. Sodium propionate stock solutions were prepared at 1 M in deionized water and stored at -21^{0}C.

Cell culture infections

Murine peritoneal macrophages RAW 264.7 (ATCC TIB-71) were kept in culture in Corning cellgro Dulbecco's Modification of Eagle's Medium (DMEM) 1X (Thermo Scientific SH30285.01) with 4.5 g/L glucose, L-glutamine, and sodium pyruvate, supplemented with 10% heat-inactivated fetal bovine serum (FBS) (JRScientific REF 4365-500, Lot N056-6). Cells were stored in a 37^{0}C incubator with 5% CO_2 and passaged every 3 to 4 days with 100 U/mL Penicillin and 100 μg/mL Streptomycin (BioWhittaker Penicillin/Streptomycin Lot 0515M10507). Caco-2 colorectal adenocarcinoma cells were cultured with Corning cellgro DMEM 1X supplemented with 20% heat-inactivated FBS. They were kept in a 37^{0}C incubator with 5% CO_2 and passaged every 4 to 6 days with 100 U/mL Penicillin and 100 μg/mL Streptomycin (BioWhittaker Penicillin/Streptomycin Lot 0515M10507).

Host cells (6 x 10^6 cells per plate) were seeded into 24-well tissue-culture plates the day before infection. The day of infection, optical densities were measured of overnight bacterial cultures. Bacterial growth was normalized and converted to volume using the conversion factor: 0.722 = 4.78 x 10^8 CFU/mL. Bacteria were harvested, centrifuged, and washed with PBS (-/-) and then added to host cells at a multiplicity of infection (MOI) of 10. Infected cells were incubated for 30 minutes at 37^0C. Cells were then washed twice with phosphate-buffered saline PBS (+/+), and 1 mL Corning cellgro DMEM 1X media containing 10 μg/mL gentamicin was added to clear extracellular bacteria. Cells were lysed after 1, 2, 4, and 8 hours using 200 μL 0.1% filter-sterilized Triton X solution per well. Intracellular bacteria were then enumerated on Luria-Bertani (LB) agar plates, and colonies were counted using an automatic colony counter (Synbiosis aCOLyte 3) after 48-72 hours of incubation in a 37^0C incubator. Percent invasion was calculated by the equation: % invasion = [(CFU at the final time point − CFU at the initial time point)/CFU at the initial time point] × 100. Intracellular growth was calculated by the equation: # colonies at time point/50 μL bacteria plated x 200 μL bacteria and Triton X solution x 100].

Immunofluorescence microscopy

RAW 264.7 macrophages were seeded onto sterile cover slips (22 x 22 mm) in 6-well plates at 1 x 10^6 cells per well and allowed to replicate for 18-24 hours. Overnight *Listeria* cultures were harvested, centrifuged, and washed with PBS (-/-) and then added to host cells at an MOI of 10. Infected cells were incubated for 30 minutes at 37^0C. Cells were then washed twice with phosphate-buffered saline PBS (+/+) and 1 mL Corning cellgro DMEM 1X media containing 10 μg/mL gentamicin was added to remove extracellular

bacteria. After 2 hours, cover slips were fixed in 4% paraformaldehyde and stored overnight at 4^0C.

Cover slips were washed once with 10 mL TBS-T (25mM Tris-HCl, 150 mM NaCl, 0.1% Triton X-100) and blocked with 1% bovine serum albumin (BSA) dissolved in 500 mL TBS-T. The blocking antibody was removed after 20 minutes and a primary anti-*Listeria* antibody (1:500 in TBS-T with 1% BSA; Thermo Scientific PA1-30487) was added. Cover slips were incubated at room temperature without light for 1 hour. They were washed with 10 mL TBS-T before adding the secondary antibodies: phalloidin-iFluor 594 (1:400, abcam ab176757) and AlexaFluor 488—goat anti-rabbit antibody (1:400, abcam ab150077) in TBS-T with 1% BSA. Cover slips were allowed to incubate for 30 minutes at room temperature without light. They were then washed 3 times with 10 mL TBS-T and suspended in 1 mL TBS-T. Sterile tweezers were used to lift individual cover slips and transfer them onto slides. Prolong Gold was used to protect the integrity of the cover slips. Slides were stored at 4^0C.

