Effects of propionate on the growth and production of listeriolyisin O by *Listeria monocytogenes* under aerobic and anaerobic conditions

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### Background

1. *Listeria monocytogenes* is one of the leading causes of death from foodborne infections. Current protective measures rely on public food recalls and individual compliance with food choice guidelines. A preventative measure will greatly protect high risk individuals against complications from *Listeria* infections.

2. The gut microbiota provides resistance against enteric infections likely through a variety of mechanisms. Better understanding of the mechanisms will enable us to develop novel preventative measures to protect high risk individuals.

3. Propionate is a fermentation product of the gut bacteria and exhibits a variety of regulatory and nutritional functions. In this study, we explored the effects of propionate anaerobically and aerobically on *Listeria* growth and production of the virulence factor, listeriolyisin O.

### Materials and Methods

**Bacteria:** *Listeria monocytogenes* strain 10403s was used for all experiments.

*In vitro Growth:* Overnight cultures started with fresh colonies were used to inoculate filter-sterilized brain heart infusion (BHI) media supplemented with different concentrations of sodium propionate. Culture optical density was measured every hour for 8 hours. Aerobic growth was performed with vigorous agitation (200rpm) while anaerobic growth was performed with static incubation inside an anaerobic chamber (Coy). All growth was conducted at 37 degrees Celsius.

**Hemolytic Assay:** To determine the effect of propionate on *Listeria* toxin production, we used a standard hemolytic assay to measure the activity of the secreted toxin, listeriolyisin O (LLO). Bacteria were cultured overnight with different concentrations of sodium propionate under aerobic or anaerobic conditions. To increase the basal level of LLO production in order to better observe potential inhibitory effects of propionate, bacteria were grown with activated charcoal-treated BHI. Culture supernatant was collected, serially diluted, and mixed with defibrinated sheep blood. The mixture was incubated at 37 degrees celsius for 30 minutes. The absorbance of the supernatant from lysed red blood cells was measured and normalized to the original culture optical density.

### Main Findings

**Growth:** Propionate at concentration as high as 25mM did not inhibit *Listeria* growth under aerobic or anaerobic conditions. However, a notable increase in doubling time and decrease in maximum growth were observed with propionate supplementation under both aerobic and anaerobic conditions.

**LLO production**: Propionate supplementation under aerobic condition leads to a dose-dependent decrease in LLO production. However, propionate supplementation under anaerobic condition leads to a dose-dependent increase in LLO production.

### Future Directions

To confirm our hemolytic assay results, we will perform western blot to determine LLO abundance and we will perform qRT-PCR to determine transcript levels of *Listeria* virulence genes. We will also test for the role of known virulence regulators, PrfA, SigB, and CodY, in mediating the effect of propionate on LLO production.

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