Determining the Role of Fatty Acid Composition in Antibiotic Resistance

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ABSTRACT

Bacterial infections that can no longer be treated by antibiotics because of bacterial mutations cause many infections and deaths each year. My research conducted aims to study how membrane fatty acid composition can affect bacterial susceptibility to antibiotics. *Listeria* monocytogenes, a gram-positive facultative anaerobe, is the bacterium that I am testing. *Listeria* has 80-90% branched-chain fatty acids (BCFAs) which allow membrane fluidity and sufficient protection against invaders. When Listeria is grown in the presence of butyrate, the BCFAs become straight-chain fatty acids (SCFAs) and make the once fluid membrane more rigid. We believe that this allows for easier antibiotic penetration of the phospholipid bilayer which lets the antibiotics affect cellular processes. By changing concentrations of butyrate I can therefore determine the minimum inhibitory concentrations of antibiotics for *Listeria* with different membrane fatty acid compositions. Moreover, as growth is a key factor in bacterial susceptibility to antibiotics. I also measure oxygen consumption rate in response to butyrate. Higher oxygen consumption rate is indicative of higher bacterial activity. Because oxygen consumption is carried out by protein complexes on the membrane, measuring oxygen consumption rate also reveal the effects of butyrate on cell membrane functionality.

MATERIALS AND METHODS

Oxygen Consumption Measurement

Suspensions of bacteria grown under different conditions were prepared and used for oxygen consumption measurement. A oxygen concentration probe was used to monitor oxygen consumption inside an anaerobic chamber where a no bacteria control sample was included to measure abiotic loss of oxygen.

Transmission Electron Microscopy (TEM)

Bacterial were grown in BHI with or without 100mM butyrate for TEM analysis. To prepare the bacterial samples for TEM, the SPI Chem SPI-Pon 812 Kit protocol was used to suspend the two different types of bacteria in resin with each type of bacteria in a separate resin. The dried samples were then cut into 100 nm sections using an ultra-microtome with a diamond blade. The sections were imaged on carbon grids by a Hitachi H-7600 Transmission Electron Microscope at 100kv and at varying magnifications between 3,000 and 80,000 x.

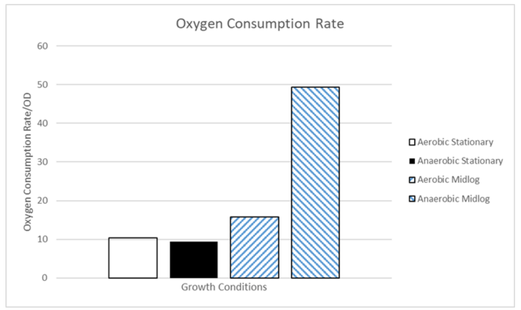
Antibiotic Susceptibility Assay

The final experimental procedure investigated that efficacy of eight different antibiotics on *Listeria* grown with and without 100 mM of butyrate. The eight antibiotics tested were chloramphenicol, erythromycin, neomycin, kanamycin sulfate, penicillin, moxalactam, streptomycin sulfate, and lysozyme. Chloramphenicol and erythromycin were only soluble in ethanol, while the other six antibiotics were soluble in water. Penicillin and moxalactam target bacterial cell wall synthesis outside the bacterial cells, while the other six antibiotics target pathways inside the bacterial cells. Each antibiotic was serially diluted and added to bacterial cultures inside sterile 96-well plates with or without butyrate supplementation. The final concentration of antibiotics increased every three wells from 8 µg/ml, 16 µg/ml, to 32 µg/ml, 64 µg/ml, 128 µg/ml, 256 µg/ml, and finally 512 µg/ml. The plates were then incubated and grown overnight aerobically at 37°C. The optical density of both types of bacteria were taken before the experiment and subtracted off the final value.

