

A laboratory setting with a microscope and a vial in the foreground. The background is slightly blurred, showing a green box with 'KIMTECH' written on it. The text is overlaid on the image in a large, white, sans-serif font.

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

An experiment in biology by Micah Forshee

Mentored by Dr. Daniel Sharda

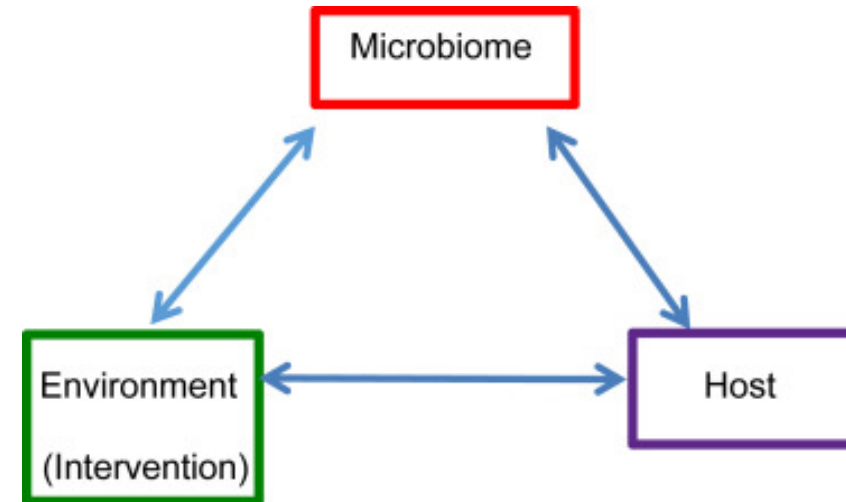
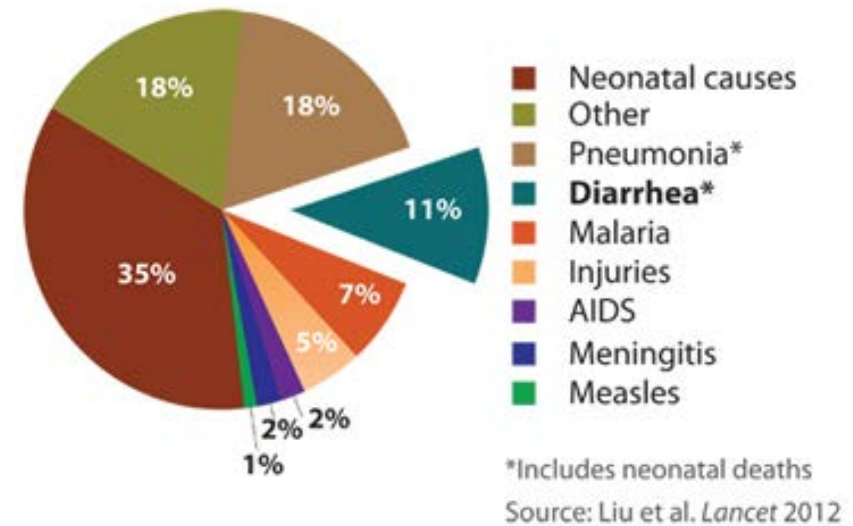
Preview

