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Microbiota Accessible Carbohydrates and Susceptibility to *Clostridioides difficile* Infection

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Introduction

The gastrointestinal (GI) microbiome in humans is instrumental in gut health and warding off disease. Loss of diversity in the GI microbiome can lead to colonization of gut pathogens. Commonly, antibiotic treatment will lead to loss of microbial diversity within the human GI tract and provide new niches for drug resistant pathogens. For example *Clostridioides difficile* has been found to be one of the pathogens that infects the GI tract following loss of microbiome diversity. For this reason it is important to investigate how microbiome diversity loss can be prevented or how diversity can be recovered following antibiotic treatment. Dietary supplemented microbe accessible carbohydrates (MACs) were investigated for their ability to help the human microbiome resist loss of diversity during/following antibiotic treatment. Understanding how dietary MACs can aid in microbiome health could help reduce unnecessary side effects of antibiotics.

Research Questions

1. Does varying the concentration of MACs in medium influence composition of fecal microbial communities and susceptibility to *C. difficile* infection?
2. Which members of the GI microbiome respond to MACs?

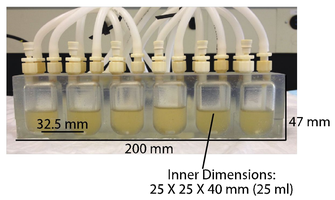
Experimental Approaches

How does the concentration of MACs influence composition of fecal microbial communities and susceptibility to *C. difficile*?

Overview:

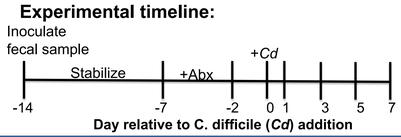
1. Culture fecal sample in replicate human fecal minibioreactors in baseline bioreactor medium
2. Shift communities to medium with variable MAC composition (n=6 replicates/media type)
3. Treat triplicate reactors with antibiotics
4. Challenge with C. difficile and monitor levels over time.
5. Assess changes in microbiome composition

Model:



Minibioreactor arrays support growth of human fecal microbial communities over time

Experimental timeline:



Experimental variables:

- **Fecal samples tested:**
  - 1 adult (18-65)
- **Antibiotics (Abx) tested:**
  - Cefaclor
  - Clindamycin
- **Media tested:**
  - Defined bioreactor medium (B; mimic conditions of colon)
    - 0.2g/L each MAC (arabinogalactan, inulin, soluble starch)
  - B/4: B + 0.05 g/L each MAC
  - B* 4: B +0.8 g/L each MAC
- **Samples collected:**
  - Levels of *C. difficile*: day 0, 1, 3, 5, 7
    - Measured by selective plating
  - Microbiome composition: day -7, -2, 0, and day 1
    - Assessed by extracting DNA, amplifying V4 region of 16S rRNA gene, sequencing on Illumina MiSeq (2X250), and analyzing with Mothur

Which members of the microbiome respond to MACs?

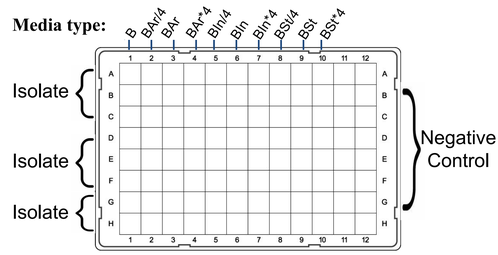
Overview:

1. Culture bacteria isolated from human fecal samples in different growth media containing MACs to determine whether they are capable of metabolizing MACs
   a. Bacterial isolates were members of the *Bacteroidaceae, Clostridaceae*, and *Ruminococcaceae* families that are dominant members of human fecal samples.

Model:

96-well absorbance spectrophotometer was used to assess growth after 24 hrs by measuring optical density at 600 nM (OD 600)

96-well Plate Configuration:



Media tested:

- Baseline medium with no MACs (B0)
- Baseline medium with 0.05 (BAr/4), 0.2 (BAr), or 0.8 (BAr*4) g/L arabinose
- Baseline medium with 0.05 (BIn/4), 0.2 (BIn), or 0.8 (BIn*4) g/L inulin
- Baseline medium with 0.05 (BSt/4), 0.2 (BSt) and 0.8 (BSt*4) g/L soluble starch

Results

MAC concentration influences microbial community composition but does not *C. difficile* susceptibility

