Carbohydrates’ effects on the production of reuterin by *Lactobacillus reuteri*

An experiment in biology by Micah Forshee

Mentored by Dr. Daniel Sharda
Preview

- Introduction to microbiome and project at hand
- Methodology
- Results
- Discussion
Introduction
Background

• “Diarrhea kills more children than malaria, measles, and AIDS combined”

• Hope and aid for these people

• So much to learn about our interconnectedness with our microbiome

Source: Xia & Sun. Genes & Disease 2017
The Microbiome

- Bacteria within the human gut
- Lots of studies but still more to learn
- Host health is influenced by its composition
  - Imbalances correlated with inflammation and heart failure
  - Good bacteria help constipation, combat obesity, and fight pathogens
Gut Community

- Probiotics – beneficial bacteria
  - Crowd out pathogens
  - Secrete inhibitory substances
- Prebiotics – indigestible compounds that probiotics can metabolize within the gut
- Pathogens – harmful bacteria
Prebiotics

• Enhance probiotic growth

• Increase antimicrobial production

• Prebiotics found in a variety of produce and milks

• How exactly do these impact probiotic workings?

https://www.thehealthyhomeeconomist.com/prebiotics-benefit-gut-health/
Inulin

Galactooligosaccharides (GOS)

https://commons.wikimedia.org/wiki/File:Inulin_strukturformel.png

https://commons.wikimedia.org/wiki/File:Galactooligosaccharide.png
**Lactobacillus reuteri**

- Probiotic that is part of a healthy gut
- Alleviates constipation, prevents pathogen colonization, prevents osteoporosis in mice
- Unique characteristic: glycerol can be converted into potent antimicrobial reuterin
Reuterin

- Induces oxidative stress to pathogens => wide antimicrobial properties
- Excreted out of the cell
- Glucose to glycerol ratio affects its production
Question of interest

• How do the metabolic consequences of prebiotics impact the activity of probiotics?

• Will addition of inulin increase the amount of reuterin produced?

• Will *L. reuteri* produce reuterin if glucose is not used as the primary carbon source?
Methodology
### Derivation of *L. reuteri* supernatants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial culture of <em>L. reuteri</em></th>
<th>+/− Glucose / Inulin / GOS</th>
<th>Glycerol added to aid reuterin production</th>
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### Culture of *Salmonella Typhimurium*

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<th>Condition</th>
<th>Description</th>
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<tr>
<td>+/−</td>
<td><em>L. reuteri</em> growth supernatant</td>
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<td><em>S. Typhimurium</em> growth assessed</td>
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**Experimental Timeline (Hours)**

- 0
- 16
- 18
- 22
- 25
Experimental Parameters

- Control for pathogen growth => SN(-)
- Supplemental inulin => inulin
- Glucose restricted with inulin => G(-)inulin
- Glucose restricted with GOS => G(-)GOS
Results
Supplemented inulin does not impact reuterin production

Dilutions of supernatant

- Glucose
- Glucose + inulin
- SN(-)

Percent growth

1 to 1 1 to 5 1 to 10 1 to 15

Graph showing the impact of inulin supplementation on reuterin production at different dilutions of supernatant.
L. reuteri growth in varied media
Inhibition of Salmonella with varied carbohydrate sources
Discussion
Supplemental Inulin

• There was no difference with additional inulin
  • Possibly due to *L. reuteri* not metabolizing inulin

• Clearly shown across the spectrum of sensitivity

• Potentially no inulin metabolism if not forced to
Glucose restricted analysis

- *L. reuteri* growth was significant with both GOS and inulin
  - Inulin metabolism is highly strain specific for *L. reuteri*
  - First time that it has been shown that *L. reuteri* PTA 6475 can metabolize inulin

- *Salmonella* inhibition
  - GOS was able to significantly reduce pathogen growth
  - Inulin supernatant did not reduce growth
Further research

- Repeat with a range of prebiotics
- Vary the concentrations of prebiotics to see if glucose to glycerol concentration would be mimicked
- Translate research into a complex modeling system to test for improved fitness
- Substitute pathogen
Summary

• We showed that in addition to the glucose to glycerol ratio, the particular carbohydrate impacts reuterin synthesis

• Inulin, though enhancing growth, did not yield reuterin synthesis

• GOS metabolism allowed for increased reuterin production
Acknowledgements

- Dr. Sharda
- Dr. Lowe
- Honors Department
- Biology Department
- Erin Olson
References

QUESTIONS?