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The Incorporation of Lipids into the Cellular Membrane of Salmonella

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The Incorporation of Lipids into the Cellular Membrane of *Salmonella*

by
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Abstract

Salmonella is a gram negative, facultative anaerobic food borne pathogen and is the leading cause of deaths related to food borne illnesses. In order to establish an infection successfully, *Salmonella* must be able to survive in the presence of various stressors that it encounters, namely changes in pH, oxygen availability, osmolarity and bile. Previous research has shown that exposure to bile causes a shift in fatty acid composition in the cell membrane of the enteric bacterium *Enterococcus faecalis.* Thus, this led to the hypothesis that *Salmonella* incorporates fatty acids into its cellular membrane following exposure to bile and thereby protects itself against bile induced damage. To determine what effect bile has on the fatty acids in the membrane of *Salmonella* and if this shift has a direct link to bile resistance, fatty acid profiles of four strains of *Salmonella* (*S. Heidelberg, S. typhimurium, S. typhi, and S. enteritidis*) were examined using fatty acid methyl esters (FAME) analysis following exposure to either 0% bile or 0.3% bile. Following exposure to bile, there was a change in the fatty acid profile in all four strains. The fatty acids that increased across all four strains were the unsaturated fatty acids oleic acid and linoleic acid. The saturated fatty acid palmitic acid increased in all strains except *S. enteriditis*. To determine if the incorporation of these fatty acids contributed to bile resistance, cultures were pre-treated with a lipid mix containing varying concentrations of lipid mix (Sigma L0288) and then exposed to 0% and 5% bile for 1 hour, and the viability was assessed using plate counts. Data from the survival analysis showed that the lipid mix had no impact on survival after bile exposure. This study shows that the fatty acid profile of *Salmonella* changed after exposure to bile and that these changes in the fatty acid profile did not correlate with increased bile survival of *Salmonella*. Further work is needed to determine how this impacts the ability of *Salmonella* to cause salmonellosis.

Dedication

This project is dedicated to my mom and dad.

Without their help, college and an Honors thesis would not be possible.

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Table of Contents

List of Abbreviations…….. viii

Chapter I: Introduction……. 1

Chapter II: Review of Literature…….. 2

Chapter III: Methodology…….. 7

Chapter IV: Results……..... 10

Chapter V: Conclusion……... 16

List of Abbreviations

TSB	Tryptic Soy Broth
TSA	Tryptic Soy Agar
GI tract	Gastrointestinal Tract
LM	Lipid Mix
PBS	Phosphate Buffer Saline

Chapter One

Introduction

According to the United States Center for Disease Control and Prevention (CDC), *Salmonella* is one of the most common food borne pathogens, causing nearly 1 million illnesses annually in the United States alone and approximately 19,000 hospitalizations and 380 deaths every year (CDC, 2012). *Salmonella* can be ingested through contaminated food sources such as poultry, eggs, and other food products made from contaminated sources. In addition, backyard chicken breeders and turtles have been known to carry the bacterium (CDC, 2012). Once consumed, this gram negative bacterium is able to resist the stressors encountered within the gastrointestinal tract (GI), including acidic conditions found in the stomach and bile found in the intestines. Bile is an essential component of the gastrointestinal system, as it breaks down and dissolves lipids and aids in the uptake of fat soluble vitamins A, E, D, and K (Jimenez, Sanches, Farina, Mongolles, and Rodrigues, 2014). Bile consists of cholesterol, proteins, bile salts, and bilirubin and is produced in the liver (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2015). Once synthesized by the liver, bile flows either directly to the small intestine or is stored in the gallbladder. Although bile is highly concentrated in the gallbladder, *Salmonella* is able to colonize this organ, demonstrating the ability of this dangerous pathogenic bacterium to survive harsh environmental conditions (Caetano et al, 2011). Not only is *Salmonella* able to colonize in the gallbladder, but it is able to grow and use nutrients from the bile, and some research suggests that *Salmonella* is able to grow almost as well in the GI tract in the presence of bile as it does in laboratory media (Caetano et al, 2011). According to previous research, bile has the potential to suppress bacteria colonization due to interruptions in homeostasis by denaturing proteins and causing damage to the DNA (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2015). In addition, bile has been shown to degrade bacterial membranes (Jimenez, Sanches, Farina, Mongolles, and Rodrigues, 2014). Because bile contains bactericidal properties, organisms that can grow and survive in the GI tract must develop processes to survive in the presence of these stressors. The mechanism by which these bacteria can overcome bile

stress has not been fully characterized, although research indicates that bacterial bile resistant properties can be due to changes in the cellular membrane composition and studies have indicated that certain bacteria can incorporate lipids found in bile to increase bile resistance (Saito, Harp, and Fozo, 2014).