Slides were later viewed with an immunofluorescent microscope (Olympus DP 71, Olympus BX-51 light), and the number of actin clouds was counted for 100 intracellular bacteria.

Hemolytic assays

Hemolytic assays were performed using the supernatant from *Listeria* cultures grown overnight. Samples were added to wells with 5 μL 0.1 M DTT and incubated at room temperature for 15 minutes. Blank BHI media was used as a negative control, and 0.4% Triton X-100 was used as a positive control for each experiment. Samples were then

serially diluted using hemolysis buffer containing 35 mM dibasic sodium phosphate and 125 mM sodium chloride, brought to pH 5.5 using acetic acid. Defibrinated sheep's blood (Hemostat Laboratories DSB050) was diluted to a hematocrit of 2% and added to samples to achieve 1% final hematocrit. Samples were then incubated at 37^0C. After 30 minutes incubation, samples were spun down at 2,000 RPM for 5 minutes to pellet intact blood cells. Supernatant lysate (120 μL) was placed into a flat-bottom 96-well plate to measure OD at 541 nm as an indicator for LLO activity. To find hemolytic unit, we calculated the inverse of the dilution factor at which half-complete lysis occurred, and normalized that value with original culture OD measured at 600 nm. The hemolytic units of samples not producing over half-complete lysis were designated as "below detection." This "below detection" designation was typical for supernatant samples from anaerobic cultures.

Measurement of acetoin and ethanol concentrations

Acetoin production in the supernatant of overnight *Listeria* cultures was quantified by an adapted Voges-Proskauer test[10]. 100 μL of supernatant or the given standard was placed into a sterile micro-centrifuge tube. Next, 50 μL of 5% creatine monohydrate (Sigma C3630-100G), 100 μL of 1-Napthol (Sigma N1000-10G), and 100 μL of 40% KOH (Chempure 831-704) in 95% EtOH were added to the tubes. Between each addition, samples were centrifuged. After the final addition, samples were incubated for 15 minutes at room temperature. Next, 200 μL of each sample was placed into a 96-well flat-bottom plate and the absorbance was read at 560 nm. Based on a standard curve, the concentration of acetoin in culture supernatant samples was calculated.

Percentage of ethanol was determined using a commercially-available enzymatic kit following manufacturer's suggested protocol (Fisher 50-489-254).

Isolation of *Listeria* from garden vegetables

A sample of garden lettuce was weighed to 25 grams and placed in a blender. The blender was washed with 70% ethanol and sterile distilled water between each sample. Each lettuce sample was blended with one bottle of Buffered *Listeria* Enrichment Broth (BLEB) for 30 seconds. The lettuce mixture was then poured into a bottle for temporary storage. This process was repeated 5 more times for a total of 6 samples. During this process, half of the samples were washed under tap water for 15 seconds before being blended with a bottle of BLEB. Next, 30 mL of lettuce mixtures were centrifuged at 10,000 RPM for 10 minutes at 3^0C. Then, 27 mL of supernatant were discarded. The tube was vortexed to re-suspend the bacteria and lettuce pellet into the 3 remaining mL of liquid. Three new culture tubes for each sample were obtained (labeled d2 d3 and d4) and filled with 3 mL of BLEB. Then 1 new culture tube per sample (labeled d1) was obtained and filled with only 2 mL of BLEB. One mL of the re-suspended mixture was added to each d1 tube. A serial dilution starting with the d1 tubes was performed by taking 300 µL from d1 and pipetting it to d2, then 300 µL from d2 to d3, and finally 300 µL from d3 to d4. The tubes were then incubated at 30^0C for 4 hours. Antibiotics were then added to each tube: 15 µL Cyclohexamide, 24 µL Naldixic Acid, and 6 µL Acriflavin. Then, the tubes were the placed back in a 30^0C incubator. After 48 hours, the bacterial suspensions were streaked onto MOX/Oxford and Chrom/Brilliance plates. Plates were placed in a 37^0C incubator. After another 48 hours, plates were checked for growth on plates selective for *Listeria*

monocytogenes. Colonies that grew were chosen at random and streaked onto Brain Heart Infusion (BHI) plates and again incubated at 37^0C for approximately 48 hours. Colonies that grew on BHI plates were run through a qPCR assay using *Listeria* and *Listeria monocytogenes* primers to test whether they were *Listeria* and, if so, whether they were *Listeria monocytogenes*. Glycerol stocks were made of all *Listeria* isolates and kept in a freezer at -80^0C.

