

University of Montana

ScholarWorks at University of Montana

Undergraduate Theses, Professional Papers, and Capstone Artifacts

2024

Assessing Lipid Composition of Cell Membrane in Escherichia Coli Under Aerobic and Anaerobic Conditions

Isabelle Johnson

ij154078@umconnect.umt.edu

Follow this and additional works at: <https://scholarworks.umt.edu/utpp>



Part of the [Biochemistry Commons](#), [Cell Anatomy Commons](#), and the [Cell Biology Commons](#)

Let us know how access to this document benefits you.

Recommended Citation

Johnson, Isabelle, "Assessing Lipid Composition of Cell Membrane in Escherichia Coli Under Aerobic and Anaerobic Conditions" (2024). *Undergraduate Theses, Professional Papers, and Capstone Artifacts*. 448. <https://scholarworks.umt.edu/utpp/448>

This Thesis is brought to you for free and open access by ScholarWorks at University of Montana. It has been accepted for inclusion in Undergraduate Theses, Professional Papers, and Capstone Artifacts by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.



Assessing Lipid Composition of Cell Membrane in Escherichia Coli Under Aerobic and Anaerobic Conditions

By

Isabelle Johnson

Presented to:

Western Regional Honors Conference

University of Montana Conference of Undergraduate Research

University of Montana ScholarWorks

By Requirement by the Davison Honors College to Graduate with University Scholar distinction from the University of Montana

Under the Supervision of:

Dr. Brian Bothner – Director of Montana INBRE – Montana State University

Bethany Applegate – Davison Honors College Capstone Advisor

Missoula, Montana

July 27th, 2023

Contents

TABLE OF FIGURES	3
ABSTRACT.....	4
SIGNIFICANCE.....	5
INTRODUCTION AND LITERATURE ANALYSIS	5
RESULTS	11
Escherichia Coli Growth and Sampling	11
Mass Spectrometry Analysis	12
DISCUSSION.....	13
METHODS	15
Escherichia Coli Growth and Sampling	15
<i>Overnight Culture Preparation</i>	15
<i>Bottle Sample Methodology</i>	15
<i>Optical Density Measurements</i>	15
<i>Cell Spin down and Wash</i>	16
Lipid Extraction Methods	16
<i>Sonication of Cells</i>	16
<i>Folch Extraction</i>	16
MALDI-MS Sample Preparation	17
<i>MALDI-MS Preparation</i>	17
ACKNOWLEDGEMENTS	17
REFERENCES	17

TABLE OF FIGURES

FIGURE 1: DIAGRAM OF ESCHERICHIA COLI CELL WALL	9
FIGURE 2: STRUCTURES OF CARDIOLIPIN, PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLGLYCEROL.	10
FIGURE 3: GROWTH CURVES OF AEROBIC AND ANAEROBIC SAMPLES.	11
FIGURE 4: MALDI-TOF-MS DATA OF ANAEROBIC CULTURES.....	12
FIGURE 5: MALDI-TOF-MS DATA OF AEROBIC CELLS	13

ABSTRACT

Escherichia coli is a highly studied model organism that is tightly tied to the mammalian gastrointestinal system. This microorganism has the capability to be a beneficial gut microbe or a life-threatening pathogen. In this study, the lipid membrane of *Escherichia coli* was investigated using Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) to observe the change in its composition in aerobic and anaerobic conditions. Evidence of desaturation was discovered in the spectra, though more investigation is needed to understand the metabolic processes and drives that result in this change. Elucidation of these pathways in the future could result in more targeted treatment of *Escherichia coli* infections. To accompany this study, a literature review of current and past knowledge of *Escherichia coli* was conducted, including information on its discovery, environmental adaptation and pathogenicity.

SIGNIFICANCE

Escherichia coli is a microorganism in the domain Bacteria and commonly found to persist in the lower gastrointestinal tract of mammals (Lupindu, 2017). Despite often being a probiotic commensal species, *Escherichia coli* is capable of pathogenicity after transference of mobile genetic elements, such as the acquisition of Shiga Toxin from bacteriophage infection (Croxen, 2010; Nakao H, 2000). As such, it has an incredible impact on the healthcare industry due to its pathogenic versatility. The pathogenic forms of *Escherichia coli* are primarily anaerobic when observing specific intra-intestinal infections (Kaper, 2004). This research aims to investigate the potential of a metabolic transformation in the *Escherichia coli* membrane while undergoing aerobic to anaerobic respiratory transition.

INTRODUCTION AND LITERATURE ANALYSIS

To underscore the importance of this research, a broad overview of the history of *Escherichia Coli* is required. First discovered by Dr. Theodor Escherich in his study of pediatric infectious disease (Escherich, 1884) (Shulman, Friedmann, & Sims, 2007), this bacterium was isolated from infant fecal samples. Dr. Escherich referred to the new microbe as *Bacterium coli commune*, which would be later renamed to *Escherichia Coli*. Basic principles of its metabolism were revealed, including the sole dependance on carbohydrates whilst under anaerobic conditions, giving the first hints at its identity as a facultative anaerobe.