The purpose of this study is to determine whether the membrane structure of *Salmonella* is altered following exposure to bile and whether this impacts bacterial survival. It is expected that the bacteria will incorporate the lipids found in bile into the cellular membrane. *Salmonella typhi*, *S. typhimurium*, *S. enteriditis*, and *S. heidelberg* will be tested; these strains represent various *Salmonella* serotypes that are known to cause infections in both humans and animals. These four were chosen because they are among the most frequent types to cause infection in a host. Each type is expected to incorporate the bile lipids into the cellular membrane; however, integration of lipids could vary in contrasting levels. In addition, some strains may incorporate certain fatty acids that other strains do not. We expect that by incorporating certain bile fatty acids, the organism will be better fit to survive in the GI tract. Therefore, fatty acid composition of the membrane will be analyzed following exposure to the bile. Strain specific incorporation of lipids will indicate whether this mechanism plays a significant role in bacterial survival following exposure to bile.

This research is important to determine which elements affect the pathogen and host susceptibility to food borne illness after contaminated food has been ingested. Understanding how bacteria can survive and grow in the GI tract is essential to developing methods to combat infections and aid in a healthy immune system.

Chapter II

Review of Literature

Salmonella is a common food borne pathogen that encounters the gastrointestinal tract (GI tract) through the consumption of contaminated foods, such as poultry and pork meats. Once consumed, this pathogen has potential to cause discomfort, such as abdominal pains, diarrhea, vomiting, nausea, and in extreme cases can cause Typhoid fever (Khan, 2014). The diseases associated with this pathogen are not usually fatal; however, the United States Centers for Disease Control and Prevention estimates 1.2 million individuals are infected by *Salmonella*, and over three hundred eighty die from illnesses associated with this disease annually in the United States (CDC, 2014). The diseases associated with this pathogen usually are resolved in as little as four to seven days, but some patients may acquire reactive arthritis as a response to the *Salmonella* infection (CDC 2014). Patients most at risk to develop disease from this pathogen are infants, children, and the elderly. Recent research suggests that *Salmonella* will become an even greater cause of concern in future years because of increased antibiotic resistance. This resistance can be attributed to widespread antibiotic uses (Khan, 2014).

Because this pathogen is food borne, once consumed, the bacteria must travel through and colonize the human gastrointestinal tract while encountering numerous environmental stressors (Fig 2.1). For instance, in order to grow in the digestive system's suboptimal conditions, *Salmonella* must compete for nutrients and space to colonize among other microbiota. The GI tract possesses many different types of intestinal microbiota that live in symbiosis with the human host. These microbes help to promote a healthy immune system and can defend against invading bacterial pathogens through a colonization resistance process (Sengupta, Ray and Chowdhury,2014). When pathogens come into contact with the gut microbiota, they must use special mechanisms to ensure growth of the pathogen while the normal microbiota work to eliminate the pathogen. In addition, the GI tract has a low pH that organisms must adapt to in order to grow and survive.

