Investigation of Mechanisms Mediating Microbial Survival, Abundance, and Pathogenicity Within Gut Microbiota

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Abstract

Abstract: The human gut microbiome is an incredibly diverse and populus environment rich with an estimated 10^{13} to 10^{14} microorganisms inhabiting the gastrointestinal tract (Gill et al., 2006). The aggregate membership of these microorganisms construct a dynamic "supraorganism" whose symbiosis, or lack thereof, with the respective human host can heavily influence human health (Kelsen and Wu, 2012). Interactions between the host and commensal bacteria native to the gut microbiome can be described as commensalism, symbiosis, and/or pathogenicity—pending on the specific context of analysis (Hooper and Gordon, 2001). These prokaryotes have the capability to synthesize metabolites, lipids, and vitamins otherwise inaccessible to the host's eukaryotic cells (Kelsen and Wu, 2012). Most notably, dysbiosis of the gut microbiome has been associated with many disorders and diseases (Rinninella et al., 2019). Recently, studies have outlined a significant role of the gut microbiome and Multiple Sclerosis (Maghzi and Weiner, 2020). This proposal aims to describe and outline a study exploring microbial dynamics within the gut microbiome as it relates to the mechanisms bacteria employ to both survive and thrive with relative abundance. This study will provide insights into mechanisms mediating the prevalence of certain bacterial communities, and insights into the potential role mimicry may have in the pathogenicity of Multiple Sclerosis. Leveraging the findings of these studies will have significant implications on human health by better understanding mechanisms underpinning crucial interactions between hosts and their most intimate gut microbial neighbors.

The Gut Microbiome: What Makes it Extreme?

The human gastrointestinal tract is an incredibly complex system with many interesting characteristics. Quite interestingly, bacteria that thrive and survive within the gut microbiome have adapted many mechanisms in response to the environment they exist in (Gomez et al., 2019). The gut microbiome is home to a diverse array of bacterial strains ranging, predominantly, from the phyla Actinobacteria, Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Verrucomicrobia —with bacteria of the phyla Firmicutes and Bacteroidetes representing 90% of gut microbiota (Arumugam et al., 2017). Obligate anaerobes dominate within the gut, despite the presence of facultative anaerobic and aerobic bacteria (Shin et al,. 2019).

With respect to the gut, these microbes are faced with a wide range of environmental stressors and challenges. Within the intestinal tract itself, pH varies from roughly 6 in the small intestine to roughly 7.4 in the terminal ileum and even 5.7 in the cecum, and ultimately reaching approximately 6.7 in the rectum (Fallingborg, 1999). In tandem with severe differences in environmental pH, in order for successful colonization, bacteria must overcome chemical barriers such as low oxygen concentrations and redox potential, as well as host physiological barriers such as gut architecture and peristalsis (de Vos et al., 2022). A healthy gut microbiome is a diverse gut microbiome—dysbiosis within the gut microbiome is associated with a wide range of gastrointestinal diseases and disorders (Rinninella et al., 2019). Gut microbiota interact quite significantly with the host's immune system and have been associated with immune responses linked to neurological diseases, most notably the immune-mediated neurodegenerative disease Multiple Sclerosis (MS) (Elsayed et al., 2022). In animal models of MS, experimental autoimmune encephalomyelitis (EAE), animals exposed to a strains *Eryspipelotrichaceae* (OTU002) and *Lactobacillus reuteri*, a member of the Firmicutes Phylum, exhibited increased progression of the disease through suspected molecular mimicry to the host's myelin oligodendrocyte glycoprotein (MOG) (Maghzi and Weiner, 2020 and Mu et al., 2018).

Fundamental Questions

What mechanisms, and characteristics adaptations favor successful survival and prevalence in the microbiome? The aims of this proposal are rooted in broad underlying factors influencing community diversity of gut microbial communities (aim 1) and investigating the prevalence and effects of gut microbial mimicry on the host (aim 2).

Aim 1. Understanding population dynamics that favor bacterial dysbiosis

In strains of *E. coli*, increased microbial diversity and evolution has previously been reported when strains were allowed to grow for an extended period of time (Finkel and Kolter, 1999, Fig.1). While many robust studies of *E. coli* have elucidated significant factors influencing population- level dynamism, such in-depth studies of these ecological principles in the gut have not been reported (Rudi and Zhao, 2021). This aim will be pursued, largely, by inspecting cultures of bacteria, inoculated from human stool samples (including individuals who are diagnosed with MS), and analyzing the changes in percent composition over a period of time (t). Nutrient conditions will be altered to mimic the relative abundance in face of certain nutrient types (e.g. high sugar concentrations versus high carbohydrate concentrations to artificially mimic the effects diet may have on relative strain abundance).