MUG assay

The GUS *Listeria* strain (p*hly-gus-neo*) was grown on a LB plate with neomycin sulfate (bioWorld Lot#0504A45) to assay for the transcriptional activity of *hly*. Colonies were selected and used to inoculate into 5mL BHI with or without 0, 5, 15, or 25 mM of propionate for growth overnight. The following day, optical density (OD) was measured in a 96-well plate at 600 nm with a volume of 200 μL per well using a 96-well plate reader (Biotek Synergy4). One mL of bacteria was then aliquoted into 3 micro-centrifuge tubes and spun at 15,000 rpm for 2 minutes at 4^0C. The supernatant was removed, and the pellet was washed twice with TBS. One hundred μL 1% Triton-X in PBS (+/+) was then used to re-suspend the pellet. Bacterial cells were then lysed using a sonicator for 3 30-second cycles. Samples were put on ice between each cycle. The mixture was centrifuged at 15,000 rpm for 5 minutes at 4^0C, and the resulting 100 μl of supernatant was collected into a 96-well plate. In the dark, 20 μL 4-Methylumbelliferyl-β-D-glucuronide (MUG; Alfa Aesar, Lot #10192480) solution (5mM MUG in dH20) was added to each well, and the plate was incubated at 37^0C. After 10 minutes, 40 μL stop solution, 0.2 M sodium carbonate (Sigma Lot#37H1219), was added in the dark. OD was then measured again at 365 excitation and

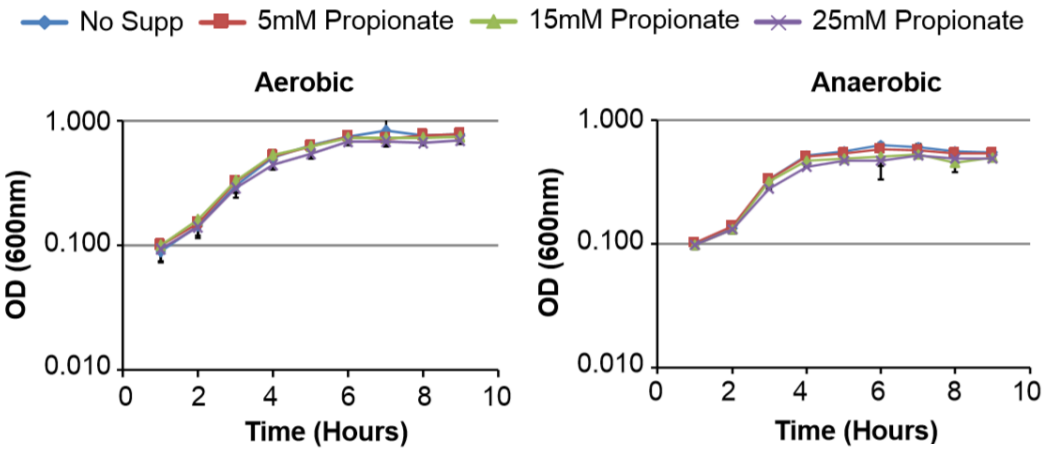
400 nm emission with a volume of 200 µL per well using a 96-well plate reader (Biotek Synergy4). A BCA assay (Pierce™ BCA Protein Assay Kit) was run using 10 µl of the supernatant to determine the protein concentration of each sample based on Bovine Serum Albumin (BSA) standards.

Statistics

Statistical analyses were done in Microsoft Excel with P-values between each pairwise comparison calculated by two-tailed Student t-tests.