As opposed to more well known strict aerobes such as *Lactobacillus*, *Mycobacterium tuberculosis*, and *homo sapiens*, facultative anaerobes are capable of persisting in environments devoid of oxygen. As such, it thrives in the anoxic human digestive tract as a commensal (Blount, 2015). Although, this bacterium takes up a paltry 0.1-5% of the entire microbial community. Its area of choice is the thin layer of mucus that lines the large intestine, forming protective biofilm colonies.

Escherichia coli, along with other important gut flora, tends to colonize the human GI tract immediately after birth from the bacteria found within the vaginal microbiome. However, with the increase of

caesarian delivery, there has been a change in the neonatal gut microbiome. These infants delivered via C-section are found to have a microbiome that more resembles bacteria found within the environment and mother's skin, while vaginally delivered infants contain the normal gut microbiome (Rios-Covian, Langella, & Martin, 2021). This initial condition corrects itself within the first few months of life but can have lasting impacts. Alteration in the gut microbiota has been correlated with food allergy, asthma, diabetes and obesity in later life. However, recent studies have indicated that treatment with probiotics could help restore this dysbiosis (Zhang, et al., 2021).

Although colonization by non-pathogenic *Escherichia coli* is conducive to a healthy gut microbiome, infection with pathogenic strains of this bacterium can range from inconvenient to life-threatening. *Escherichia coli* can infect the human body via wounds in the skin, contact with mucus membranes, or ingestion. The latter often occurs due to contamination of food products along the course of their production and distribution (Yang, Lin, Aljuffali, & Fang, 2017). When ingested, pathogenic *Escherichia coli* often establishes biofilms to increase the success of the infection.

Biofilms line about every surface imaginable. This bacterial structure coats the water fountains in educational settings (Liu, 2020), thrives in the acidic rivers of Yellowstone National Park (Skorupa, 2014), and clogs the water filtration system of the International Space Station (Justiniano, et al., 2023). *Escherichia Coli* is no exception, forming biofilms in the mucus of the gastrointestinal tract in order to maintain this advantageous position. Biofilms are formed via four general steps: initial adhesion in favorable environments, early development and gene expression, maturation, and dispersion (Sharma, et al., 2016).

Adhesion surfaces for biofilms can be biotic or abiotic, as long as other environmental conditions, such as pH, temperature, ionic forces, repulsive electrostatic forces and hydrodynamic conditions are favorable as well. In early development, the cells convert from a motile to a sessile state, and the gene expression converts to promote fimbriae and curli fimbriae (Sharma, et al., 2016). Maturation is a far more complex process. Activation of genes such as Antigen 43, which promotes cell-cell adhesion, allow for the

colonies to build upwards as well as outwards to form the biofilm mat. The Extracellular Polymeric Substances (EPS) are produced by the bacteria in order to provide structure and protection for the biofilm. This substance is comprised of water, proteins, nucleic acids, nutrients, lipids and more (Flemming & Wingender, 2010). Then, the biofilm begins to increase quorum sensing by releasing autoinducers to nearby cells. These factors can increase virulence, motility, heat/stress resistance and fimbriae strength (Sturbelle, Marco, Guiral, & Vila, 2015).

After the biofilm matures, it begins the dispersion process. These bacteria regain their planktonic character in order to propagate to new locations. This can occur due to quorum sensing decisions, degradation of the EPS, or via some external force that dislodges it (Sharma, et al., 2016). Thus, if there is a pathogenic infection of *Escherichia coli* within the human digestive tract, it can travel from the upper colon to the lower colon, creating a worsening infection.

Although *Escherichia coli* is ever-present as a commensal in the human gut microbiome, several pathogenic strains can infect various parts of the body, including the gastrointestinal tract, urinary tract, blood, mucous membranes, and even prostatic implants (Parham, et al., 2010). *Escherichia coli* is classified into several pathotypes, including enterohemorrhagic, enteropathogenic, uropathogenic, septicemic-associated, enteroaggregative, and meningitis-associated (Blount, 2015). Many of these properties are gained from mobile genetic elements of the genome, such as via conjugation and bacteriophage transmission. Shiga-like toxin can be produced by an enterohemorrhagic strain of *Escherichia coli*. It gains its life-threatening properties from a bacteriophage that inserts genomic elements into the cell's genome. In particular, it is capable of causing hemorrhagic/gangrenous colitis, hemolytic uremic syndrome and central nervous system complications (Bitzan, 2009).