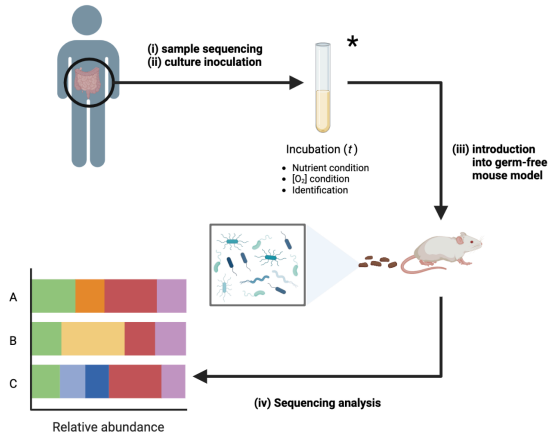

Figure 1. Workflow diagram of in vivo and in vitro analyses. After bacteria are collected from human stool samples, they will be subsequently sequenced and inoculated in liquid cultures. After which, they will be incubated for 30 days under different nutrient and oxygen-level conditions and introduced into mice models. As denoted (*), small aliquots of the liquid cultures will be sent for sequencing analysis after each day (without introduction into mice models) and the relative population abundance will be tracked.

In tandem with *in vitro* studies, *in vivo* approaches will also be employed using germ-free mice model systems. After identifying strains who exhibit a high degree of competitiveness, survival, and prevalence in liquid cultures, these strains will be engraphed into mice containing no gut microbiome (i.e. germ-free), stool samples will be collected to observe changes, if any, in population composition (Figure 1)—while specifically tracking the relative abundance of *L. reuteri*.

While difficult to predict which strains and bacterial species will fluctuate over the course of the experiment, we hypothesize that there will be some level of community homeostasis achieved whereby populations reach equilibrium at some distinct time point. The results from this study will lay foundational knowledge in understanding how to engineer the human gut microbiome by characterizing factors that may influence favorable population dynamism between strains. These findings will also support a correlation, if any at all, with *L. reuteri* and MS versus non-MS individuals. As this experiment is rooted in population-level changes, an interesting shift towards a strain-specific approach may reveal interesting factors that contribute to successful survival at the transcriptomic level — exploring if there are certain genes of which expression pattern changes yield increased community prevalence.

Aim 2. Profiling and characterization of *L. reuteri* host mimicry protein in MS versus non-MS individuals

Germ-free animals co-colonized with OTU0002 and *L. reuteri* have been demonstrated to display intense synergistic progression of EAE through an enhanced T helper 17 cell (Th17) response and molecular mimicry to myelin oligodendrocyte glycoprotein (MOG), respectively (Maghzi and Weiner, 2020). Interestingly, *L. reuteri* the protein exhibiting mimicry with MOG is UvrA (Maghzi and Weiner, 2020). UvrA is cross-reactive with MOG-specific CD4 T cells that, in tandem with the Th17 response, cause demyelination of the Central Nervous System (Maghzi and Weiner, 2020). UvrA is a well-characterized component of the nucleotide excision repair mechanism and has been demonstrated to aide in survival of the facultative anaerobe *Bacillus subtilis* when exposed to stressors such as UV-exposure (Smith et al., 2002, Figure 1). To better characterize the potential link of UvrA and MS *L. reuteri* strains will be isolated from both MS and non-MS individual stool samples and perform western

blot analysis to assess levels of UvrA. The lysed samples will be transferred to a membrane, after being ran on an SDS–PAGE gel, use anti-uvrA antibodies (Abs) with a secondary Ab, containing a fluorescent reporter, to quantitatively and visually measure changes in UvrA levels between the two groups. While not certain, increased levels of UvrA in patients with MS would likely be observed, operating under the hypothesis linking it to the disease. Further experimentation would include *in vitro* competition experiments to explore if strains containing hyperactive UvrA perform better than their knockout counterparts long term (following a similar experimental setup Figure 1). To further assess the inducibility of increased UvrA production, multiple *L. reuteri* strains will be collected and isolated, grown under an array of nutrient conditions, and analyzed via western analyses as mentioned above. 3 liquid culture growth conditions, *+sugar, +fat,* and *–nutrient*, will be tested in addition to a 4th control condition to artificially mimic dietary conditions. *+sugar* and *+fat* have an increased concentration of sugars and fats, respectively, to mimic dietary trends. *–nutrient* will have a decreased concentration of readily available nutrients to mimic starvation (in context, an individual with an eating disorder or who engages in intermittent fasting). While unable to asses the *+sugar* and *+fat* conditions, as UvrA acts as a DNA repair mechanism, it is expected that the *–nutrient* growth condition will likely have a decreased amount of UvrA abundance when compared to the control on the basis of energy likely being shunted to mechanisms only crucial for survival (ex. metabolism and anabolism), rather than DNA repair. Implications of this study lie heavily in the potential impacts of behavioral patterns of individuals with MS by providing insights into dietary effects of UvrA protein abundance from *L. reuteri*.

Concluding Remarks

The results produced by these experiments would not only provide information on the effects an individual's diet has on their gut microbiome, but they could also provide insight on how the microbiome's journey back to homeostasis can lead to microbial diversity. Bacterial strains that are deemed beneficial in certain diet conditions could be isolated and further researched for implementation into the gut microbiome of individuals whose gut microbiomes are unable to adapt to their diets. Furthermore, research into the journey towards homeostasis in the gut microbiome could uncover "areas of concern" with the bacteria within individuals with bacteria dysbiosis, gastrointestinal diseases caused by an imbalance in the gut microbiome. Elucidating the potential connection between mimicry and MS will also provide novel insights into the molecular mechanisms underlying its causes.

Works Cited

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