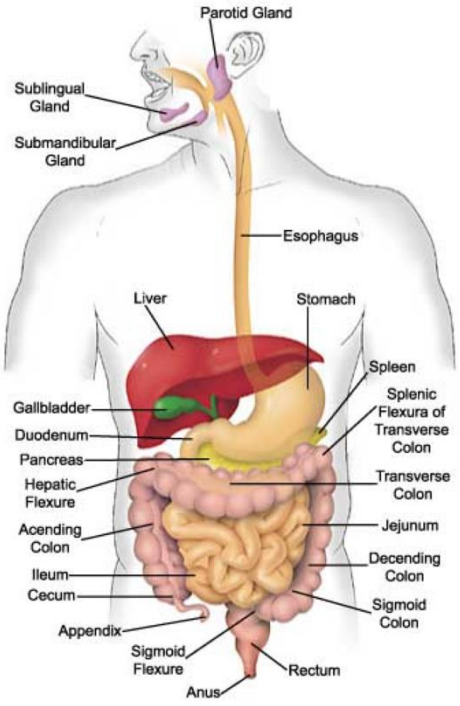

Figure 2.1 Diagram of the human gastrointestinal tract. Adapted from

http://cephalicvein.com/2016/05/digestive-system-diagram/

Bile is another stressor found in the GI tract and is composed of cholesterol, proteins, bile salts, and bilirubin. Bile is produced by the liver and is either stored in the gallbladder or released to the small intestine upon ingestion of food (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). Bile is essential to the host because it helps to break down and dissolve lipids and is important for the absorption of the fat soluble vitamins A, D, E, and K (Jimenes, 2014). In addition, bile demonstrates antimicrobial properties and is thought to cause DNA, protein, and cell wall damage to bacteria (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2015). Once in contact with the bacteria, these bile salts can denature proteins and dissolve membrane lipids (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). Certain enteric bacteria recognize bile as a host specific cue, which in turn regulates the expression of several virulence factors (Sengupta, Ray and Chowdhury, 2014). *Salmonella* are able to survive in the

presence of high concentrations of bile. Additionally, these bacteria can survive within the gallbladder, which typically does not have colonization of bacteria due to the high bile concentration (Dowd, Joyce, Hill, and Gahan, 2011). Research suggests that *Salmonella* can not only survive in the presence of bile, but can use nutrients from the bile to aid in their growth processes (Caetano, 2011). The processes in which *Salmonella* survive bile and use its nutrients is essential to the bacteria's ability to cause disease.

Cell envelope structures are known to be involved in bacterial bile resistance properties. Figure 2.2 shows an illustration of a gram negative bacterial cell membrane. The composition of the membrane of *Salmonella* is important to the viability of the cell. The membrane composition has the ability to act as a barrier against the bile salts or allow for some salts to enter into the cell. According to previous research, there are many proteins embedded within the cell envelope that impede the uptake of bile salts into the cell (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). The protein that is most affected is a penicillin binding protein. In the presence of bile, this protein may either change its activity or its amount in the cell. If these changes occur, the cell membrane may be more affected by bile (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). These proteins are important to ensure that the cell can function properly. Bile may also enter the bacterial cell through diffusion; mechanisms must then reduce the concentration of bile in order to keep the cell functioning properly (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). The cell uses the process of down regulation to reduce the effect of genes that are bile sensitive. This processes allows the cell to reduce the concentration of bile salts following the bile exposure (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2015). In addition, the cell has efflux pumps that work to reduce the concentration of bile salts found intracellularly. (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2015). Even though *Salmonella* can grow and survive in the presence of bile, these cell regulators are important to ensure that the cell can continue to grow in the physiologically relevant conditions.

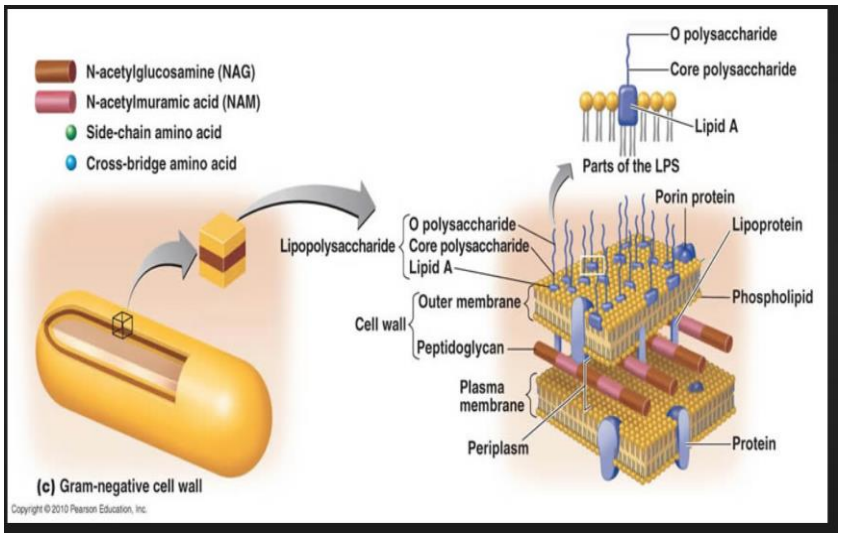

Figure 2.2 Illustration of a gram negative bacterial membrane. Adapted from http://immunepath-ip.vn/chi-tiet-tin-tuc/54/the-cell-wall.html

