



The Effects of Short Chain Fatty Acids on the Ability of *Listeria monocytogenes* to Form Biofilm



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ABSTRACT

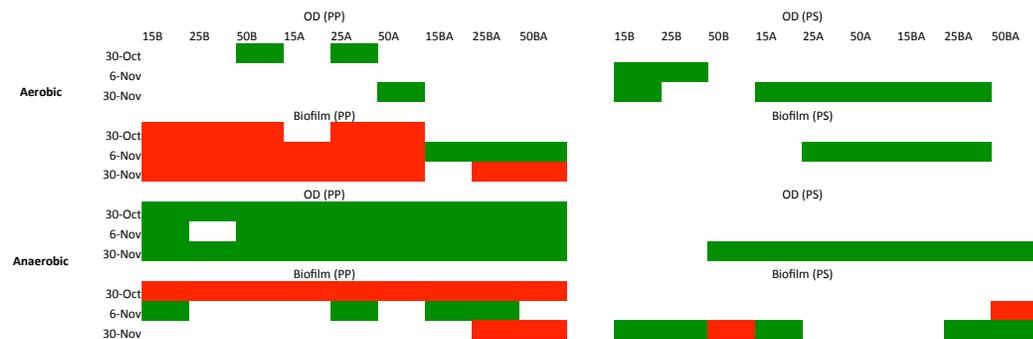
Introduction. *Listeria monocytogenes* is a common food borne pathogen that can be fatal to those who with compromised immune systems. To combat food borne pathogens, short chain fatty acids are added as food to preservatives. Purpose. The purpose of this experiment was to determine the effects of short chain fatty acids (SCFAs) on the growth of *Listeria monocytogenes*. Specifically, this research looked at the impact of varying amounts of acetate, butyrate, and propionate on the ability of *Listeria* to grow in a biofilm, both aerobically and anaerobically, in polypropylene (PP) and polystyrene (PS) in microwell plates. Results. Under anaerobic, but not aerobic, conditions, *Listeria* planktonic growth increased when SCFAs were added in the culture medium. However, I did not detect consistent results when *Listeria* was growing in the microwell plates. Conclusion. As indicated by these results, *Listeria monocytogenes* planktonic growth can be increased when grown anaerobically with SCFAs. However, future research is necessary to assess the effects of SCFAs on biofilm growth.

BACKGROUND INFORMATION

A common method of extending the time before a food spoils is the use of food preservatives. These preservatives can either be of organic or inorganic nature, with those used in this study being of organic nature. The short chain fatty organic acids used throughout the duration of this experiment were acetate, propionate, and butyrate.

We used *Listeria monocytogenes* as a model organism to test the ability of these bacteria grow in the presence of acetate, butyrate, and propionate. *Listeria* is a common food borne pathogen. This bacteria is particularly dangerous to the elderly, pregnant women, newborns, and the immunocompromised. These groups have a mortality rate of 30% upon infection with *Listeria monocytogenes*. Many food companies have fallen victim to *Listeria* outbreaks, with notable companies being Dole, Northwest Naturals, Zoologics, and The Maiden's Creamery.

RESULTS



The green represents a statistically significant increase while the red represents a statistically significant decrease in growth. White represents no statistically significance. OD indicates planktonic growth. PP represents bacteria grown in a polypropylene well and PS represents bacteria grown in a polystyrene well.

MATERIALS AND METHODS

Listeria monocytogenes was placed in 3 mL of BHI media and grown aerobically in an incubator set at 37 °C. After a 24 hour incubation period, 1 mL of bacterial solution was spun down in a microcentrifuge tube for three minutes at 10,000 RPM. The bacteria was then resuspended in 1 mL of BHI and aliquoted into sterile 96-well plates with or without 15, 25, or 50 mM of either butyrate, acetate, a combination of butyrate and acetate. The plates were incubated at 37°C for 72 hours under aerobic or anaerobic conditions.

After 72 hours of incubation, the plates were removed from the incubators first for optical density reading. Then, the plates were washed five times with sterile water and air dried until no visible moisture remained. Dried plates were then stained with 150 uL of crystal violet for 45 minutes followed by washing five more times with water. Following the wash, plates were dried again until no visible moisture remained. Once dry, 200 uL of EtOH was pipetted into each well that held crystal violet. After this, 100 uL was pipetted into corresponding wells in a new flat-bottom 96-well plate and optical density was recorded at 550 nm.

MAIN FINDINGS

My findings suggest that under anaerobic, but not aerobic, conditions, *Listeria* growth is increased when SCFA were added. Despite this, I was not able to obtain consistent results when determining the effects of SCFA on growth in 96-well plates.

FUTURE RESEARCH

Further research is needed to look at the effects of SCFA on *Listeria*'s ability to form biofilm. This should include *Listeria* grown aerobically and anaerobically. Different types of 96-well plates should also be used to detect differences that may occur due to *Listeria*'s ability to adhere to the plate.

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