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Introduction

Objective: To investigate a novel therapeutic for the treatment of Type I Diabetes (T1D).

The therapeutic would not kill, but just retard the growth of bacteria that are members of the genus *Bacteriodes* while not effecting other bacterial species.

Why: T1D is a progressive autoimmune disorder characterized by the destruction of the insulin secreting Beta cells in the pancreas (Harrison *et al.* 2008).

While it has been previously acknowledged that there are genetic factors responsible for the onset of T1D, there is evidence to suggest environmental causes as well (Achenback *et al.*, 2005; Gale, 2002).

Not everyone that has the genetic predisposition to developing the disease will have T1D.

Our interest lies in the observation that just before the onset of T1D in an individual, there is a bloom of bacteria from the Bacteroidetes phyla. (Giongo *et al.*, 2011).

How: We are investigating therapeutics that can inhibit the Starch Utilization System (SUS) specific to members of *Bacteriodes*. Removing the SUS as a method of gaining biomass and energy for the cell would not kill the cell but slow its growth. The cell will have to find alternative and less effective methods of metabolism.

Current Work: We have investigated the effects of the drugs Acarbose and Miglitol on the growth of members from the *Firmicutes* and *Bacteroidetes* phyla.
Acarbose has been used previously in diabetes treatments because of its ability to retard the degradation of starch via inhibiting human α-amylases. α-amylases, similar to the ones observed in humans, can be found within the Sus.

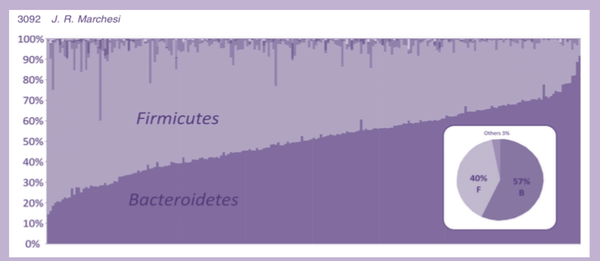

Figure 1. Variation of ratios of the two major phyla of bacteria, Firmicutes and Bacteroidetes, found in 386 fecal samples
(Figure from: **Marchesi, J.R. 2011**. Human distal gut microbiome. Environmental Microbiology **13**: 3088-3102)

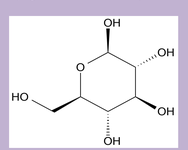

Figure 2. Molecular structure of D-glucose

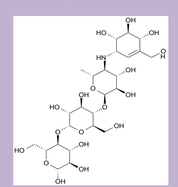

Figure 3. Molecular structure of Acarbose

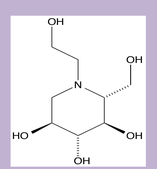

Figure 4. Molecular structure of Miglitol

Methods and Materials

- *Bacteroides thetaiotaomicron* was grown on tryptone yeast glucose (TYG) agar anaerobically.
- Colonies from plates were used to prepare overnight cultures of *B. thetaiotaomicron* TYG broth (grown anaerobically).
- Test tubes were filled with minimal media containing either 0.5% glucose, maltose, or pullulan as a carbon source, cysteine, *Bacteroides* culture, and varying amounts of acarbose.
- An anaerobic environment was created by inserting a cotton ball in the tube and lighting it on fire.
- Upon completion of burning, sodium bicarbonate and pyrogallol were added.
- The tube was stoppered, sealed, and incubated at 37°C for 24 hours.
- Inhibition was indicated the following day by measuring the optical density at a wavelength of 600nm.
- *Lactobacillus reuteri* strains were grown on de Man, Rogosa and Sharpe (MRS) agar aerobically.
- Colonies from plates were used to prepare overnight cultures of *L. reuteri* MRS broth (grown aerobically).
- Test tubes were filled with MRS broth containing either 0.5% glucose, maltose, or pullulan as a carbon source, cysteine, *Lactobacillus* culture, and varying amounts of acarbose.
- The test tubes were incubated at 37°C for 24 hours.
- Inhibition was indicated the following day by measuring the optical density at a wavelength of 600nm.