Escherichia coli exhibits adaptability when presented with various environmental conditions. When presented with an aerobic medium, such as agar plates and Luria Broth, they exhibit planktonic characteristics including tumbling motility and flagellar structures (Swiecicki, Sliusarenko, & Weibel, 2013). Aerobic respiration is highly productive, producing 26 ATP per glucose with the use of oxygen as

a terminal electron acceptor in the electron transport chain. However, this process generates Reactive Oxygen Species (ROS) that cause damage to cellular structures including DNA (Finn, Shewaramani, Leahy, Janssen, & Moon, 2017). Strangely, the mutation rate of anaerobic *Escherichia coli* is found to be greater than aerobic *Escherichia coli*. The reasoning for this difference in mutation rate is not yet understood (Shewaramani, et al., 2017). Anaerobic metabolism is far less fruitful than aerobic metabolism, netting a comparatively paltry 3 ATP per glucose. Without the presence of alternative electron acceptors to oxygen, anaerobic *Escherichia coli* undergo fermentation, the conversion of sugars to acetate, ethanol, lactate, formate, and small amounts of succinate (Clark, 1989). In both aerobic and anaerobic conditions, *Escherichia coli* cells tend to form colonies whether that be in the form of swarms or in biofilms, respectively (Swiecicki, Sliusarenko, & Weibel, 2013).

The cell membranes of *Escherichia coli* are of utmost importance for the integrity of the cell. These membranes and thin peptidoglycan layers make up the cell wall of this bacteria. *Escherichia coli* has two major membranes, the inner membrane (IM) and the outer membrane (OM), with periplasmic space between. These two membranes are present in gram-negative bacteria. The IM is primarily composed of phospholipids and α -helical proteins. The OM, on the other hand, is composed of phospholipids, glycolipids and β -barrel proteins (Rowlett, et al., 2017). While the two leaflets of the IM are fairly similar to each other, the leaflets of the OM are quite different. The inner leaflet is primarily composed of phospholipids. The outer leaflet contains the glycolipids, most notoriously the lipopolysaccharides. This

notoriety arises from the endotoxic shock caused by this structure in gram-negative bacteria (Silhavy, Kahne, & Walker, 2010).

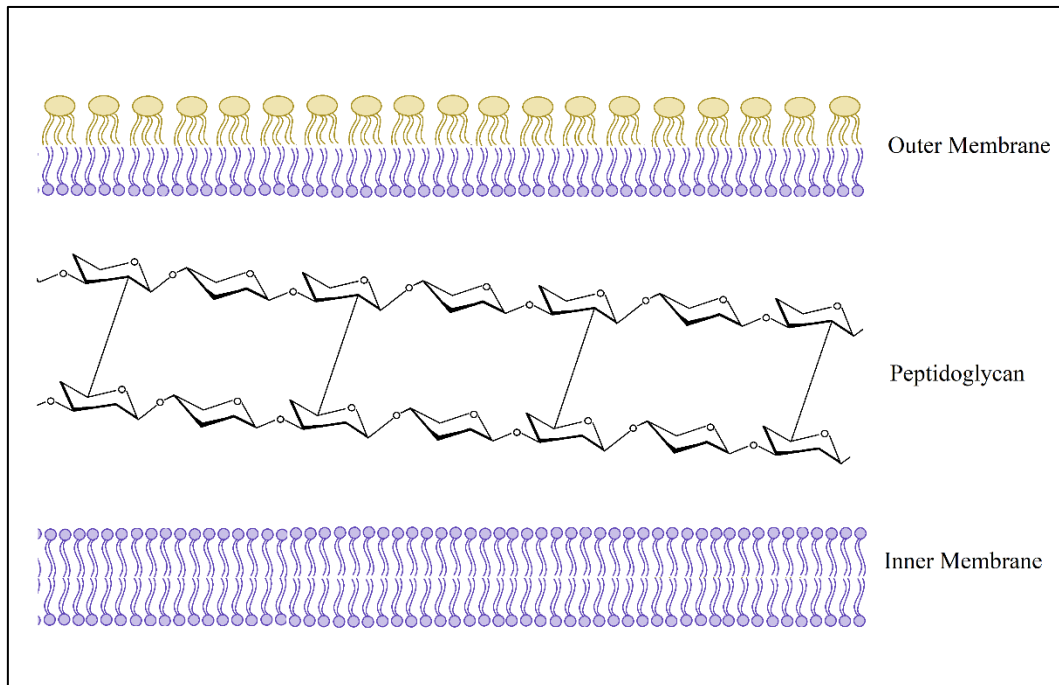


Figure 1: Diagram of *Escherichia coli* cell wall

Alterations of this cell membrane understandably alter the cell's survivability. Changes in the phospholipids of the cell membranes can result in impairment of cellular envelope structure and function, lack of biofilm formation, reduction in overall fitness and increased susceptibility to environmental stress (Rowlett, et al., 2017). In their study, Rowlett et. al. mutated each gene in the phospholipid biosynthesis pathway past the first step, including the genes *dgk*, *pssA*, *psd*, *pgsA*, *pgpABC*, *mdoB*, and *clsABC*. This eliminates the *Escherichia coli* phospholipids phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL). These changes result in temperature sensitivity, cellular envelope disorders, defective chemotaxis, reduction in outer protein synthesis, cell division, energy metabolism and osmoregulation (Rowlett, et al., 2017). These phospholipids are vital for the proper functioning of the cell and their elimination greatly decreases fitness. Therefore, therapies that disrupt the expression of phospholipid genes could result in treatment of pathogenic *Escherichia coli*.