Previous studies indicate that the adaptations to the cell's structure greatly impact the pathogen's ability to survive within a host. One of the adaptations to *Salmonella's* cell structure include changes in the peptidoglycan layer. Recent studies have indicated that the peptidoglycan layer of *Salmonella* is chemically altered depending on stage of growth of the culture, in response to environmental conditions, and in case of eukaryotic cell infection (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). Presence of bile in the GI system is one of the environmental conditions that influences *Salmonella's* effect on the peptidoglycan layer of the pathogen. The peptidoglycan layer remodeling may effect proteins that are able to bind to the cell wall of the bacteria, and the changes in the binding of the proteins may positively effect the survival of the cell (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). The use of bile as a signal to peptidoglycan layer remodeling is important in the growth of *Salmonella* in the GI system. *Salmonella* can recognize the bile as an environmental stressor and can alter the peptidoglycan layer to ensure cell survival. Research suggests that the peptidoglycan layer remodeling may effect the uptake of bile salts into the cell, causing an increase in the resistance of the bacteria after exposure to bile (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2015). Other research indicates the

bacteria's bile resistant properties can be due to cellular membrane construction and makeup (Saito, Harp, and Fozo, 2004).

Additionally, studies have shown that certain bacteria can incorporate lipids found in bile to increase bile resistance (Saito, Harp, and Fozo, 2014). This mechanism is the focus of our experiment. In a 2014 study by Saito et al, fatty acid composition after exposure to bile was analyzed in *Enterococcus faecalis*. *E. faecalis* is a Gram-positive bacteria that is a part of the normal intestinal flora of most mammals. This study found that the cellular membrane of *E. faecalis* was altered in response to bile stress. The alteration in the membrane was due to the uptake of fatty acids from bile. Bacteria capable of growing in low amounts of bile incorporate fatty acids into the cellular membrane as a protection for higher stressors. This study showed that most of the fatty acids incorporated after the exposure of bile were saturated fatty acids. Unlike *E. faecalis*, *Salmonella* is a Gram-negative pathogen. The comparison between these two bacteria lead us to consider the effect of bile on *Salmonella's* cellular membrane. We expect that *Salmonella* will also alter the fatty acid composition of the cellular membrane, but are unsure of the extent to which these alterations will affect the bacteria's survival.

This study focuses on four stains of *Salmonella*. Each of the strains will be accessed for the changes in the fatty acid composition of the cellular membrane. The changes in composition will then be used to determine if the change has an effect on the bacterium's survival when exposed to bile. In addition, performing the experiment on four different strains will lead to constructive replication and more accurate experimental data. This research is important to determine which elements effect the pathogen and host susceptibility to food borne illness after contaminated food has been ingested. Understanding how bacteria can survive and grow in the GI tract is essential to developing methods to combat infections and aid in a healthy immune system.

Chapter III

Methods

Bacterial strains and cultivation conditions.

The four strains of *Salmonella* used in this study are *Salmonella typhimurium, S. typhi, S. enteriditis, and S. Heidelberg.* Stocks of each strain were made by freezing cultures in 20% glycerol at -80°C. Overnight cultures were prepared by inoculating the four different strains into individual tubes contain 2 mL of Tryptic Soy Broth (TSB). The strains were then placed in the shaker incubator overnight to allow for growth at 37°C.

FAME analysis.

After overnight incubation, the strains were the taken out of the incubator and prepared for logarithmic growth by inoculating the bacteria into tubes containing 5 mL of TSB. The cultures were placed in the incubator and grown until they reached mid-logarithmic growth. The cultures were checked for mid-logarithmic phase based on reading from a nanodrop measuring the optical density of light at a wavelength of 600 nm (OD 600). Once in midlog, the tubes were then centrifuged and supernatants decanted. The cultures were then suspended in TSB supplemented with either using 0% or 0.3% porcine bile (Sigma #B8631). After suspension, the strains were placed in the 37˚C incubator and allowed to grow for one hour. After the hour exposure, the strains were washed using PBS and frozen in the -80˚C incubator. After three replicates of all strains were attained, the samples were sent to Microbial ID to be assessed for their fatty acid composition.

When the Microbial ID company evaluated our samples, they first streaked for a pure culture and extracted that pure culture for analysis. They then prepared the samples to assess their fatty acid methyl esters by saponification and methylation. After these steps, the company extracted and washed the fatty acid methyl esters to be identified. The samples are assessed using gas chromatography and identification of the fatty acid was based on Sherlock Pattern Recognition Software database of a known species. Figure 3.1 is an outline of the process that Microbial ID produced.