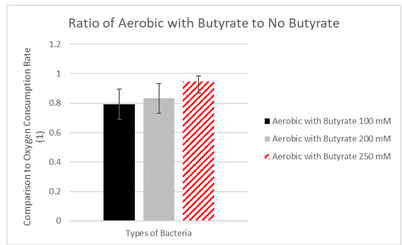
REFERENCES

Center for Disease Control and Prevention

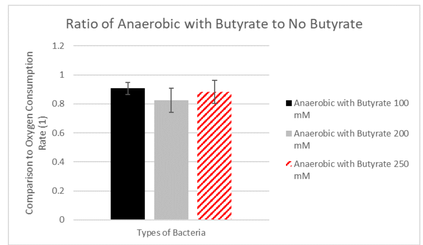
RESULTS



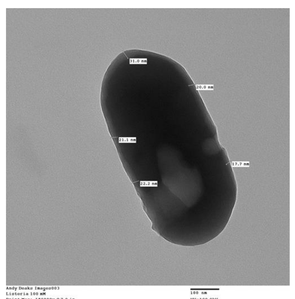
Preliminary data on the oxygen consumption rate of aerobically and anaerobically grown bacteria



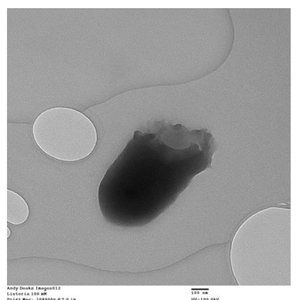
Ratio of aerobically-grown bacteria supplemented with butyrate compared to aerobically-grown bacteria with no butyrate supplementation. No butyrate supplementation is the baseline (1). Any bar lower than 1 means a lower oxygen consumption rate.



Ratio of anaerobically-grown bacteria supplemented with butyrate compared to anaerobically-grown bacteria with no butyrate supplementation. No butyrate supplementation is the baseline (1). Any bar lower than 1 means a lower oxygen consumption rate.



Listeria monocytogenes without butyrate supplementation

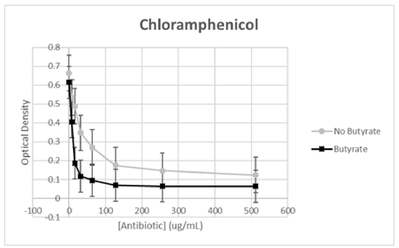


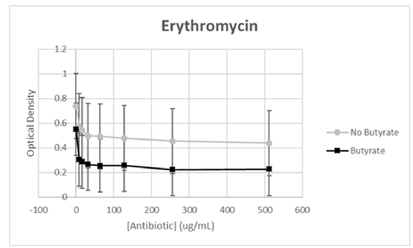
Listeria monocytogenes with 100 mM butyrate supplementation

ACKNOWLEDGEMENTS

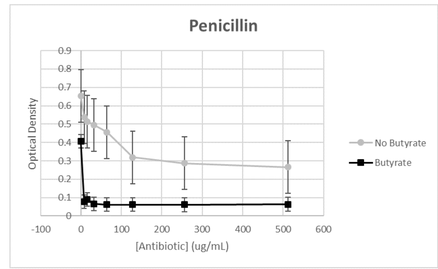
I would like to dedicate this project to Dr. Yvonne Sun for all her hard work throughout my collegiate experience. I would also like to acknowledge the entire microbiology lab team, especially Nathan Wallace, the University of Dayton Honors Program, the College of Arts and Sciences Dean's Summer Fellowship, the Chair of the Biology Department Mark Nielsen, Ryan McEwan, Kevin Custer, and Barbara Miller.

RESULTS





The average optical density of *Listeria* across various concentrations of two different antibiotics. *not statistically significant*



The average optical density of Listeria across various concentrations of penicillin. *statistically significant*

MAIN FINDINGS

The average optical density of the butyrate-supplemented Listeria was lower than the Listeria with no butyrate at every concentration tested. Additionally, the average rate of change between the beginning optical density and the final optical density, as well as the average rate of change between each concentration were both lower in the bacteria that was grown with butyrate. **My thesis research identified that butyrate supplementation, which is known to increase the amount of straight chain fatty acids in Listeria, lowers the oxygen consumption rate of Listeria monocytogenes, with the largest decrease in oxygen consumption rate at a butyrate concentration of 100 mM. Moreover, butyrate supplementation also resulted in an altered morphology as well as increased susceptibility to penicillin**.

FUTURE RESEARCH

This research can be used by the pharmaceutical industry in an effort to combat the issue of antibiotic resistant bacteria in the United States and the rest of the world. Instead of increasing the dosage of antibiotics prescribed or spending billions of dollars to develop new and unique antibiotics, the pharmaceutical industry could use this research to investigate how changing the composition of the bacteria can increase the efficacy of antibiotics. Further research in this field is necessary in order to confirm these findings. Further research should investigate the effects of butyrate on other types of bacteria, other types of antibiotics, and the effects of the supplementation of other short-chain fatty acids besides butyrate.