Results

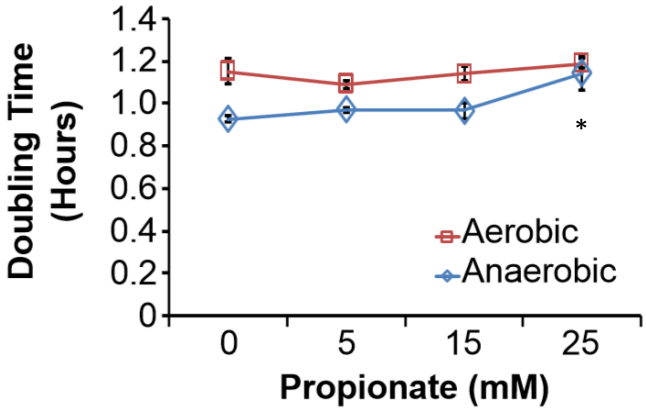
Previous research had shown that *Listeria* grown under anaerobic conditions exhibits less overall growth than *Listeria* grown under aerobic conditions. However, the effect of propionate supplementation on *Listeria* growth had not been studied. Experiments conducted by Eric Newton demonstrated that propionate supplementation (0, 5, 15, and 25 mM) to overnight cultures did not significantly change *in vitro* growth over 9 hours **(FIG 1A)**.



Eric Newton

Figure 1A: Propionate supplementation does not significantly impact *Listeria* growth levels.

However, propionate supplementation (25 mM) did lead to an increase in doubling time for both aerobically- and anaerobically-grown *Listeria*. (**FIG 1B**).



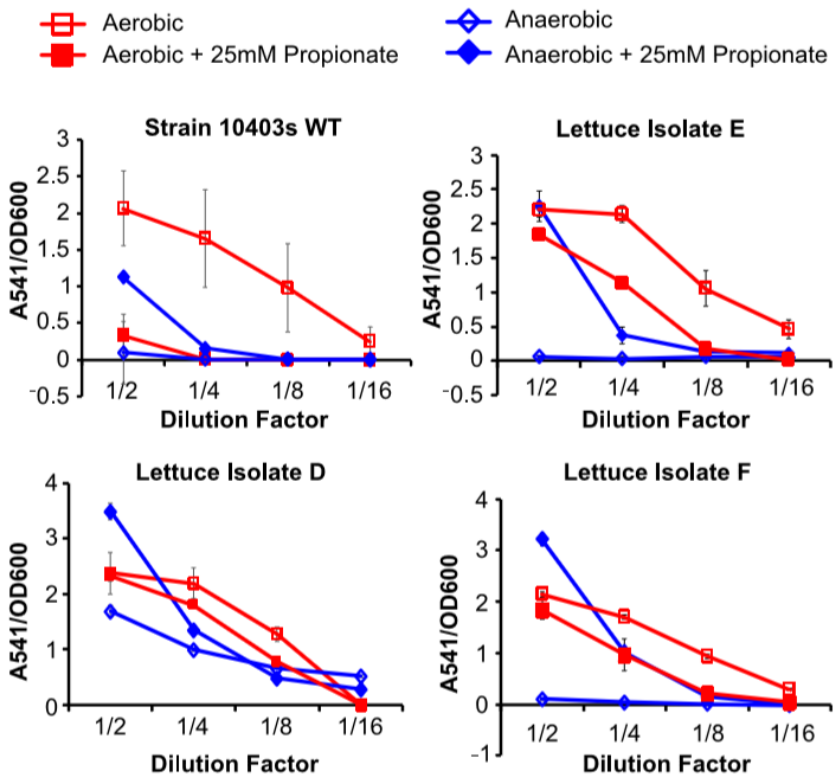
Eric Newton

Figure 1B: Propionate supplementation significantly increases doubling time for anaerobically-grown *Listeria*.

We next examined the effect of propionate supplementation on *Listeria* toxin secretion. We assayed for listeriolysin O (LLO), the toxin protein *Listeria* uses to escape the phagosome once it is in the cell. Hemolytic assay results showed that propionate supplementation (25 mM) to aerobically-grown *Listeria* resulted in decreased LLO

secretion. For anaerobically-grown *Listeria*, however, propionate supplementation (25 mM) led to increased LLO secretion (**FIG 2A**).