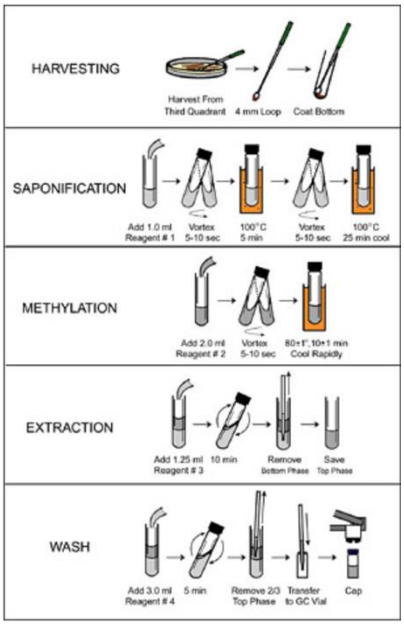

Figure 3.1 Illustration of the process of FAME analysis from Microbial ID. Adapted from http://www.microbialid.com/services/fatty_acid_materials.html

Survival assays.

Overnight cultures of each strain were prepared as stated above. After incubation, the cultures were then removed from the incubator and prepared for mid-logarithmic growth by inoculating the cultures in TSB (1:100 dilution). The survival assay was completed in three conditions: 1) control (5 mL of TSB), 2) LM (4 mL of TSB and 1 mL of lipid mix LM), and 3) PBS (4 mL of TSB and 1 mL of PBS). Each replicate of the strains was inoculated into a tube of each condition. After preparing the samples, they were placed in the 37ºC incubator and incubated until they reached mid-logarithmic growth phase. The midlogs were assess based on a nanodrop reading using the optical density of light at a wavelength of 600 nm (OD 600). Once in mid-log, the bacteria were removed from the incubator and centrifuged for five minutes at 10,000 x g for 5 minutes. Each condition was then divided into two tubes, centrifuged at 10,000 x g for five minutes and washed twice using PBS. This was done to ensure that the lipid mix had washed off properly, and we were accounting for the most controlled experiment possible. After washing, the cultures were suspended in TSB supplemented with either 0% or 5% porcine bile (Sigma #B8631).

The 5% bile was used in this portion of the experiment because 5% bile concentration is more closely related to the concentration found in the gallbladder. To dissolve the bile, 0.1% methanol was added. After the addition of bile, the cultures were incubated for 1 hour at 37ºC. After an hour incubation period, the cultures were serially diluted on Tryptic Soy Agar plate (TSA). The plates were then incubated in at 37 ºC incubator for 24 hours and then were assessed for viable plate counts.

Chapter IV

Results

FAME analysis showed an alteration in the fatty acid composition of all four strains' cellular membrane.

The samples were exposed to 0% and 0.3% bile, which mimics the concentration of bile found in the small intestines. After exposure to bile, all of the strains showed a change in their fatty acid profiles. As expected, there was a significant decrease in a large number of fatty acids. Most notably, the concentration of palmitoleic acid (16:1 w7c) was decreased in all four strains after exposure to the bile. In addition, all four strains of *Salmonella* that were tested, the unsaturated fatty acids oleic acid (18:1 w9c) and linoleic acid (18:2 w6,9c) were increased after bile exposure. Concentrations of linoleic acid more than double after exposure to bile. The saturated fatty acids palmitic acid (16:00) increased after exposure to bile in all four strains, except *S. enteritidis,* which showed only a small decrease. In addition to the unsaturated fatty acids that were incorporated or increased, the saturated stearic acid (18:00) was increased in all four strains.

S. typhimurium was altered as stated above, but did show some trends that were not consistent with the other three types of *Salmonella.* The fatty acid 17:0 cyclo was present in a smaller concentration in *S. typhimurium* before and after exposure to bile when compared to the other strains. Also, stearic acid was shown to have the greatest increase in *S. typhimurium* following exposure to bile. This increase was more than double the increase seen in the other four strains. Table 4.1 represents the fatty acid gas chromatography peak numbers and their corresponding common names. The table also shows whether the acid is saturated or unsaturated. Table 4.1 below is a representation of the fatty acid composition in each strain. The strains are labelled under the graph and the graph is arranged in percent fatty acid composition of the membrane. This chart shows the strain with exposure to 0% bile as well as 0.3% bile.

