Determining the Role of Membrane 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 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 100 mM oxygen consumption probe was used to monitor oxygen consumption inside an anaerobic chamber where no bacteria control sample was included to measure abiotic loss of oxygen.

Transmission Electron Microscopy (TEM)
Bacterial were grown in BM with or without 100 mM butyrate for TEM analysis. To prepare the bacterial samples for TEM, the SPI Chem SPI-Pan 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 ultramicrotome 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, minocycline, streptomycin sulfate, and tetracycline. Chloramphenicol and erythromycin were only soluble in ethanol, while the other six antibiotics were soluble in water. Penicillin and minocycline 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 the bacterial cultures inside sterile 96 well plates with or without butyrate supplementation. The final concentration of antibiotics increased every three wells from 0.0 µg/mL, 0.16 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.

RESULTS

Oxygen Consumption Rate
The average oxygen density of Listeria across various concentrations of different antibiotics is shown in the figure. The average oxygen consumption rate is 0.035 ± 0.005 µmol O2/mg protein for bacteria grown with butyrate and 0.015 ± 0.002 µmol O2/mg protein for bacteria grown with no butyrate. The average oxygen consumption rate for bacteria grown with butyrate supplemented with 100 mM butyrate was 0.045 ± 0.006 µmol O2/mg protein.

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REFERENCES

Center for Disease Control and Prevention

ADDENDUM

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.