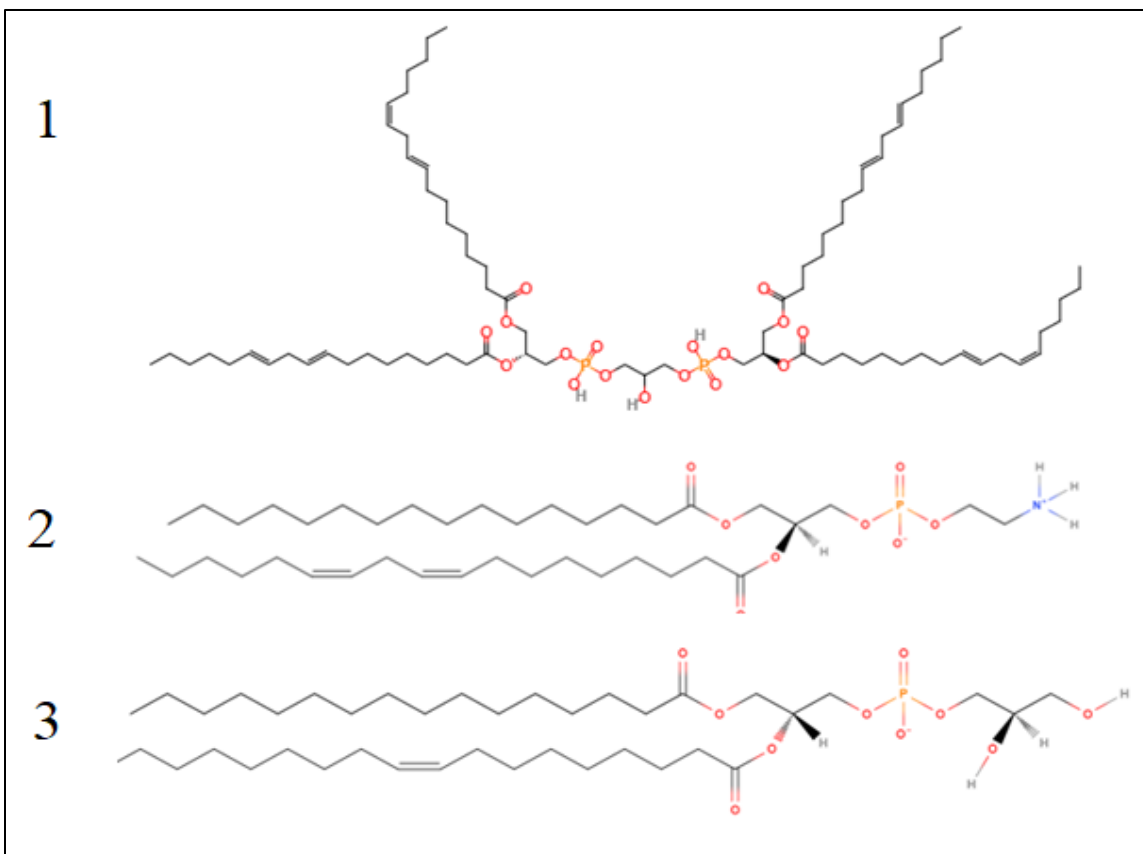


Figure 2: Structures of Cardiolipin(1), Phosphatidylethanolamine(2) and phosphatidylglycerol(3). Images generated with MolView.

Current therapies against pathogenic *Escherichia coli* are numerous but losing efficacy against the rapidly adapting bacterium. Antibiotics are the most commonly used treatment for bacterial infections. However, antibiotics are not specific to pathogenic bacteria and consequentially wipe out beneficial microbiota as well (Langdon, Crook, & Dantas, 2016). This can prevent the microbiome from adequately absorbing nutrients, producing vitamins, and protecting against bacterial and non-bacterial pathogens. In addition, overuse of antibiotics has placed strong evolutionary pressure on bacteria to become resistant to these treatments (Ventola, 2015). Therefore, it is advisable to reduce use of antibiotics to prevent further acquisition of antibiotic resistance in not only *Escherichia coli*, but also other pathogenic microorganisms.

RESULTS

Escherichia Coli Growth and Sampling

Optical Density Growth Curves: To establish the growth patterns of *Escherichia coli* when placed under aerobic and anaerobic conditions, LB media was inoculated with overnight *E. coli* growths and incubated for a period of 6 hours total for each replicate. Optical density measurements were taken at increments of either 30 minutes or 60 minutes and several growth curves were constructed. Figure 1 best exemplifies these data. It was found that the anaerobic and aerobic cells exhibited a decrease in density (thus a decrease in growth) close to $t = 4.5$ hrs. This indicates that the anaerobic cells must be undergoing a switch in metabolism to adapt to the anoxic environment. The aerobic cells were not anticipated to have the decrease in density as well, however, this can be attributed to experimental design. For the aerobic cells, they were grown in Erlenmeyer flasks covered with tinfoil, which could have subsequently decreased their access to environmental oxygen and resulted in a growth curve similar to the anaerobic cells.

