Testing the Effects of Mucin and Nisin on the Susceptibility of *Listeria monocytogenes* to Antimicrobial Peptides

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Background

Listeria monocytogenes is a foodborne pathogen that can survive and cause infections in the human gastrointestinal tract. In susceptible populations, such as those immunocompromised, *L. monocytogenes* is able to cross the intestinal barrier and cause diseases such as meningitis that are much higher in mortality. During *L. monocytogenes* transit through the GI tract, it is exposed to the mucosal barrier rich with mucus and antimicrobial peptides (AMPs)--two major innate defense mechanisms against foreign pathogens. Moreover, the endogenous microbes produce large quantities of fermentation acids that also assist in reducing pathogen colonization.

Main Objectives

In this study, we examined the effects of mucus and propionate, one of the major fermentation acids found in the human GI lumen, on the susceptibility of *L. monocytogenes* to AMPs.

Research Methods

- Prepared anaerobic and aerobic cultures of *Listeria monocytogenes* wild type and ΔsigB mutant overnight at 37 degrees celsius
- After cultures grew the optical density was measured and equal concentrations of bacteria were formed
- Using a 96 well plate different conditions (bacteria, water, nisin, musin, propionate) were applied to each well and then were incubated at 37 degrees celsius for an hour
  - 18 wells with different conditions were used for both WT and ΔsigB
- After incubation dilutions were performed with water on each well up to $1{:}10^6$
- Dilutions $1{:}10^3$ $1{:}10^4$ $1{:}10^5$ $1{:}10^6$ were used to plate on LB plates and incubated at 37 degrees celsius for 48 hours
- The results shown here are from trial 3

Results: Control

Added to each well	mucin	nisin	propionate	bacteria	H_2O	strain	Results 2/5/2020
1.	No mucin	No nisin	No prop	50 µL $+O_2$	30 µL water	WT	
1.	No mucin	No nisin	No prop	50 µL $+O_2$	30 µL water	sigB	
2.	No mucin	No nisin	No prop	50 µL $-O_2$	30 µL water	WT	
2.	No mucin	No nisin	No prop	50 µL $-O_2$	30 µL water	sigB	

Nisin Only: Reference for Results 1, 2, and 3

7.	No mucin	10 µL nisin	No prop	50 µL $+O_2$	20 µL water	WT	
7.	No mucin	10 µL nisin	No prop	50 µL $+O_2$	20 µL water	sigB	
8.	No mucin	10 µL nisin	No prop	50 µL $-O_2$	20 µL water	WT	
8.	No mucin	10 µL nisin	No prop	50 µL $-O_2$	20 µL water	sigB	

RESULT 1: Propionate Effect on Listeria Susceptibility to Nisin

11.	No mucin	10 µL nisin	10 µL prop	50 µL $+O_2$	20 µL water	WT	
11.	No mucin	10 µL nisin	10 µL prop	50 µL $+O_2$	20 µL water	sigB	
12.	No mucin	10 µL nisin	10 µL prop	50 µL $-O_2$	20 µL water	WT	
12.	No mucin	10 µL nisin	10 µL prop	50 µL $-O_2$	20 µL water	sigB	

RESULT 2: Mucin Effect on Listeria Susceptibility to Nisin

13.	10 µL mucin	10 µL nisin	No prop	50 µL $+O_2$	20 µL water	WT	
13.	10 µL mucin	10 µL nisin	No prop	50 µL $+O_2$	20 µL water	sigB	
14.	10 µL mucin	10 µL nisin	No prop	50 µL $-O_2$	20 µL water	WT	
14.	10 µL mucin	10 µL nisin	No prop	50 µL $-O_2$	20 µL water	sigB	

RESULT 3: Propionate and Mucin Effect on Listeria Susceptibility to Nisin

15.	10 µL mucin	10 µL nisin	10 µL prop	50 µL $+O_2$	No water	WT	
15.	10 µL mucin	10 µL nisin	10 µL prop	50 µL $+O_2$	No water	sigB	
16.	10 µL mucin	10 µL nisin	10 µL prop	50 µL $-O_2$	No water	WT	
16.	10 µL mucin	10 µL nisin	10 µL prop	50 µL $-O_2$	No water	sigB	

RESULT 4: Anaerobic Growth Effect on Listeria Susceptibility to Nisin

7.	No mucin	10 µL nisin	No prop	50 µL $+O_2$	20 µL water	WT	
7.	No mucin	10 µL nisin	No prop	50 µL $+O_2$	20 µL water	sigB	
8.	No mucin	10 µL nisin	No prop	50 µL $-O_2$	20 µL water	WT	
8.	No mucin	10 µL nisin	No prop	50 µL $-O_2$	20 µL water	sigB	

yellow: aerobic
red: anaerobic

Conclusions

Using nisin as a model AMP, we found that propionate and mucin alone increased the susceptibility of *L. monocytogenes* to nisin. With the exception of the *L. monocytogenes* ΔsigB mutant, in which propionate alone decreased susceptibility to nisin. We found that propionate and mucin together seemed to have no effect on the susceptibility of *L. monocytogenes* to nisin. From our results we also determined that anaerobic growth only increased *L. monocytogenes* susceptibility to nisin in the ΔsigB mutant. Further research is to be done with the human antimicrobial peptide LL-37 to see if similar results are found.

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