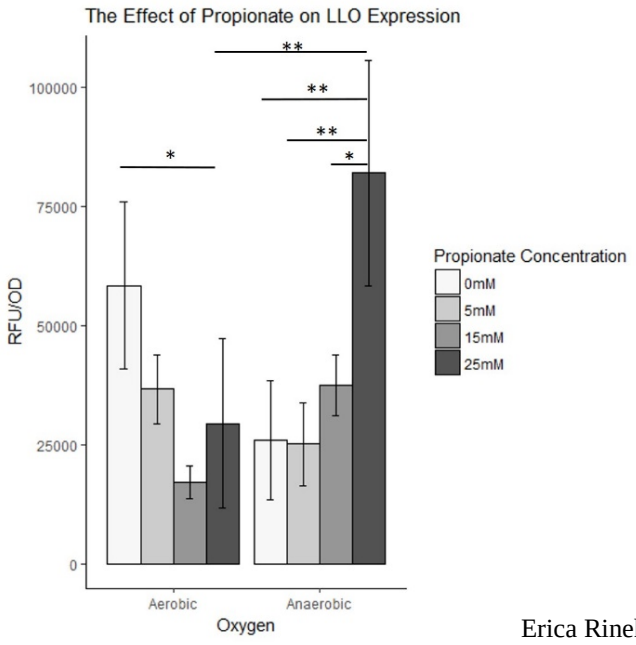
We then aimed to extrapolate our toxin secretion data to *Listeria* strains found in the environment. While our *Listeria* lab strain 10403S is widely-used to study the pathogen, we tested for environmental relevance by isolating *Listeria* from garden vegetables. We then ran hemolytic assays using these new strains to determine whether they exhibit similar behavior as the lab strain. Measurements of LLO secretion by the environmental strains mirrored those of the lab strain (**FIG 2B, 2C, 2D**).



Eric Newton. Kaitlin Beemiller

Figure 2A: (top left) Propionate supplementation significantly increases LLO secretion for anaerobically-grown *Listeria*, but decreases LLO secretion for aerobically-grown *Listeria* using the 10403S wild-type lab strain. **Figure 2B, 2C, 2D:** (top right, bottom left, bottom right, respectively) The same trend in LLO secretion is seen using strains isolated from garden lettuce.

Listeria LLO production is controlled by the *hly* gene. Therefore, we further verified the hemolytic assay results using a MUG assay to measure *hly* transcription. The transcription of *hly* drives the production of the enzyme GUS, whose activity can be quantified to represent *hly* transcription level. Data showed that propionate supplementation (0, 5, 15, 25 mM) increased *hly* transcription of *Listeria* grown aerobically, but decreased *hly* transcription of *Listeria* grown anaerobically (**FIG 2E**).

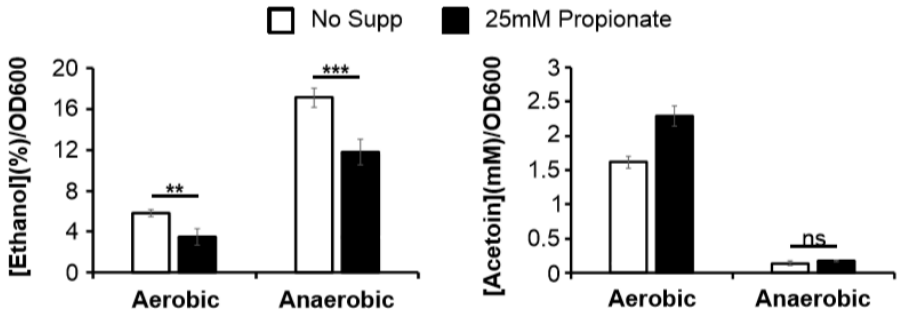


Erica Rinehart

Figure 2E: Transcription of *hly* in anaerobically-grown *Listeria* is increased with propionate supplementation. Transcription of *hly* in aerobically-grown *Listeria* is decreased with propionate supplementation.

