# Prebiotic and Probiotic Pairing to Inhibit the Growth of Pathogenic Gut Microbes

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## Introduction

***Context***

According to the World Health Organization (WHO), nearly 1.7 billion cases of diarrheal disease are reported per annum. Diarrhea is a leading cause of malnutrition, morbidity, and death amongst children under five years old. Pathogenic gut microbes are primarily responsible for such cases of diarrheal disease; thus, antibiotics are traditionally used to diarrheal disease induced by pathogenic gut microbes. However, antibiotic resistance amongst these microbes calls for a new solution. Probiotics, beneficial live microorganisms, and their prebiotic stimulants, non-digestible food components, could potentially be utilized to treat diarrheal disease amongst children.

***Purpose***

This study sought an efficacious prebiotic-probiotic pairing to inhibit the growth of specific pathogenic gut microbes.

***Two-Part Hypothesis***

- Pairings with prebiotics or prebiotic combinations would result in greater probiotic growth and more effectual probiotic antimicrobial activity than parings with controls.
- Antimicrobial activity effects would be directly proportional to the amount of time the probiotic is grown with prebiotics.

## Methods

***Phase 1 Methodology***

1. Prebiotics, prebiotic combinations, or glucose were added to autoclaved Phenol-Red Broth Base at 1% wt./v (Marhamatizadeh and Sayyedi 2018).
2. 240 µl of each Phenol-Red Broth Base was aliquoted into a 96-well plate, and each well was then inoculated with 10 µl of probiotic cultures.
3. Probiotic cultures were incubated at 37°C for 24 hours, growth was evaluated through optical density readings at 600 nm, and absorbance values were compared with a Mann-Whitney test.

***Phase 2 Methodology***

1. Two groups (Group 1 and Group 2) of probiotic cultures were inoculated into each Phenol-Red Broth Base (similar to Phase 1) and then incubated in an anaerobic environment at 37°C for 24 hours (Group 1) or 96 hours (Group 2).
2. After cultures were incubated, CFCSs were isolated (Campana et al. 2017).
3. For each CFCS, Hektoen Enteric Agar (Group 1) and Tryptic Soy Broth (Group 2) plates were prepared following the Agar Well Diffusion Method (AWDM).
4. All plates (216 in total) were incubated and zones of inhibition were measured.

## Materials

Probiotics: *Lactobacillus casei* and *Bifidobacterium bifidum*

Pairings: Prebiotics fructooligosaccharides (FOS), galactooligosaccharides (GOS), and inulin from chicory root; prebiotic combinations FOS:inulin and GOS:inulin; carbohydrate control glucose

Pathogenic gut microbes: *Shigella sonnei*, *Escherichia coli*, and *Salmonella enterica subsp. enterica*

## Results

***Phase 1 Results***

- *Lactobacillus casei* expressed the greatest growth with GOS - 8682 absorbance units.
  - GOS obtained a statistically significant difference from PRB (control), $U(N_{GOS} = 6, N_{PRB} = 6) = 0$, $z = -2.88$, $p < 0.004$.
- All prebiotic treatments on *Bifidobacterium bifidum* obtained a statistically significant difference from PRB (control), $U(N_{Prebiotic\ Treatment} = 6, N_{PRB} = 6) = 0$, $z = -2.88$, $p < 0.004$.

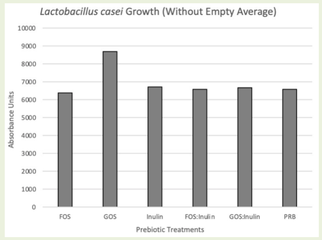


***Figure 1***: Growth of *Lactobacillus casei* cultures under different treatments after 24 hours of incubation (OD 600 nm).

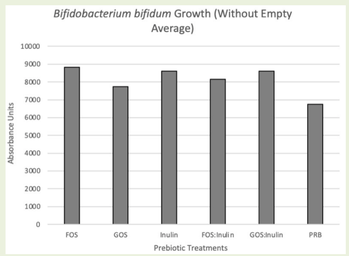


***Figure 2:*** Growth of *Bifidobacterium bifidum* cultures under different treatments after 24 hours of incubation (OD 600 nm).

***Phase 2 Results***

- All pathogenic gut microbes grown indicated proficient growth (excluding *Shigella sonnei* on Hektoen Enteric Agar).
- All CFCSs obtained did not exhibit antimicrobial activity as zones of inhibition were reported to be 0 mm.

## Conclusions

***Significance***

- Prebiotics - particularly GOS for *Lactobacillus casei* and FOS for *Bifidobacterium bifidum* - suggest to greatly enhance probiotic growth.
- In the future, GOS-*Lactobacillus casei* (ATCC 393) and FOS-*Bifidobacterium bifidum* (Fisher Scientific 23-001-911) pairings could hold applications against diarrheal disease through the "prebiotic effect."
  - Prebiotic Effect: Prebiotics are thought to alter the composition of the gut microbiota by increasing beneficial bacteria concentrations (e.g., probiotics) and decreasing pathogenic concentrations.
- To my knowledge, no previous investigation has examined the antimicrobial activity of the probiotics I utilized, let alone in pairings with prebiotics.

***Limitations***

- This study was limited in its number of trials, variety of bacterial strains, and human error.
- This *in vitro* investigation does not accurately reflect the environmental experiences the pairings and pathogenic gut microbes would encounter in a mammalian host.

***Suggestions for Future Research***

- Analyze CFCS compounds through a short chain fatty acid analysis to determine antimicrobial activity potential.
- Analyze alternative mechanisms of inhibition (e.g., increasing pH, preventing adherence, immunomodulation).

## References

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