Table 4.1: Fatty Acids

Fatty Acid Number	Fatty Acid Name	Saturated or Unsaturated
Unknown 1	Unknown	N/A
16:00	Palmitic	Saturated
12:0 aldehyde		
12:00	Lauric	Saturated
17:0 cyclo		
18:0 ante		
14:00	Myristic Acid	Saturated
18:2 w6,9c	Linoleic Acid	Unsaturated
18:1 w9c	Oleic Acid	Unsaturated
Unknown 2	Unknown	N/A
18:1 w7c	Cis-Vaccenic acid	Unsaturated
17:00	Margaric Acid	Saturated
14:0 3 OH		
18:00	Stearic acid	Saturated
15:00	Pentadecylic acid	Saturated
16:1 w7c	Palmitoleic acid	Unsaturated
19:0 cyclo w8c		

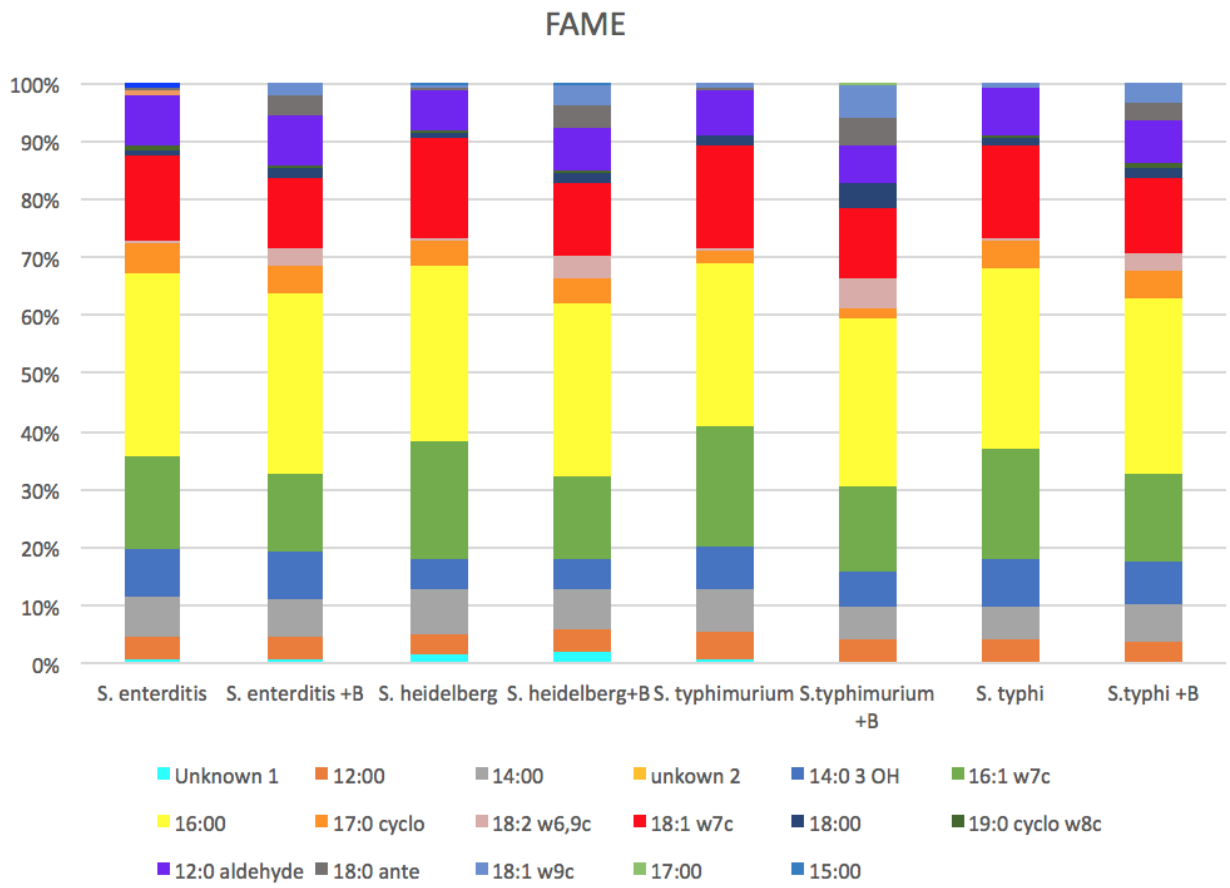

Figure 4.1 Aerobic FAME Results. This graph shows the composition of the cellular membrane before and after exposure to bile. The strains are denoted with "+B" to reflect exposure to bile.