To determine whether the results we observed were due to an effect of propionate on *Listeria* carbon metabolism, we measured concentrations of two metabolic byproducts: ethanol and acetoin. We observed a decrease in ethanol concentration in *Listeria* culture supernatant when supplemented with propionate (25 mM) under both aerobic and

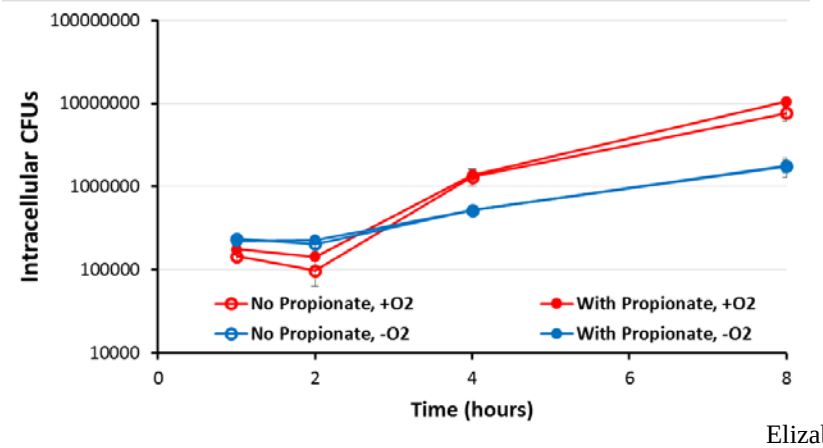
anaerobic conditions (**FIG 3A**). Propionate supplementation (25 mM) also resulted in increased acetoin supernatant concentrations under aerobic, but not anaerobic conditions (**FIG 3B**).



Eric Newton, Nathan Wallace

Figure 3A: (left) Propionate supplementation led to a decrease in ethanol production in both aerobically- and anaerobically-grown *Listeria*. **Figure 3B:** (right) Propionate supplementation led to an increase in acetoin production in aerobically-grown *Listeria*.

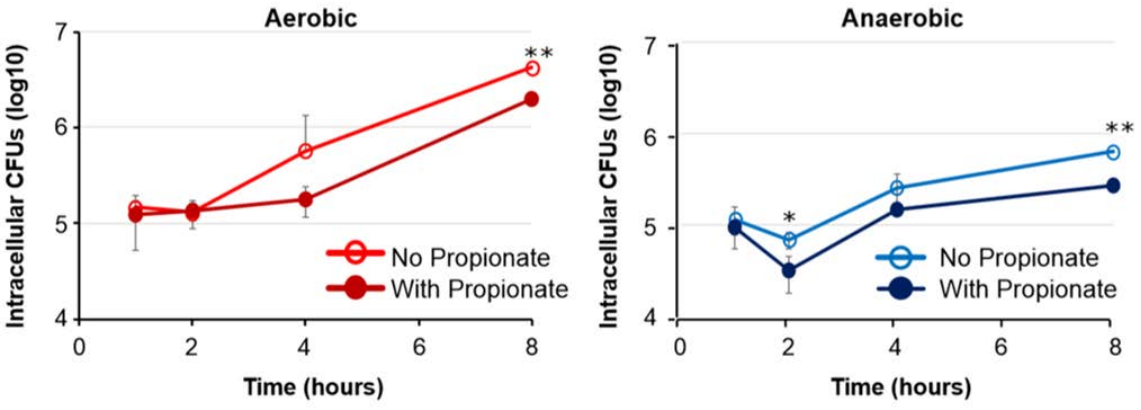
Next we tested the effect of propionate supplementation on cell infections. RAW 264.7 murine macrophages were infected with *Listeria* grown aerobically or anaerobically, with or without propionate added to overnight cultures (0, 5, 15, 25 mM). We observed intracellular growth of *Listeria* over an 8-hour time course. Previous work showed that anaerobically-grown *Listeria* exhibited less intracellular growth than aerobically-grown *Listeria*[11]. This effect was observed, but propionate supplementation did not significantly affect intracellular growth (**Fig 4A**).



Elizabeth Abrams

Figure 4A: Anaerobically-grown *Listeria* exhibits higher initial invasion but lower intracellular growth over time than aerobically-grown *Listeria.* Propionate supplementation does not have a significant effect on intracellular growth.