- 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



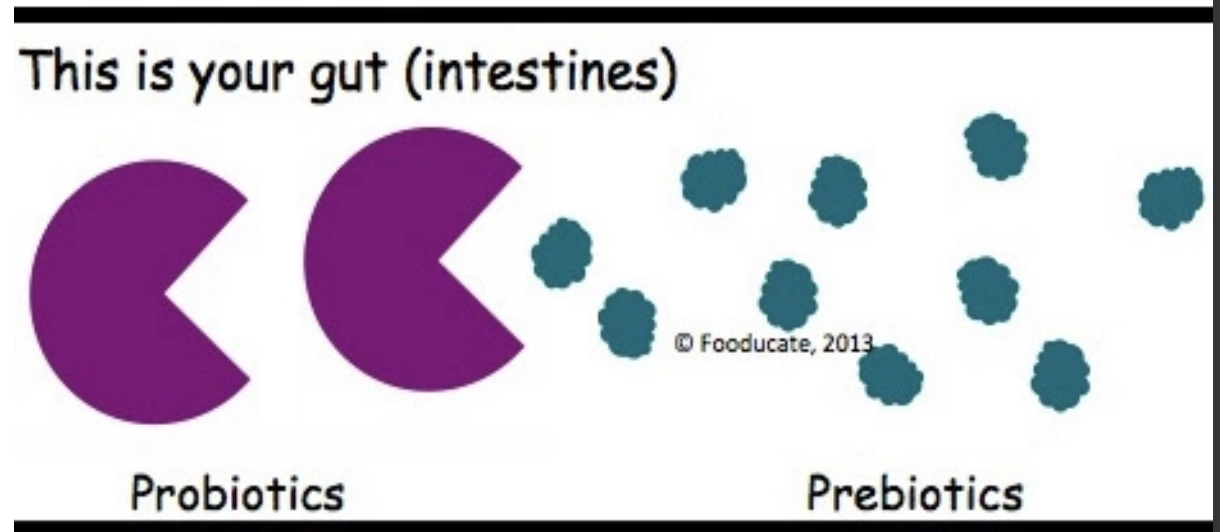
The Microbiome

- 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

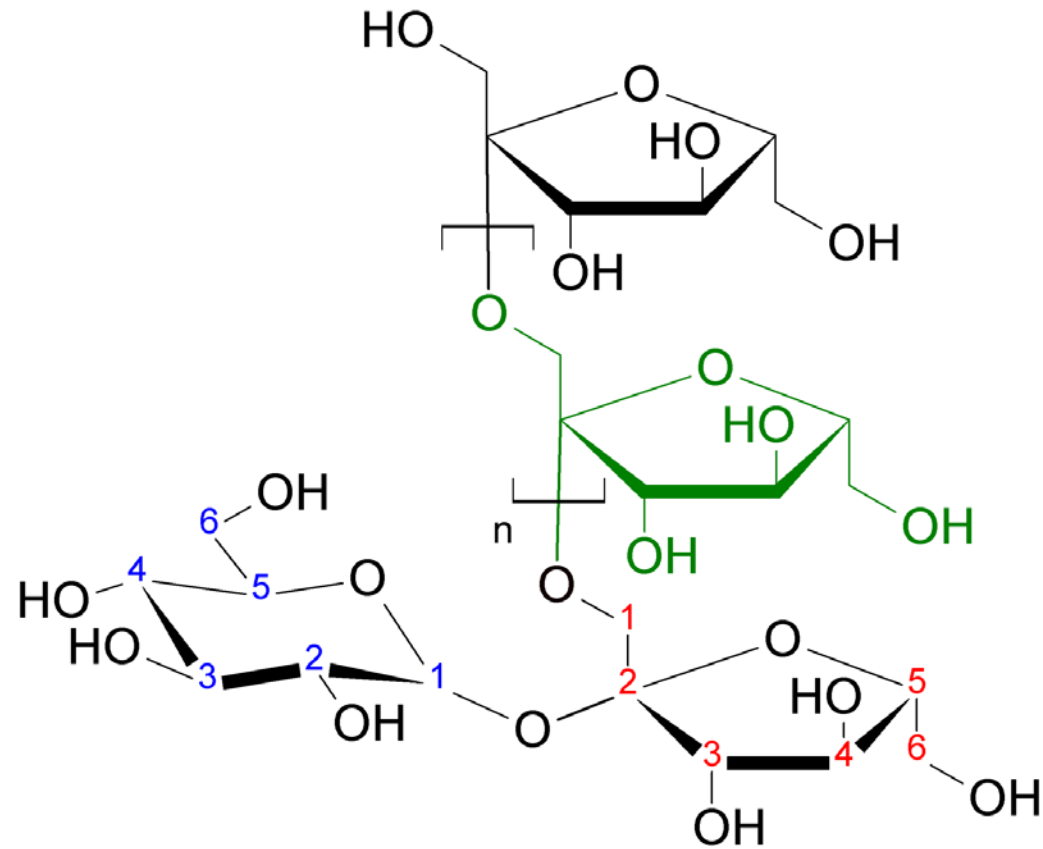
The Benefits of Prebiotics



- Enhance probiotic growth
- Increase antimicrobial production
- Prebiotics found in a variety of produce and milks
- How exactly do these impact probiotic workings?

