Unraveling the process of polysaccharide utilization in complex bacterial ecosystems

Intellectual Merit – Introduction:

Bacteria do not exist in isolation in nature; they form complex communities in which they must recognize, compete over, and, share nutrient resources.[1,2] The gut microbiome is an ideal model to study this type of ecosystem, as we know what nutrients these bacteria are exposed to and have access to powerful tools to study taxonomy and metabolism. Gut bacteria break down polysaccharide nutrients and convert them to health-beneficial end products of metabolism called short-chain fatty acids (SCFAs) that are taken up and used for energy by host intestinal cells.[1,2] **However, there is a gap in our understanding of what factors give rise to the emergent phenomenon of SCFA production.** The leading hypothesis in the field is that communities that fail to respond to a given polysaccharide lack certain "keystone species" that are integral to metabolism.[1] The search for keystone species has focused on primary polysaccharide degraders,[1] but findings in our lab indicate that these are abundant even in samples otherwise characterized as non-responders (Fig. 1). In contrast with the notion of a single species as a keystone, **I hypothesize that community-level polysaccharide metabolism is driven by the presence of core communities of multiple species that function as an assembly line.** This would explain how samples can fail to produce SCFAs despite the presence of primary degraders. By shifting the focus to species that consume degradation byproducts, I will reveal novel cross-feeding interactions and develop a model to predict polysaccharide response from community composition. Our lab has a collection of stool samples from multiple healthy donors that have been characterized for SCFA production (Fig. 1), making me well situated to conduct this research. I will address my hypothesis in the following aims:

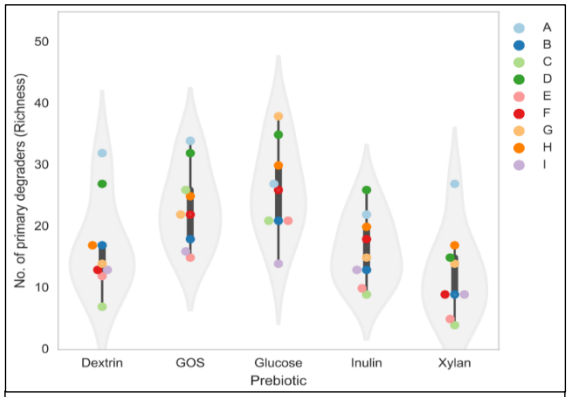

Figure 1: Bacterial communities derived from stool samples from 9 donors (A-I) contain primary degraders of all tested polysaccharides.[3]

Intellectual Merit – Research Plan:

Aim 1: Isolate the core communities from multiple stool donors.

I will grow bacterial communities from stool samples on minimal media with inulin as the sole carbon source in our lab's "artificial gut," a set of eight bioreactors.[4] Inulin is a well-studied polysaccharide, a polymer of fructose with a single terminal glucose residue. The species that persist after three weeks of growth in this media, as measured by 16S sequencing, will include all species that consume inulin or inulin byproducts. By reducing complex communities to only those species involved in inulin metabolism, **I will test the hypothesis that core community composition is directly related to SCFA production.** I will next quantify expression of genes encoding select carbohydrate active enzymes and transporters for all species present using RT-qPCR. I hypothesize that degradation will occur in waves, with primary degrader species upregulating genes of interest at early time points, followed by secondary degraders. While I expect all samples to exhibit a robust first wave of upregulation, **I hypothesize that the magnitude of the second wave will correlate with SCFA production**. If there is no correlation, this would support the alternative hypothesis that strain-level variation drives differences in polysaccharide response. In this case, I would expect the gene expression experiments to reveal an increased number of species that upregulate the genes of interest in SCFA-producing samples.

Aim 2: Differentiate primary, secondary, and tertiary degraders.

Our lab has developed a microfluidics growth assay that allows for the isolation of single cells in droplets (Fig. 2). Species that grow in this assay are defined as primary degraders, since cross-feeding interactions are prevented. I will identify secondary and tertiary degraders by taking conditioned media from primary degraders, which will contain partially broken down polysaccharides and byproducts of their breakdown, and using this media as the sole carbon source in a subsequent assay. Conditioned media will be characterized using the lab's GC and HPLC to measure SCFAs and polysaccharides, respectively. **I hypothesize that while stool samples from all donors will contain some primary degraders, samples unable to produce SCFAs will lack key secondary and/or tertiary degraders.** Growth will be determined by 16S sequencing to measure relative abundance and flow cytometry to calculate the total number of cells. The use of 16S will also allow me to determine which bacteria are involved at which stage of polysaccharide utilization. I hypothesize that the primary degraders will comprise of both generalists (*Bacteroides ovatus*) and specialists (*Roseburia inulinivorans*, for inulin), secondary degraders will include acetate producers (*Bifidobacteria* and *Lactobacilli*), and tertiary degraders will include acetate- and lactate-utilizing butyrate producers (*Eubacterium* and *Anaerostipes*).

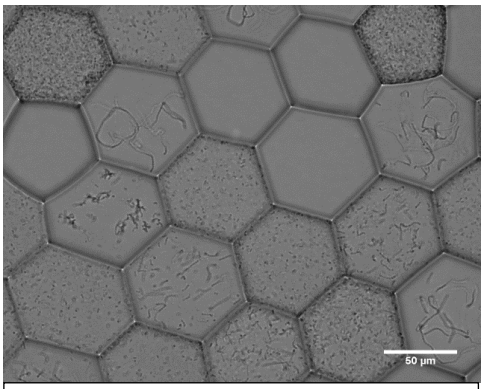

Figure 2: Droplets containing bacterial cultures each derived from single cells.[5]

Broader Impacts – Disseminating Research:

To date, **I have shared my research findings with diverse audiences from kindergarteners to faculty** and will continue to use the results of this project as a means of promoting science education. I am making my research accessible to middle and high schoolers by teaching for a Saturday program called Duke Splash, where I have previously taught Intro to Microbiology and Bread Science. **I am in the process of developing two new classes to debut this November, Nutrition Science and Data Science, both of which incorporate data from my own research on polysaccharide metabolism.** At Duke, I have many opportunities to share my research, such as the monthly Duke Microbiome Center seminar, where I recently presented my findings on transcriptional memory of polysaccharides in gut bacteria. To reach undergraduates, I will be giving a talk at the annual University Scholars Program (USP) Symposium, the theme of which will be "(in)dependence." With this theme in mind, I will be presenting on how humans are dependent on gut bacteria to help metabolize dietary fiber as it relates to my research. Previously, I spoke at the USP Graduate Research Seminar on my work as a rotation student. Beyond Duke, I plan to present my research at conferences such as the 2019 Keystone Microbiome Conference.

Broader Impacts – Importance and Innovation:

Emergent properties of complex systems can be difficult to deconstruct, and this project provides a methodology for doing so in a novel, high-throughput manner that can be applied in future studies. **By determining how emergent polysaccharide metabolism phenotypes arise, I will address a key gap in our understanding of how microbial ecosystems respond to nutrients.** These results may be applied in human diet research to predict individual polysaccharide response and in bioremediation to develop co-culture methods for effective degradation of pollutants. Moreover, my findings will raise exciting new questions related to evolutionary biology as to how polysaccharide utilization came to be an assembly line process.

References: 1. Makki et al. 2018. *Cell host & microbe*. 2. El Kaoutari et al. 2013. *Nature reviews Microbiology*. 3. Villa. 2018. Unpublished. 4. Silverman et al. 2018. *bioRxiv*. 5. Bloom. 2018. Unpublished.