Preliminary Results

Bacteroidetes representative: *B. ovatus, B. theta, B. fragilis*
Firmicutes representatives*: L. reuteri* ATCC 55730 and *L. reuteri* PTA 6475

Monosaccharide representative: Glucose
Disaccharide representative: Maltose
Polysaccharide/Glycan representative: Pullulan

Table 1. Inhibition of Bacterial Growth by Acarbose

Species	Glucose	Maltose	Pullulan
B. theta	None	At 200µM	At 50µM
B. fragilis	None	Not tested	At 10µM
L. reuteri ATCC 55730	None	None	None
L. reuteri PTA 6475	None	None	None

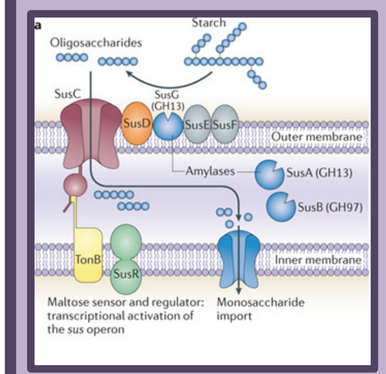

Figure 5. Starch Utilization System (Sus) for *B. thetaiotaomicron*. This is the pathway responsible for recognition, degradation, and importation of complex carbohydrates. (Figure from: Koropatkin, N.M., E.A. Cameron, E.C. Martens. 2012. Nature, **10**: 328.)

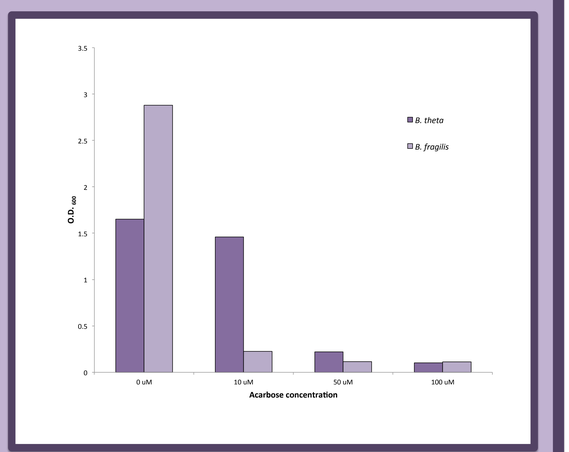

Figure 6. Representative growth assay demonstrating acarbose inhibition of *B. theta* and *B. fragilis* grown with pullulan.

Conclusions

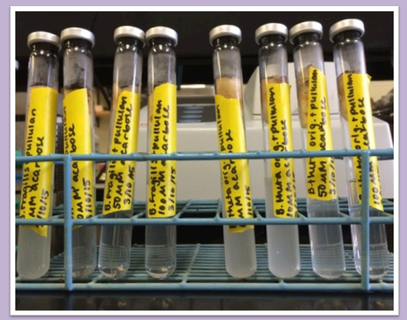

Figure 7. Anaerobic cultures of *B. theta* and *B. fragilis* with varying levels of acarbose.

What we have found:
- Acarbose inhibits the growth of *Bacteroides* spp. and is not lethal to the cells.
- Acarbose does not inhibit *Lactobacillus* growth.
- Acarbose has structural similarities to many glycan molecules and is most likely effecting the Sus and therefore prohibiting its ability to breakdown available glycan molecules.

These findings have the possibility of providing a novel T1D treatment by preventing the bloom of Bacteroidetes in the large intestine.

Our future directions include:
- Assay inhibition on other complex carbohydrates
- Investigate inhibitory effects with another *Bacteroides sp.* and a non-*Bacteriodes sp.* within the Bacteroidetes phylum
- Establish exact mode of inhibition of Sus system in *B. thetaiotaomicron (i.e.* where does it bind to inhibit?)
- Determine if correlation is an indicator of causation by conducting *in vivo* experiments using the Non-obese diabetic (NOD) mouse model to examine the effectiveness of acarbose administration at delaying or preventing the onset of T1D.

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