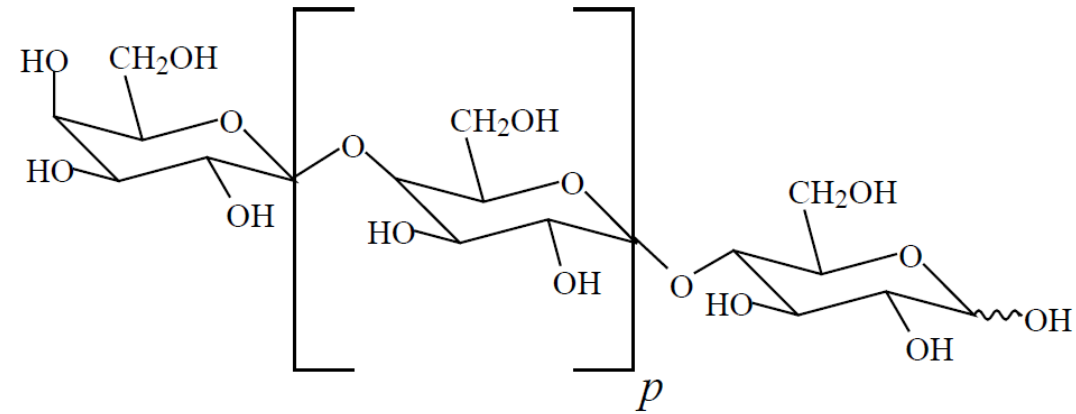
<https://www.thehealthyhomeeconomist.com/prebiotics-benefit-gut-health/>

Inulin



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

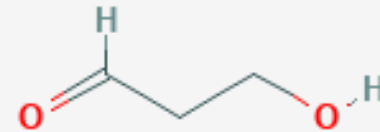
Galactooligosaccharides (GOS)