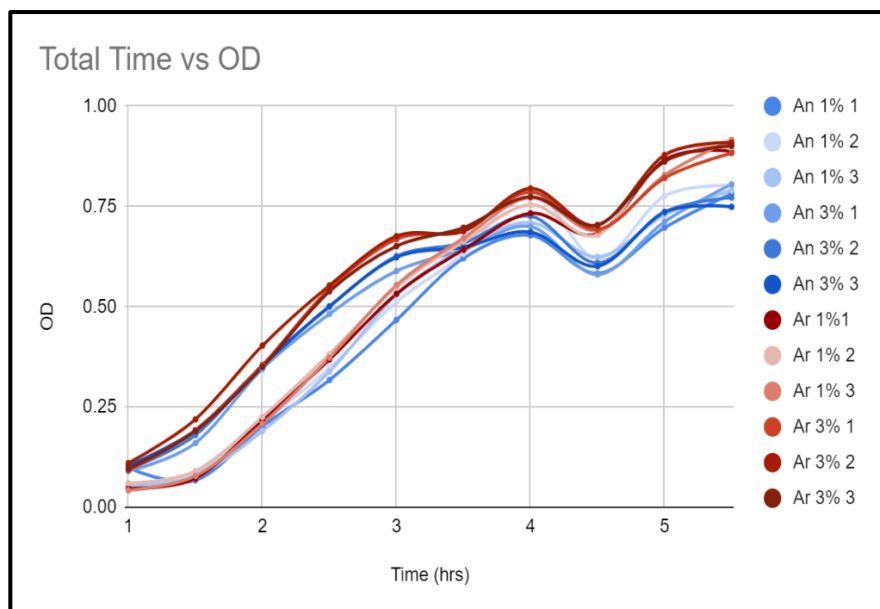


Figure 3: Growth Curves of aerobic and anaerobic samples. Samples were grown for a period of 6 hours total, with OD measurements taken at specific time increments.

Mass Spectrometry Analysis

MALDI-TOF-MS Spectra: At timepoints 3hrs, 4.5hrs, and 5hrs, samples of *Escherichia coli* were collected from the incubator and spun down to form cell pellets. These pellets were rinsed with deionized water to remove any impurities. The whole cell samples were analyzed with Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS). Figure 4 and Figure 5 display the data for the anaerobic and aerobic cultures, respectively. Lipids are found within the range of 678-902 M/Z (Zemski Berry, et al., 2012). Within these data, a peak was found at 739 M/Z that stayed constant in the aerobic cultures but decreased to 736 M/Z after 4.5hrs in the anaerobic sample. This is indicative of the unsaturation process occurring in the phospholipid membrane of the anaerobic cultures.

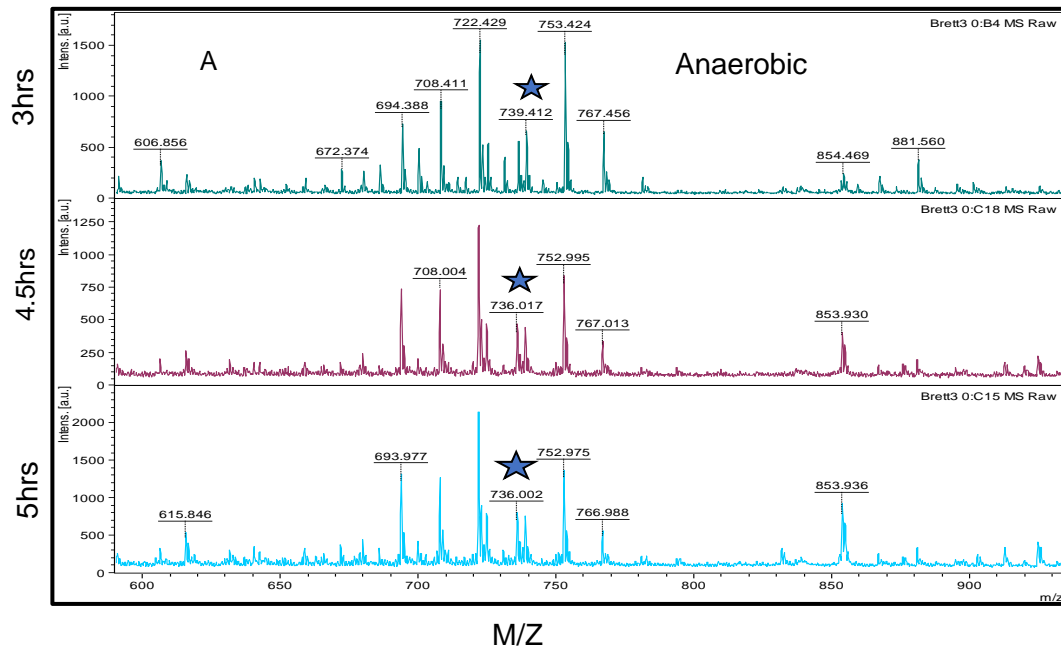


Figure 4: MALDI-TOF-MS Data of Anaerobic Cultures. Lipids such as membrane phospholipids appear in the 700 M/Z range. As compared to Figure 3, the starred peak value of M/Z is decreased.