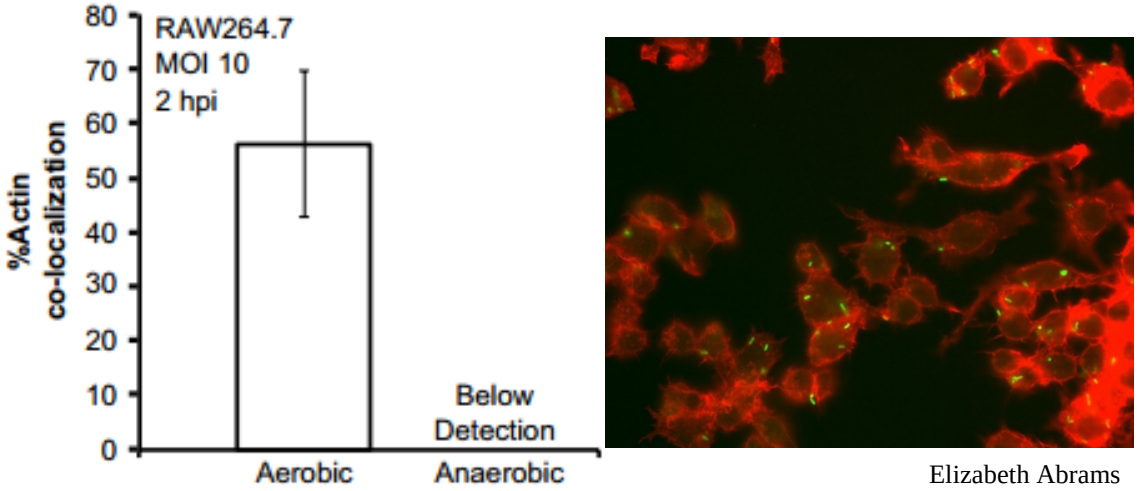
Because propionate is consistently present within the environment of the intestinal lumen, we next added propionate (5 mM) to the macrophages during cell infections to determine the impact of host exposure to propionate on *Listeria* infections. When aerobically- and anaerobically-grown *Listeria* supplemented with propionate (25 mM) was added to the macrophages, propionate supplementation resulted in decreased intracellular CFUs (**FIG 4B**).



Elizabeth Abrams

Figure 4B: Both aerobically- and anaerobically-grown *Listeria* exhibit lower intracellular growth when propionate is added.

In order for *Listeria* to grow inside the macrophages, it must escape the phagosome and move around the cytosol using actin tails. Therefore, measuring actin co-localization with *Listeria* is another method of quantifying intracellular bacteria. We used fluorescence microscopy to determine the proportion of co-localization (**FIG 5B**). We found that anaerobically-grown *Listeria* showed significantly less actin co-localization than aerobically-grown *Listeria* (**FIG 5A**). However, propionate supplementation (25 mM) did not affect actin co-localization.



Elizabeth Abrams

Figure 5A: (left) Anaerobically-grown *Listeria* exhibit significantly lower actin co-localization than aerobically-grown *Listeria.* **Figure 5B:** (right) Composite fluorescence microscopy image showing aerobically-grown *Listeria* (in green) supplemented with 25 mM propionate at 2 hours post-infection inside macrophages labeled by actin filaments (in red).

Discussion

Listeria must adapt to a range of intestinal conditions in order to break the intestinal wall and successfully move to the bloodstream and other areas of the body. In order to prevent this bacterial spread, we aim to understand how encountering propionate affects the functionality of *Listeria.* We first measured bacterial growth and discovered that while propionate supplementation did not have an impact on *Listeria* overall growth, it did

produce an increase in doubling time for *Listeria* grown under both aerobic and anaerobic conditions. This means propionate is slowing down, but not stopping, bacterial growth.