<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

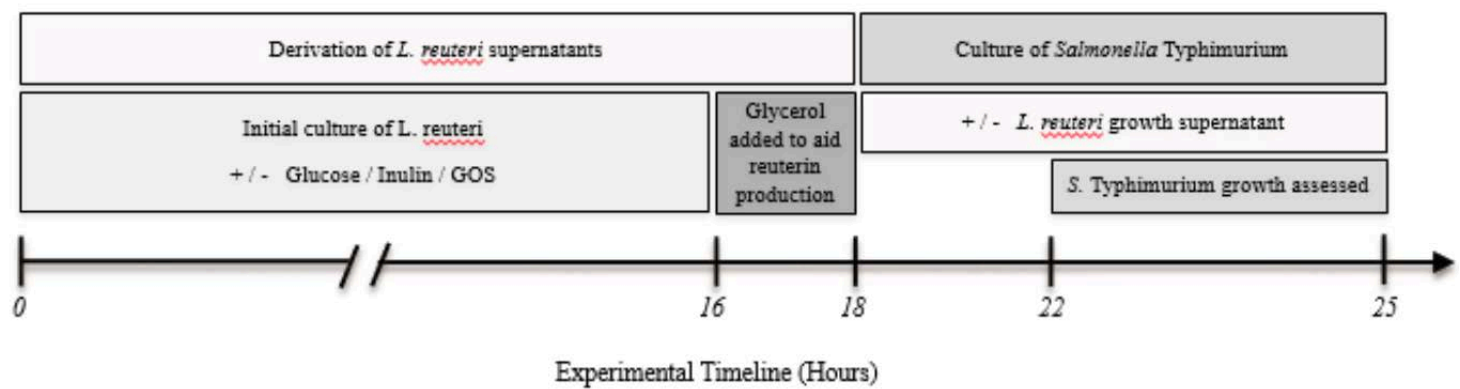
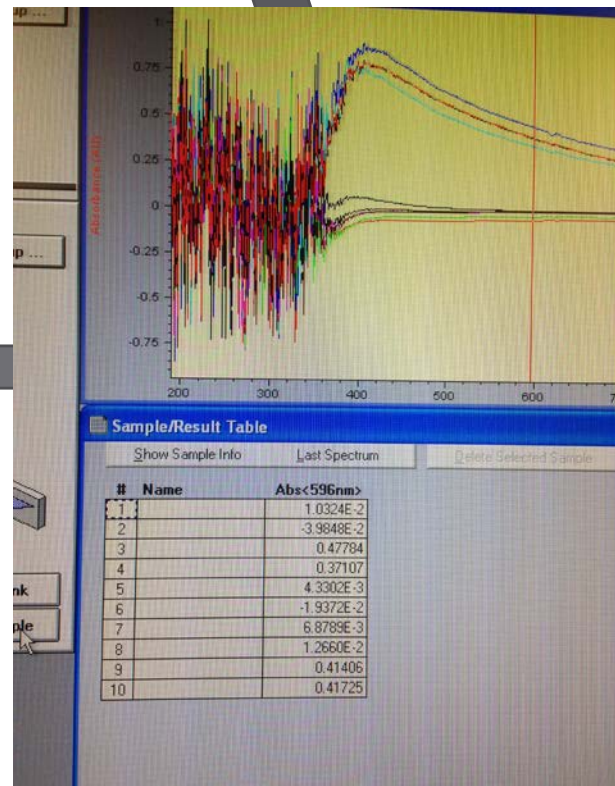
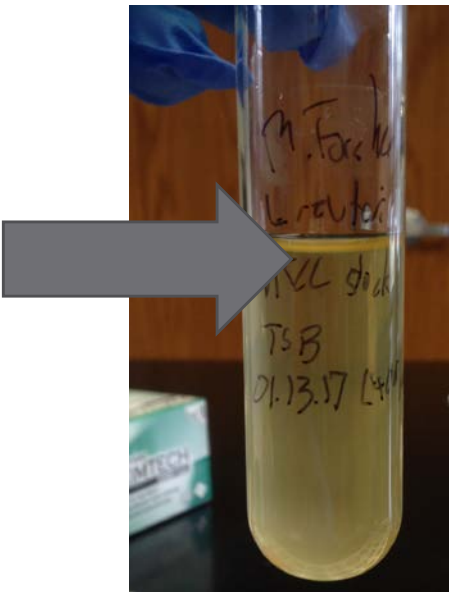
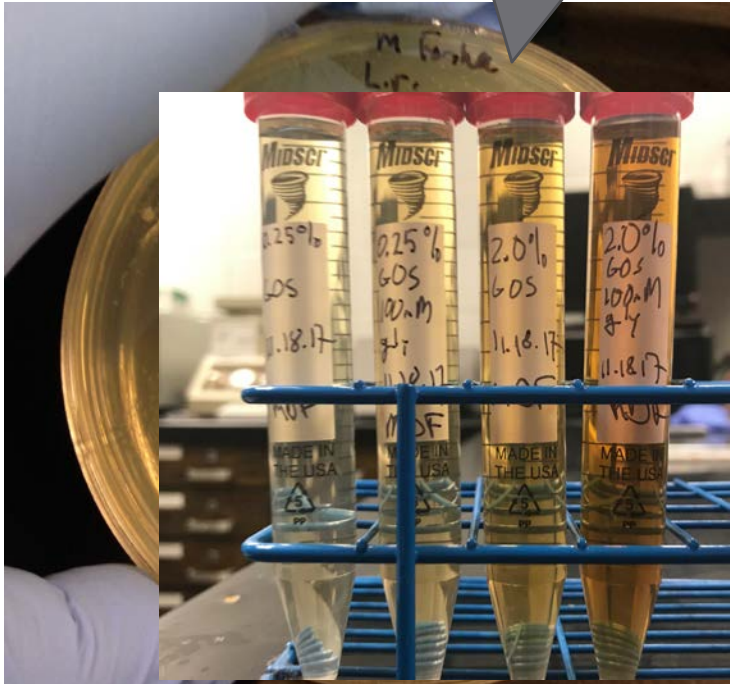
- Induces oxidative stress to pathogens => wide antimicrobial properties
- Excreted out of the cell
- Glucose to glycerol ratio affects its production



