1. **RESEARCH QUESTIONS ADDRESSED (3-5 Pages)**

This proposal seeks to decode the diet-microbiome relationship using a powerful new tool - fecal mini-bioreactor arrays

(MBRAs). First used to study *Clostridium difficile* infection, the MBRA allows for continuous cultivation of complex fecal microbial communities to study infection. The bacteria in the gut, collectively termed the microbiome, is most strongly affected by our diet. Our understanding of how this diet-microbiome relationship impacts response to infection is rudimentary. Several promising studies indicate that fermentable dietary fibers (prebiotics) are paramount in maintaining a healthy microbiome that is resistant to disease (REF). Dietary fiber is one of the main sources of carbohydrates the microbiome uses for fuel in the production of short chain fatty acids (SCFAs), which supports gut barrier function. Without fiber, the gut bacteria feed off the mucus lining the gut, leaving the host susceptible to infection and disease (REF). Little is known; however, about *which types* of dietary fiber are responsible for these protective effects nor their mechanisms. Using the MBRA system, developed by collaborator Dr. Robert Britton, we seek to answer several outstanding questions surrounding the diet-microbiome relationship, focusing on the effects of two types of fermentable fiber, inulin and resistant starch.

Our long-term goal is to develop the MBRA system for use with other investigators at Baylor in microbial studies (Dr. Chris Kearny), and continue to support our diet-microbiome research**.** The goal of this proposal is to create a functional MBRA system to provide preliminary results for future studies from testing two types of dietary fibers in healthy stool communities**.** This study is connected with our current research on the fiber supplement Prebiotin (enriched inulin), which is currently being used in 2 NIH-funded clinical trials. Our randomized-placebo controlled trial of Prebiotin (URC-funded) is testing the effect of this fiber on appetite hormones, body composition, and perceived stress in medical residents. Building on this research, and other ongoing complimentary host-microbiome research, we seek to synergize and expand these efforts to answer outstanding questions with regard to the impact of individual nutrients on the microbiome structure and function. Further, we will be able to train graduate and undergraduate students in using the MBRA system, which will give them critical skills to enhance learning and their ability to be competitive in the STEM fields. To address these goals, we have developed the following specific aims:

**A.1. Specific Aim 1: Determine changes in model stool community composition and metabolism after exposure to inulin or resistant starch.** The gut microbiome is reliant upon dietary fiber for energy, which in turn supplies energy for the intestinal epithelial cells. Data show that lack of dietary fiber results in dysbiosis, changes in metabolites (e.g. SCFAs), and increased disease risk. Further, human dietary fiber intervention studies with inulin or resistant starch show heterogeneity in their effects on the microbiome and SCFA production. *Thus*, *we hypothesize that the gut microbiome composition and SCFAs production will be differentially altered in response exposure to inulin or resistant starch, which will be dependent upon the donor community supplied.*

**A.2. Specific Aim 2: Measure ability to resist pathogen colonization in fiber-exposed stool communities.** The ability of microbial communities to resist pathogen colonization involves mechanisms that are, in part, reliant upon fermentation of prebiotic fibers. Specifically, commensal *Bacteroides spp.* confer colonization resistance again *Salmonella* infection through the production of the SCFA propionate (REF). Further, a combination of two prebiotic fibers plus the probiotic *Lactobacillus reuteri* 1063 prevented colonization of the pathogenic strain of *E. coli* (REF) by limiting mucus adhesion. *Thus*, *we hypothesize that inulin and resistant starch will confer differential resistance to the pathogens, enterotoxic B. fragilis (ETBF), and C. difficile.*

Our research plan, supported by a highly-experienced team of scientists (REFs), will provide the foundation for an externally funded research program dedicated to elucidating the diet-microbiome relationship. Completion of this research will provide the basis for defining the causative relationship between dietary fiber, the microbiome, and infection. *These findings will impact our field by not only answering outstanding questions that remain with regards to microbiome resilience and stability to infection, but also lead to novel dietary and microbial engineered interventions (synbiotics) to prevent infection and disease.*



**Fig. 1.** Study Design Schema for Phase I.

**A.3. Research Approach**

Approach: To initiate our study, we (Dr. Greathouse and a doctoral student) will begin by training with Dr. Britton on setup and running the MBRA system at Baylor College of Medicine. This training will allow us to fully operationalize the MBRA system in the laboratory of Dr. Greathouse and begin experiments **(Fig. 1)**.

To address **Aim 1,** we will collect donor stool from three healthy individuals according the methods previously described (REF). Three replicate reactors will be used for each of the three donor stool samples (n=9 reactors). Reactors will be inoculated with 25% fecal supernatants by adding them to pre-reduced growth medium for 16hrs, and then fresh medium will be continuously flowed in through the MBRA system. To determine changes from baseline in community composition we will extract 1mL from each reactor on day 0 (inoculation), and daily thereafter for 20 days and store at -80 degrees. To compare the effects of inulin vs. resistant starch, 0.2 g of inulin or resistant starch will be added to each donor sample reactor with fresh medium flow provided for 20 days. Samples will be collected in the same manner as at baseline. DNA will be extracted from each sample using bead beating and the Qiagen DNeasy Tissue Kit, and will be quantified with QuantIT. Samples (n=594) will be sent to Baylor College of Medicine (Dr. Joseph Petrosino) for 16S rRNA sequencing (V4 region) as previously described (REF). For analysis of metabolites, including SCFAs, a separate 1mL sample will be collected at all time points previously indicated. Samples from each donor will be pooled together by time point and sent to Metabolon ([www.metabolon.com](http://www.metabolon.com)) for global and SCFA metabolite analysis (n=36).

To address **Aim 2,** we will perform invasion studies using *C. difficile* (CD2015 from Dr. Britton) or ETBF (Dr. Greathouse), both of which are responsible for life-threaten infectious diarrhea, chronic disease, or colon cancer. Prior to inoculation we will test samples for *C. difficile* or ETBF presence. Using an experimental design similar to the one described previously (REF), we will inoculate three reactors with either CD2015 or ETBF at a dilution of 1:100 from a culture grown to an OD600 of 1.5 to measure CFU/mL in pure culture by plating and by qPCR. We will test the ability of the pooled communities to resist pathogen invasion by pretreating with antibiotics, inulin + antibiotics, resistant starch + antibiotics, or control media. To measure resistance to invasion, we will take 1 mL samples at 15 min and 3 hours post-inoculation, and once daily for 15 days, and measure CFU/mL by plating and by qPCR. To determine community structure changes, we will also collect 1mL samples for 16S rRNA sequencing (n=405).

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| Specific Aim | Year 1 Benchmark | Year 2 Benchmark | Year 3 Benchmark |
| Aim 1 | Trained/Initialize MBRA  Model communities growing | Samples collected from inulin/starch exposure | Community/metabolite  analysis finalized |
| Aim 2 | CD2015/ETBF growing in MBRA reactors | Samples collected from pathogen exposure | Community/invasion analysis finalized |
| Manuscripts/Grants |  | Grant funding identified/manuscript outlined | Grant/manuscript submitted |