Because toxin secretion is an integral part of *Listeria* pathogenesis, we next quantified LLO secretion of *Listeria* supernatant. LLO is the protein *Listeria* uses to break out of the phagosome once it is in a cell; without it, *Listeria* has a limited ability to spread. Therefore, we wanted to understand how the presence of propionate affected secretion of this protein. Hemolytic assay results demonstrated an oxygen-dependent change in LLO secretion: aerobically, the presence of propionate decreased *Listeria* LLO secretion, while anaerobically, propionate supplementation increased LLO secretion[11]. Similar results were observed of three different *Listeria* strains isolated from garden lettuce. This affirms the applicability of our results using the 10403S lab strain. Further, we confirmed the effects of propionate on LLO by measuring transcription of the gene that encodes the LLO protein, *hly*. This was done using a *hly* transcription reporter strain, which showed that, when propionate was added to overnight cultures, transcription of the *hly* gene encoding LLO decreased when *Listeria* was grown under aerobic conditions and increased when *Listeria* was grown under anaerobic conditions. Together, these data suggest that the effects of propionate are modulated by the presence of oxygen. *Listeria* encounter an oxygen gradient as they move from the lumen, through the epithelium, and into the bloodstream, so knowing that oxygen changes the interaction of propionate and *Listeria* is significant and relevant to authentic intestinal conditions.

We wanted to understand why propionate has a unique effect on bacterial growth and toxin levels. Even though propionate doesn't seem to affect the overall growth curves, the increase in doubling time signals that the bacteria are growing slower. One way to test

this is by examining the carbon metabolism process. During carbon metabolism, pyruvate is broken down into both ethanol and acetoin. We used ethanol and acetoin assays, respectively, to measure the output of those byproducts. Results showed that propionate supplementation led to a decrease in ethanol concentration in the culture supernatant under both aerobic and anaerobic conditions. Propionate supplementation also led to an increase in acetoin concentration under aerobic, but not anaerobic conditions. This data suggest that carbon may be being directed more toward acetoin than ethanol when propionate is present. That shift in carbon metabolism could, in turn, be the signal causing the difference in LLO secretion.

We also examined how propionate supplementation affects the growth of *Listeria* in macrophages. When we added propionate to overnight *Listeria* cultures and used them to infect cells (MOI=10, 30 minutes), we did not observe an change in intracellular colony forming units (CFUs) between *Listeria* that had been grown with propionate and that which did not grow with propionate. However, we did see a difference in intracellular growth between cultures grown aerobically versus cultures grown anaerobically. At 1 hour post-infection, anaerobically-grown *Listeria* had a higher number of intracellular CFUs. However, by 4 hours post-infection, aerobically-grown *Listeria* grew more inside the cell, and this difference was even more exaggerated at 8 hours post-infection[11].

Since the intestinal area is likely continuously coated with propionate, however, we decided to conduct cell infections in which we not only added propionate to the *Listeria* overnight cultures, but also to the macrophages themselves during infection. These conditions did result in a significant change based on propionate supplementation. *Listeria* grew more inside the cell when propionate was *not* added to either the macrophages or the

bacteria than it did when propionate was added to *both* the macrophages and the bacteria. Therefore, it seems that propionate is somehow protecting the macrophages by limiting *Listeria* intracellular growth. Further work should be done to better understand the mechanism for this.

To further understand the interaction between *Listeria* and host cells, we quantified co-localization of actin with *Listeria.* Actin co-localization was significantly compromised in anaerobically-grown *Listeria* compared to aerobically-grown *Listeria*[11]. This may be due to decreased ActA expression in anaerobically-grown bacteria, but this needs to be confirmed with additional assays. ActA is the protein that produces actin, and it is possible that the lack of oxygen limits its presence or functionality. No significant difference was noted in actin co-localization due to the presence of propionate during overnight *Listeria* growth.

Going forward, we aim to continue this work by studying how propionate changes the composition of *Listeria* by fatty acid methyl ester (FAME) analysis. This data will tell us whether propionate is affecting fatty acids in the cell wall of *Listeria* and thus disrupting bacterial structure. Additionally, we want to study the role of propionate using strains that are mutants for key transcription factors in *Listeria* virulence regulation (PrfA, CodY, SigB). Finally, we will also perform similar analyses using butyrate, another SCFA produced in the intestinal lumen.

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