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



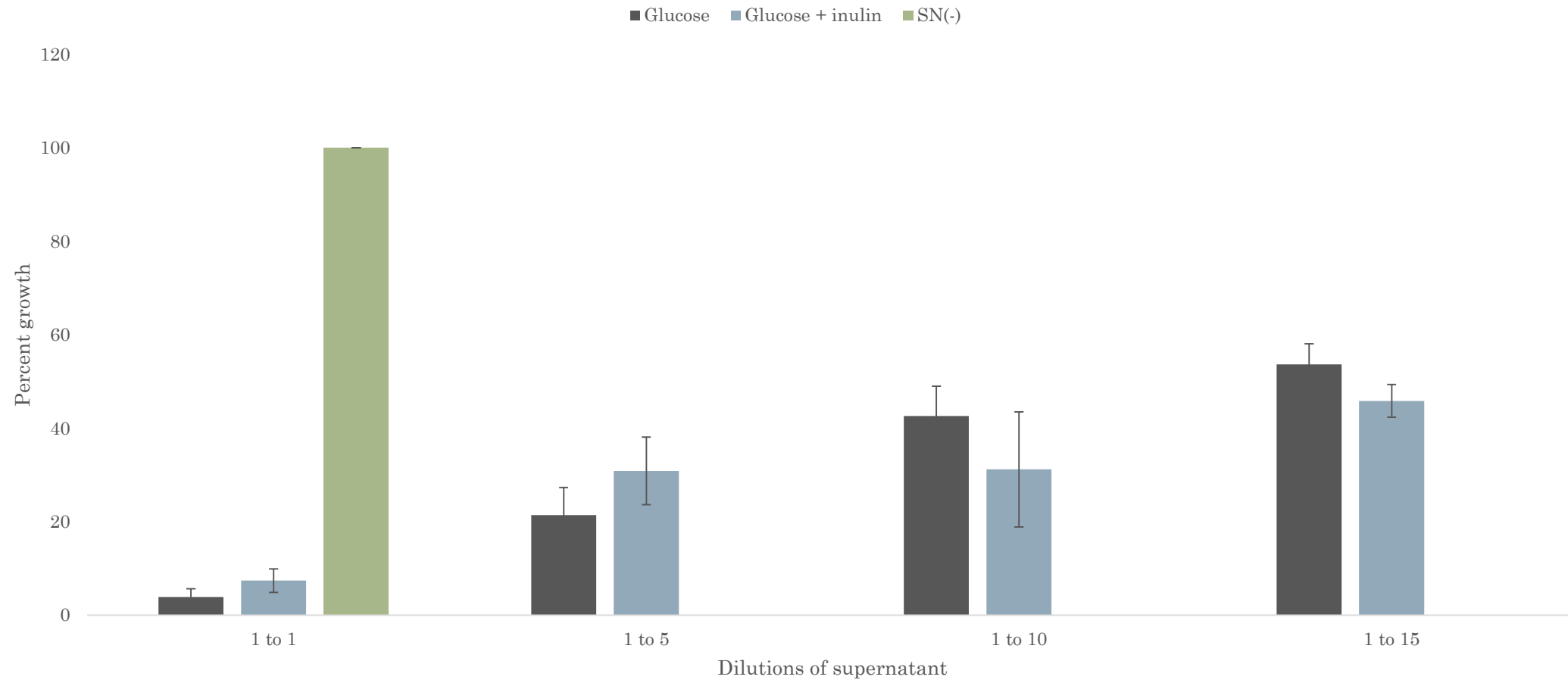
Experimental Parameters

- Control for pathogen growth => SN(-)
- Supplemental inulin => inulin
- Glucose restricted with inulin => G(-)inulin
- Glucose restricted with GOS => G(-)GOS

