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

An experiment in biology by Micah Forshee

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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

- Initial culture of *L. reuteri*
- +/− Glucose / Inulin / GOS
- Glycerol added to aid reuterin production

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Culture of *Salmonella Typhimurium*

- +/− *L. reuteri* growth supernatant
- S. Typhimurium growth assessed

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

- Glucose
- Glucose + inulin
- SN(-)

Percent growth

Dilutions of supernatant

1 to 1  1 to 5  1 to 10  1 to 15
L. reuteri growth in varied media

Glucose Glucose (-) G(-) inulin G(-) GOS

Absorbance (OD596)
Inhibition of Salmonella with varied carbohydrate sources

*Percent growth of Salmonella inhibition with different 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

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- Erin Olson
References


https://cookwithkathy.wordpress.com/tag/microbiota/

QUESTIONS?