Survival Assay

The survival assay contained cultures prepared in three conditions. The three conditions included 5 mL of TSB, which is indicated as "0". The second condition contained 4 mL of TSB and one mL of lipid mix and is indicated as "LM". The final condition included 4 mL of TSB and one mL of PBS and is indicated by "PBS". The samples were then treated with either 0% or 5% bile and plated to assess viable plate counts from a minimum of three independent replicates. In the chart below, the cultures that were exposed to the 5% bile are indicated as "+B". The results from the viable plate counts are charted below. The astrics (*) on the graph represent a level of significance of $p \leq 0.005$.

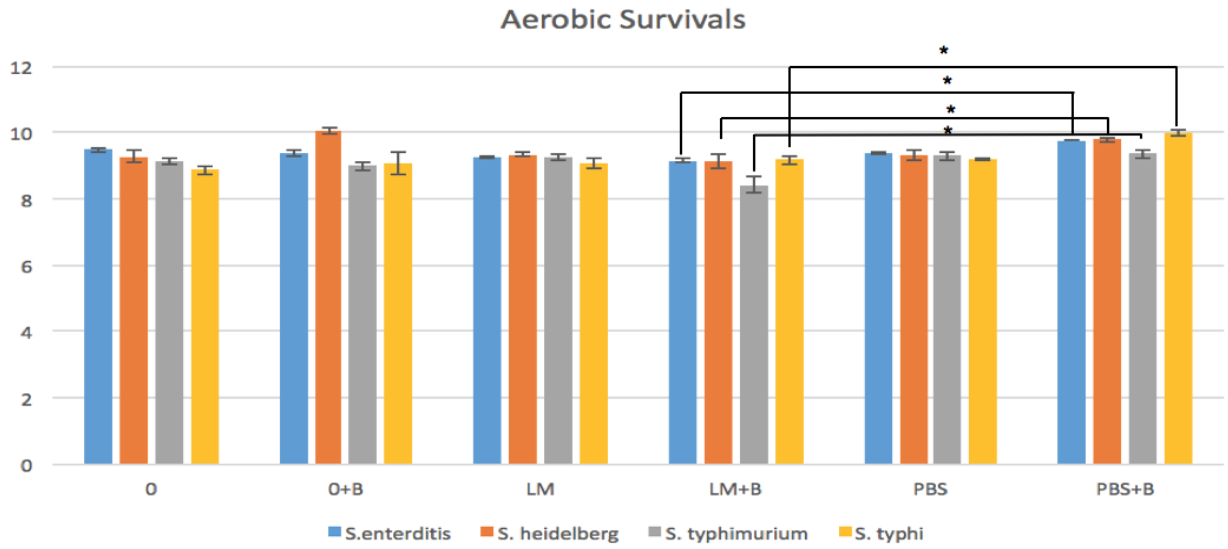

Figure 4.2 Survival Assay Results. *S. enteriditis, S. Heidelberg, S. typhimurium,* and *S. typhi* were exposed to 0% or 5% bile (represented as "0" and "0+B", respectively). Strains were also exposed to a lipid mix prior to exposure to bile (represented as "LM" and "LM + B", respectively). A control for alterations in the availability of nutrients was depicted as "PBS" control, with exposure to bile depicted as "PBS +B". Values represent the average of three independent replicates. * indicate a $p < 0.05$.

After exposure to bile, there was a decrease in *S. enterditis* and *S. typhimurium*, but *S. heidelberg* and *S. typhi* both increased following exposure to bile. Lipid mix was used to see if pre-exposure to lipids would aid in bile survival of the cells. The lipid mix that was selected for the survival analysis contained the same fatty acids that increased in *Salmonella's* cellular membrane as seen in our FAME analysis. However, when comparing the survival of strains tested that received the lipid mix prior to bile in comparison to the control of PBS and bile, there was a significant decrease in the viability among strains pre-exposed to lipid mix and then exposed to bile. This indicates that the pre-exposure to lipid mix decreased survival after exposure to the bile. This also shows that the lipid mix did not play a key role in the survival. In both the LM and PBS conditions, the survival of the cells is about the same. This indicates

that the increase in the survival of the *Salmonella* following exposure to bile must be attributed to different mechanisms.