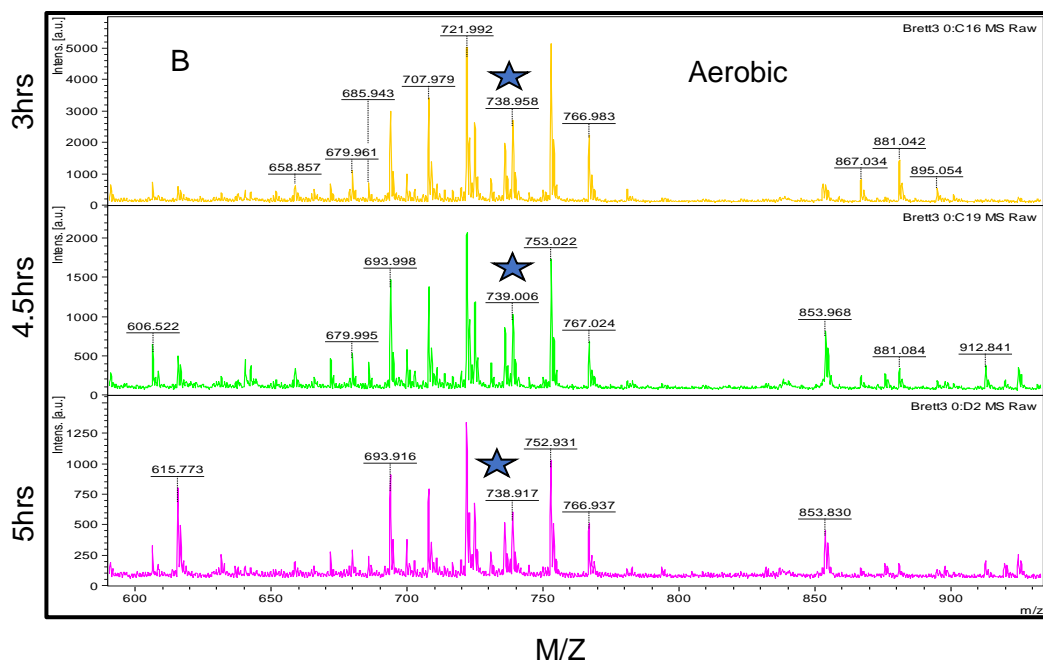


Figure 5: MALDI-TOF-MS of aerobic cells. Starred peak stays consistent over the course of 5hrs, indicating no large change in the phospholipid bilayer.

DISCUSSION

Escherichia Coli Growth and Sampling

The growth curves produced results that partially confirmed the hypothesis of an observable drop and resurgence of growth for *Escherichia coli* cells under anaerobic conditions. The anaerobic cultures exhibited a noticeable decrease in growth at the 4.5 hour timepoint. This drop can be explained by the complete consumption of the oxygen of the flask causing in a switch in metabolic strategy. The flasks themselves were not flushed with nitrogen before inoculation and incubation, resulting in normal aerobic metabolic function up until this transference point. Therefore, a metabolic change must be taking place that causes a measurable decrease in the *Escherichia coli* population once available resources are depleted. These experiments laid the foundation for the MALDI-TOF-MS experiments, defining critical timepoints where samples could be taken.

Although this observation is consistent with the anaerobic data and previous literature, the data for the aerobic cultures appears to contradict these findings. While it was anticipated that a normal

logarithmic growth curve would result from the aerobic environment, this was not observed. Instead, a similar sigmoid shape was seen in the aerobic cultures. This is inconsistent with the literature and indicates an error in the methodology. One possible explanation is that the experimental setup of the aerobic cultures, an Erlenmeyer flask with tinfoil over the opening, could have prevented proper aeration of the liquid media, resulting in an optical density graph similar to the anaerobic cultures. Another explanation is that during the course of the research, difficulty was had with contamination of the cultures, which could have skewed the resulting data. Given that the growth curve of *Escherichia coli* is well characterized (Pletnev, Osterman, Bogadanov, & Dontsova, 2015), we can consider this data to be in error. Despite this, the aerobic cultures displayed greater numbers than the anaerobic cultures, lending credence to the hypothesis.

Mass Spectrometry Analysis

The MALDI data reveal important characteristic changes of the transition of aerobic bacteria to anaerobic bacteria. The aerobic *Escherichia coli* exhibited fairly consistent M/Z values for their peaks over the course of the experiment. The consistent environment allowed for little change in the composition of the lipid membrane. Each of these spectra were generated in triplicates and each displayed similar data. These samples were collected after the initial growth curve defining experiments and thus are thought to be without error.

The anaerobic *Escherichia coli*, on the other hand, consistently had decreases in the M/Z values over the course of the experiment. For example, Figure 4 displays peaks decreasing from 694 M/Z to 693 M/Z, 739 M/Z to 736 M/Z, and 767 M/Z to 766 M/Z, to name a few. This is indicative of desaturation of the phospholipids present in the bacteria cell wall. When a lipid is desaturated, a hydrogen atom is consumed and a double bond forms. These hydrogen atoms could potentially be used as an energy source during the transition of aerobic bacteria to anaerobic bacteria while the organism prepares to undergo anaerobic respiration. If this process could be inhibited in some way, pathogenic *Escherichia coli* could be eliminated from the intestine during an infection. Such a treatment would need to be specific in order

to prevent attack of benevolent *Escherichia coli*. Further investigation into the specific enzymes and molecular processes that drive the transition of the cell wall could yield results beneficial to medical treatment and healthcare.