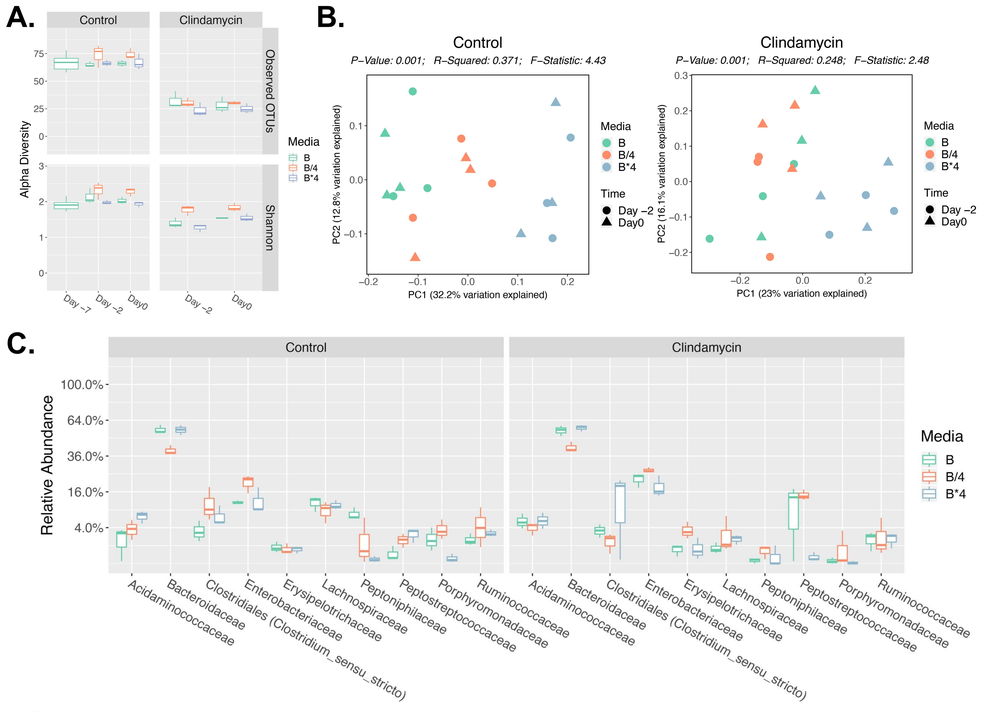


Fig 1. Effect of MAC concentration on microbial community structure in the presence and absence of antibiotic treatment. Microbes from a fecal sample were grown in replicate reactors containing baseline medium (B), baseline medium with ¼ concentration of MACs (B/4), and 4X concentration of MACs (B*4). Community composition was assessed at day -7, day -2 and day 0 relative to *C. difficile* challenge. Triplicate reactors for each condition were treated with clindamycin or mock-treated with water as a control. **A.** Alpha diversity of cultures (number of OTUs and Shannon diversity) **B.** Principle Coordinates Analysis of Bray-Curtis dissimilarities (A). **C.** Distribution of ten most-abundant bacterial classes in control and clindamycin-treated reactor communities.

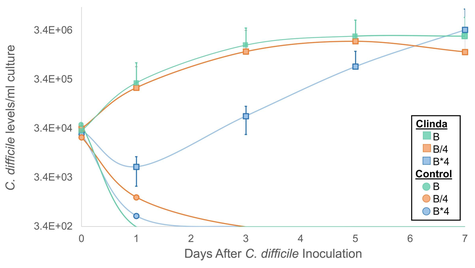


Fig 2. *C. difficile* colonization resistance is not significantly influenced by MAC concentration. *C. difficile* levels were measured at the indicated time points in *Clostridium* difficile Colonization occurred to the same degree in BRMX and BRM+4. BRM-4 colonization occurred in a different capacity leading to an investigation of the colony composition of reactors in the low MACs environment.

MACs stimulate the growth of some *Clostridiaceae, Lachnospiraceae* and *Ruminococcaceae* isolates

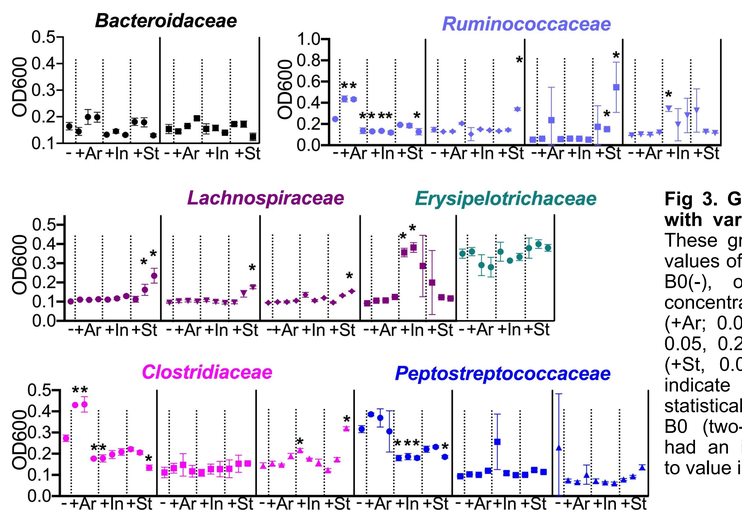


Fig 3. Growth of isolates in media with variable MAC concentrations. These graphs represent final OD600 values of isolates grown in triplicate in B0(-), or in B0 with increasing concentrations of arabinogalactan (+Ar; 0.05, 0.2, 0.8 g/L), inulin (+In; 0.05, 0.2, 0.8 g/L) or soluble starch (+St, 0.05, 0.2, 0.8 g/L). Asterisks indicate conditions that were both statistically significant from growth in B0 (two-tailed student's t-test) and had an increased/decreased relative to value in B0 by ≥1.5-fold.

Conclusions and Future Directions

- Although MACs influence overall community composition, the changes that they cause are not sufficient to restore colonization resistance to human fecal communities treated with clindamycin.
- Soluble starch promotes robust, dose-dependent stimulation of isolates in *Ruminoccocaceae*, *Lachnospiraceae* and a *Clostridiaceae* families.
- Most isolates exhibit enhanced growth in the presence of one type of MAC. Some isolates exhibit lower final OD600 values in the presence of MACs
- Further studies will be needed to determine if specific MAC inhibit growth or cause rapid robust growth with death phase/cell density loss by 24 hrs of growth
- Additional MACs will need to be tested to determine if they are able to restore *C. difficile* colonization resistance.

Acknowledgements

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