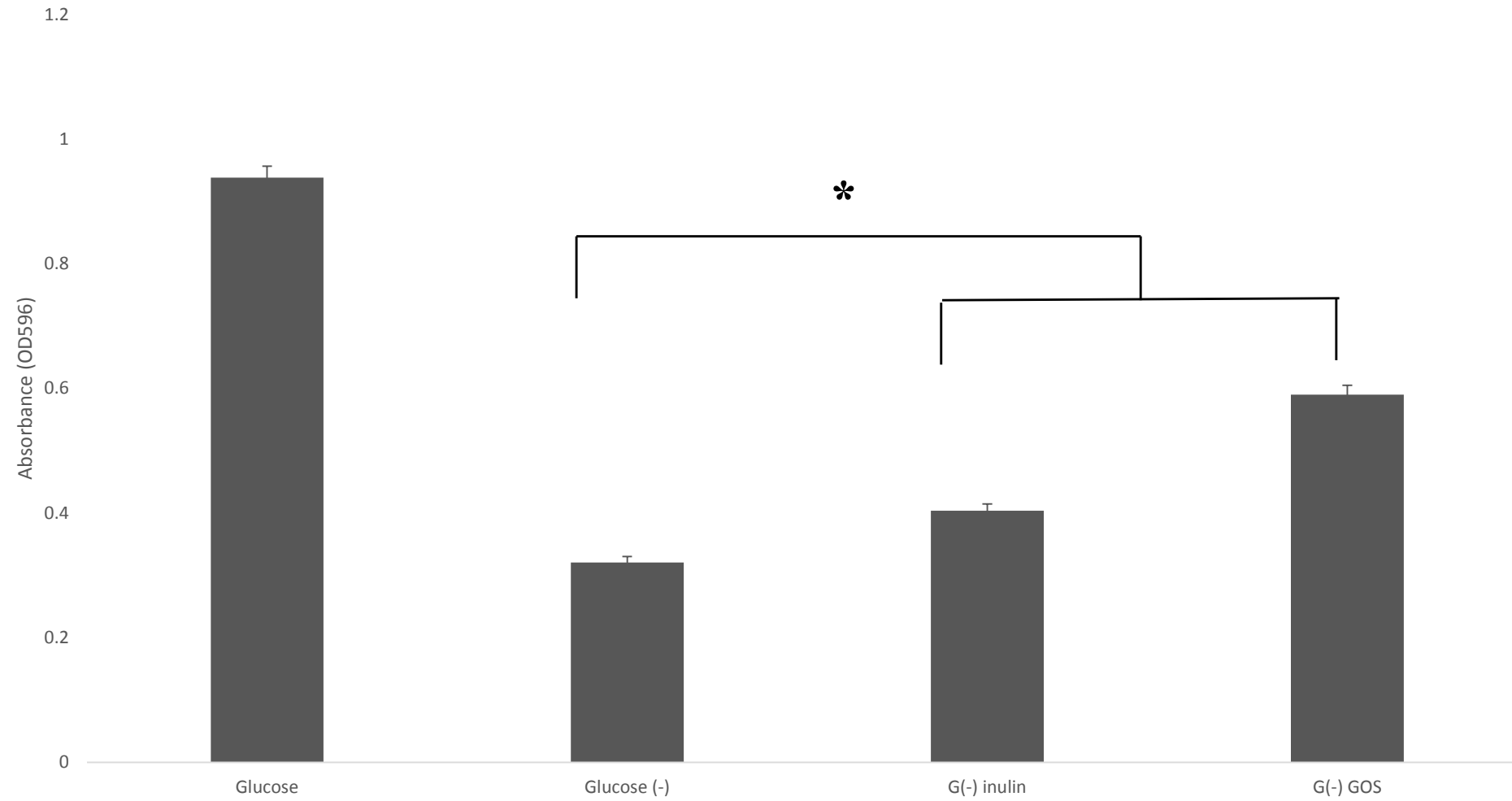
Results



Supplemented inulin does not impact reuterin production

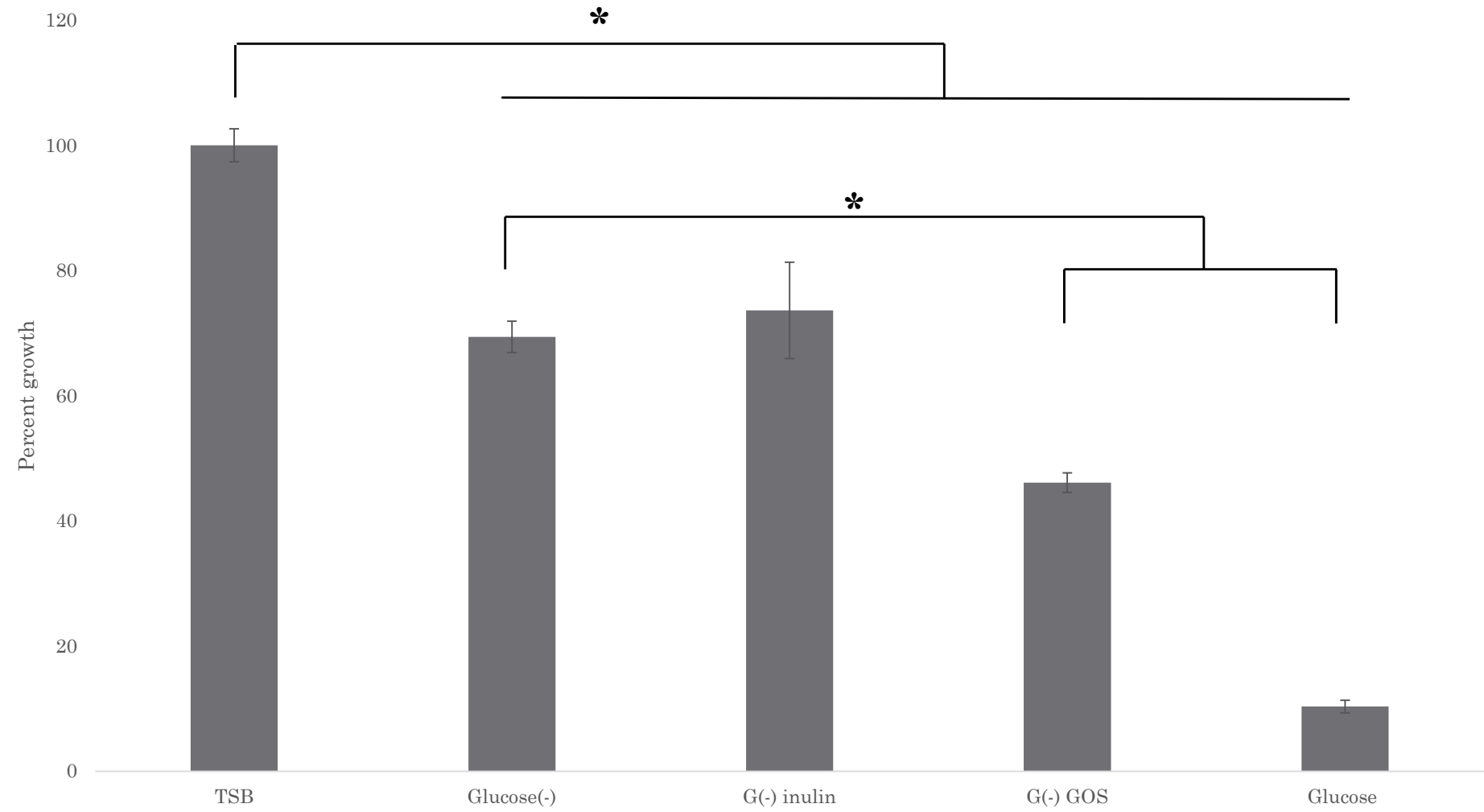


L. reuteri growth in varied media





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

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QUESTIONS?