METHODS

Escherichia Coli Growth and Sampling

Overnight Culture Preparation: Refrigerated sample of K12 *Escherichia coli* previously plated was used for the overnight growths. 3 autoclaved 50mL Erlenmeyer flasks were filled with 20mL of LB media via automatic pipette within a fume hood after sterilization with UV light. The flasks were then inoculated each with a single colony of *Escherichia coli*. The plates were resealed for later use and returned to the refrigerator. The 50mL Erlenmeyer flasks were then placed into an incubator at 165 rpm and 37 °C overnight. Overnight cultures were prepared at 4pm the previous day and used to inoculate LB media at 9am the day of the experimentation.

Bottle Sample Methodology: For each round of experimentation, an equal amount of autoclaved 250mL Erlenmeyer flasks and 250mL glass sealable bottles were filled with 100mL of LB media within a fume hood. Each container was inoculated with the *Escherichia coli* overnight solution at either 3% or 1% concentration. Each was labeled in equal groups for timepoints 3hrs, 4.5hrs, and 5hrs. The Erlenmeyer flasks were topped with tinfoil and the sealable bottles were closed with a rubber stopper and aluminum collar. These samples were then incubated at 180 rpm and 37 °C.

Optical Density Measurements: At timepoints 3hrs, 4.5hrs, and 5hrs, 1mL samples were collected from each flask with the respective labeling scheme. The aerobic samples were gathered with an automatic pipette and the anaerobic samples were gathered with a sterile syringe. The spectrophotometer was blanked at 550nm with a cuvette filled with deionized water. Each sample was measured and the data was graphed to observe a growth curve.

Cell Spin down and Wash: 10mL of sample was collected at 3hrs, 4.5hrs, and 5hrs for each duplicate. These samples were pipetted into 10mL conical tubes and stored in the centrifuge. These samples were then centrifuged for 5 minutes and the supernatant was discarded. The resulting pellets were then resuspended with deionized water. The samples were centrifuged and suspended twice more. The samples were stored in an -80°C freezer.

Lipid Extraction Methods

Sonication of Cells: Frozen samples were defrosted briefly at room temperature and resuspended with 5mL 200mM ammonium acetate. Samples were placed into a 250mL beaker and surrounded by frozen steel pellets. The samples were sonicated for a period of 10 minutes. Between each sample, the sonicator was rinsed with deionized water to prevent cross-contamination. Samples were then centrifuged for 20 minutes and the supernatant was discarded. The resulting pellets were then stored in the -80°C freezer.

Folch Extraction: Using glass pipettes, the samples were resuspended with 1.3mL of HPLC grade methanol and transferred into a 7mL glass test tube nested within a 15mL falcon tube. 1mL of HPLC grade MTBE and 1mL of HPLC grade CHCL₃ was to each test tube and the solution was vortexed briefly. The samples were incubated at 225 rpm and 22°C for 1 hour. The samples were briefly vortexed and then centrifuged at 17500g for 10 minutes. 1.9mL of supernatant was collected from the tubes, taking care to not pierce the buffy layer. This supernatant was placed into a 7mL glass test tube and dried in a vacuum centrifuge until no liquid remained. This was then frozen at -80°C.

MALDI-MS Sample Preparation

MALDI-MS Preparation: The cell pellets were thawed and resuspended in phosphate-buffered saline. 1 microliter of slurry was then spotted onto a MALDI plate. 1 microliter of DHB matrix was applied after the slurry was dry. This plate was then analyzed with MALDI-TOF MS for lipid composition.

ACKNOWLEDGEMENTS

I would like to thank the Bothner Lab for giving me this opportunity to explore this subject of study and experience research in-depth. Dr. Bothner, Brett Sather and all those in the Bothner lab made this whole project a wonderful experience that fuels my interest in research and biochemistry. The research was conducted with a National Science Foundation grant that provided a stipend, room and board, and much more. I would like to thank Brynne Miller as well, who I worked equally with to gather the data for this project and helped to edit the drafts. Montana State University made this possible by hosting the program, with Dana Skorupa's mentorship providing not only academic and career tools, but exploration of the beautiful Bozeman area. I would also like to thank the Davison Honors College for this opportunity to dive deeper into this area of study.

REFERENCES

- Bitzan, M. (2009). Treatment options for HUS secondary to Escherichia coli O157:H7. *Kidney International*, S62-S66.
- Blount, Z. D. (2015). The unexhausted potential of E. coli. *eLife*.
- Clark, D. P. (1989). The fermentation pathways of Escherichia coli. *FEMS Microbiology Reviews*, 223-234.
- Croxen, M. F. (2010). Molecular mechanisms of Escherichia coli pathogenicity. In *Nature Reviews Microbiology* (pp. 26-38).