Chapter V

Discussion

Salmonella causes approximately 380 deaths every year in the United States (CDC, 2012). Research on this pathogen is essential in potentially lowering the number of illnesses and deaths annually. This research also provides some insight into other food borne pathogens and may influence studies and future research in this field. The ultimate goal of research on food borne pathogens is to determine how pathogens cause disease in the host so that we may be able to stop the growth of the organism

The purpose of this study was to determine if the fatty acid composition of *Salmonella's* membrane was altered following exposure to bile and determine if that alteration would increase the survival of the cell. This study utilized cells that were in mid-logarithmic growth phase. This phase of growth was used because the cells are most susceptible in this phase. After exposing the cells to bile, we saw an alteration in the fatty acid composition of the membrane. The acids that were incorporated were consistent with some of the acid changes seen in resent literature. One of the fatty acids that increased during this experiment was oleic acid. Oleic acid was also seen to be increased in the cellular membrane of *E. faecalis* following exposure to bile (Saito, Harp, and Fozo, 2014). Research on other enteric bacteria may show incorporation or increase of oleic acids as well. The acids were also increased in 0.3% bile, indicating that the cell will respond to bile stress even at very low concentrations of bile.

S. typhimurium was different than the other bacteria tested in this study. Before and after exposure to bile, it had the greatest percentage of stearic acid when compared to the other strains. After exposure to bile, 17:0 cyclo was present, but in smaller concentrations. In the other three strains, 17:0 cyclo was decreased following exposure to bile, but the decrease was not as large as that observed in *S. typhimurium*. This is interesting because in all three conditions, after exposure to bile the viability for *S. typhimurium* was less than that of the other strains. The differences in the fatty acid changes may have an effect on the survival of the cell and could be a possible explanation for the decreased viability. Though the fatty acid changes were observed, further research is needed to confirm the full effect on the survival of the cell.

The incorporation of the different saturated and unsaturated fatty acids can play a potential effect on the fluidity of the cellular membrane of the bacteria as well as how the cell handles the stress of the environment (Taranto, Murga, Lorca and de Valdez, 2003). Research indicates that *Salmonella* has proteins and other structures in the cell membrane that are responsible for maintaining homeostasis for the cell (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). The protein that is most effected is a penicillin binding protein. In the presence of bile, this protein may either change its activity or its amount in the cell. If these changes occur, the cell membrane may be more effected by bile (Hernandez, Cava, Pucciarelli, Portilla, and de Pedro, 2012). Incorporation of different fatty acids has the ability to affect the cellular proteins in assisting with homeostasis of the cell. Because of this, the *Salmonella* strains tested may have a decrease in the survival if the fatty acid composition did not allow for the cellular proteins to function properly.

The bacteria's fatty acid composition of the cellular membrane changed after exposure to bile. The changes in the fatty acid composition can be attributed to a stress response within the cell and incorporation of these acids is an energy expensive step for the cell. Because this is an energy expensive step, the incorporation of the fatty acids may have an energy benefit on the cell. The study shows that the incorporation did not increase the survival of the cell, but the cell could possibly be using the fatty acids for a different purpose.

This study is limited because of the growth conditions of the bacteria. Food borne pathogens encounter many stressors inside the GI tract. These stressors include acidic pH, oxygen availability changes, and bile (Caetano et al, 2011). This study was completed at a neutral pH, and is not representative of changes that may been seen at a lower pH. In addition, this study was completed in aerobic conditions. The alterations in the cellular membrane and the survival of the cell may differ in conditions of lower pH and in anaerobic conditions. While altering the conditions would be more physiologically relevant, this study did provide some insight into the effects of the bile on the cell membrane of *Salmonella*.

This project demonstrated that the fatty acid composition of *Salmonella's* membrane did alter after exposure to bile; however, further research is needed to determine what role this alteration has for the cell. This is an energy expensive alteration for the cell, and future research should investigate this role. Also, this study could benefit from being conducted in a lower pH and anaerobic environment to analyze conditions that may be more physiologically relevant.

Chapter VI

Conclusion

In conclusion, *Salmonella* encounters bile stressors inside the gastrointestinal tract and must have mechanisms to survive this bile stress. This study focused on alterations of the fatty acid composition of the cellular membrane to identify what type of role the alterations may have played in cell survival. The study concluded that the fatty acid composition of all strains' cellular membrane did change after exposure to bile. Each strain increased the unsaturated fatty acids oleic and linoleic acids. The saturated palmitic and stearic acids were also increased in all strains. The survival assay indicated that the pre-exposure to lipid mix had an adverse affect on the bacteria's survival in aerobic conditions. We saw a significant decrease in the survival of the cell when comparing the lipid mix and bile condition and the lipid mix and PBS condition. Because of this decrease, we concluded that the change in the fatty acid composition of the membrane did not allow better survival for the cell, and future research should focus on determining the reason the bacteria incorporated the acids.

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