- Escherich, T. (1884). The intestinal bacteria of the neonate and breast-fed infant. 1884. *Rev Infect Dis*.
- Finn, T. J., Shewaramani, S., Leahy, S. C., Janssen, P. H., & Moon, C. D. (2017). Dynamics and genetic diversification of *Escherichia coli* during experimental adaptation to an anaerobic environment. *PeerJ*, 10.7717/peerj.3244.
- Flemming, H., & Wingender, J. (2010). The biofilm matrix. *Nat Rev Microbiol*, 623-633.
- Justiniano, Y.-A. V., Goeres, D. M., Sandvik, E. L., Kjellerup, B. V., Sysoeva, T. A., Harris, J. S., . . . Lott, K. (2023). Mitigation and use of biofilms in space for the benefit of human space exploration. *Biofilm*.
- Kaper, J. N. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology*, 123-140.
- Langdon, A., Crook, N., & Dantas, G. (2016). The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome Medicine*, Article number 39.
- Liu, Y. (2020). A New Spatially-Resolved Method to Sample Biofilms from Drinking Water Fountains. *McKelvey School of Engineering Theses and Dissertations*.
- Lupindu, A. M. (2017). Isolation and characterization of *Escherichia coli* from animals, humans, and environment. In A. Samie, *Escherichia Coli-Recent Advances on Physiology, Pathogenesis and Biotechnological Applications* (pp. 187-206). London, United Kingdom: IntechOpen Limited.
- Nakao H, T. T. (2000, August 9). *Escherichia coli* Shiga toxin. *Journal of Natural Toxins*, pp. 299-313.
- Parham, S., Frei, R., Maurer, T. B., Tramuz, A., Zimmerli, W., & Graber, P. (2010). *Escherichia coli* Variants in Periprosthetic Joint Infection: Diagnostic Challenges with Sessile Bacteria and Sonication. *Journal of Clinical Microbiology*, 1720-1725.

- Pletnev, P., Osterman, I., Bogadanov, A., & Dontsova, O. (2015). Survival Guide: Escherichia coli in the stationary phase. *Acta Naturae*, 22-33.
- Rios-Covian, D., Langella, P., & Martin, R. (2021). From Short- to Long-Term Effect of C-Section Delivery on Microbiome Establishment and Host Health. *Microorganisms*, 10.3390/microorganisms9102122.
- Rowlett, V. W., Mallampalli, V. K., Karlstaedt, A., Dowhan, W., Taegtmeier, H., Margolin, W., & Vitrac, H. (2017). Impact of Membrane Phospholipid Alterations in Escherichia coli on Cellular Function and Bacterial Stress Adaptation. *Journal of Bacteriology*, 10.1128/JB.00849-16.
- Sharma, G., Sharma, S., Sharma, P., Chandola, D., Dang, S., Gupta, R., & Gabrani. (2016). Escherichia coli biofilm: development and therapeutic strategies. *Journal of Applied Microbiology*, 309-319.
- Shewaramani, S., Finn, T. J., Leahy, S. C., Kassen, R., Rainey, P. B., & Moon, C. D. (2017). Anaerobically Grown Escherichia coli Has an Enhanced Mutation Rate and Distinct Mutational Spectra. *PLOS Genetics*, 10.1371/journal.pgen.1006570.
- Shulman, S. T., Friedmann, H. C., & Sims, R. H. (2007). Theodor Escherich: The First Pediatric Infectious Diseases Physician? *Clinical Infectious Diseases*, 1025-1029.
- Silhavy, T. J., Kahne, D., & Walker, S. (2010). The Bacterial Cell Envelope. *Cold Spring Harbor Perspectives in Biology*, 10.1101/cshperspect.a000414.
- Skorupa, D. J. (2014). In situ gene expression profiling of the thermoacidophilic alga Cyanidoschyzon in relation to visible and ultraviolet irradiance. *Environ Microbiology*.
- Sturbelle, R., Marco, F., Guiral, E., & Vila, J. (2015). Biofilm formation in uropathogenic Escherichia coli strains: relationship with urovirulence factors and antimicrobial resistance. In A. Nikibakhsh, *Clinical Management of Complicated Urinary Tract Infection* (pp. 159-170). Croatia: Intech.

- Swiecicki, J.-M., Sliusarenko, O., & Weibel, D. B. (2013). From swimming to swarming: *Escherichia coli* cell motility in two-dimensions. *Integrative Biology*, 1490-1494.
- Ventola, C. L. (2015). The Antibiotic Resistance Crisis. *Pharmacy and Therapeutics*, 277-283.
- Yang, S.-C., Lin, C.-H., Aljuffali, I. A., & Fang, J.-Y. (2017). Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. *Archives of Microbiology*, 811-825.
- Zhang, C., Li, L., Xu, X., Zuo, X., Li, Y., & Li, Z. (2021). The Effects of Delivery Mode on the Gut Microbiota and Health: State of Art. *Frontiers in Microbiology*, 10.3389/fmicb.2021.724449.