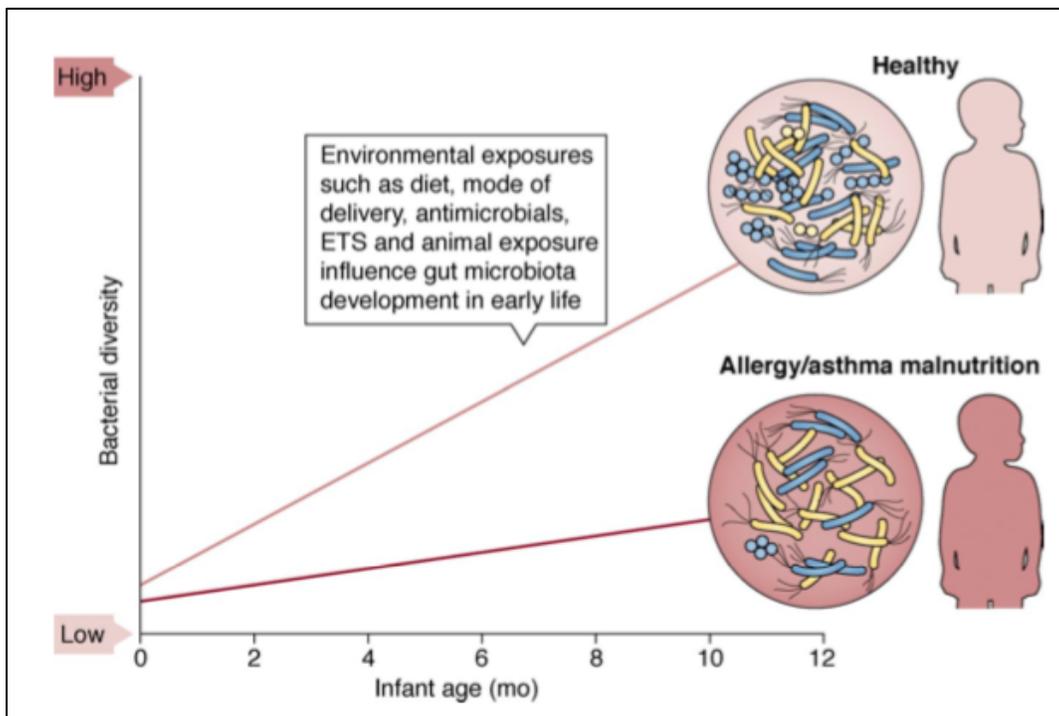


# Exploring Gut Microbiome-Based Therapies for Gastrointestinal and Metabolic Disease

## The Gut Microbiome and Disease

We, as humans, host an entire ecosystem of microbes that outnumber our own cells by a factor of ten (Durack and Lynch 2018). This community living on and within our bodies is known as our microbiome. We have developed a symbiotic relationship with these bacteria over evolutionary time, but recognition of the sheer diversity of bacteria and their transcriptomic capability has only recently been recognized with the development of high-throughput sequencing platforms (Durack and Lynch 2018). Although these microbes provide beneficial functions to humans such as facilitating the development of the immune system, detrimental life events and physical stresses such as asthma, diet, and antibiotic use can threaten bacterial diversity in the gut, contributing to disease later in life (Figure 1) (Durack and Lynch 2018).



**Figure 1:** As the gut microbiome and the resulting immune system develop early in life, environmental and lifestyle elements can inhibit microbial diversity in the gut. This may lead to a variety of diseases. Diagram obtained from Durack and Lynch 2018.

Irregularity in the composition of the microbiome, known as dysbiosis, contributes to several gastrointestinal and metabolic diseases, such as celiac disease, irritable bowel disease, and obesity (Shreiner et al. 2015; Scher 2016). Given the strong relationship between dysbiosis and disease states, recent research on the microbiome has focused on how the composition of the microbiome can be modified from an irregular state to a normal state, hopefully working to alleviate disease symptoms. Several strong associations have been drawn between disease and dysbiosis, and researchers have developed a variety of therapeutics for the correction of the microbiome. While many of these methods have proven to be effective at relieving disease symptoms and altering the microbiome, there are several limitations and potential dangers linked to each technique, so scientists and the United States Food and Drug Administration are still struggling to weigh the potential options for microbiome-based therapy and optimize techniques to reduce risk of adverse effects (Suez and Elinav 2017). This paper will review three of the most popularly proposed and studied techniques for curing disease through the microbiome, emphasizing the safety and efficacy of each method.

## **Bacteria-Based Therapies**

Fecal microbial transplant (FMT) and probiotic administration are therapies involving the direct transplantation of living microbes into the gut microbiome in hopes of outcompeting pathogenic or foreign bacterial species.

### *Fecal Microbial Transplant*

FMT involves the transplant of gut bacteria retrieved from a stool sample of a healthy donor into a patient via oral ingestion or enema (Lan, Ashburn, and Shen 2017). A clinical trial by Cui et al. (2015) presents very promising results for the use of FMT in the treatment of Crohn's disease (CD). Of 30 human CD patients, 86.7% experienced clinical improvement and 76.7% went into clinical remission within one month of FMT (Cui et al. 2015). These patients also experienced associated physical recovery, including increased body weight, improved lipid profile, and fast and continuous relief of abdominal pain according to the Harvey-Bradshaw Index (Cui et al. 2015). However, the severity of an individual patient's symptoms may affect the susceptibility of FMT to achieve clinical remission. A study by Wang et al. (2018) reported that patients experiencing adverse events related to CD, including fever, abdominal pain, and bloody stool, also experienced lowered clinical response and remission rates after FMT, with 45% and 20% of patients exhibiting response and remission versus 75.6% and 63% in the group without adverse events (Wang et al. 2018). This suggests that inter-patient variability in symptom severity may create harsher conditions for bacterial colonization in some cases, reducing the efficacy of treatment by FMT. In either case, there is agreement that FMT provides at least some level of beneficial microbiome modification.

Despite the efficacy of FMT, many studies have emphasized the need for proper donor-patient matching. A case report by Alang and Kelly (2014) describes a female patient at a healthy weight who, within 16 months of FMT by a healthy but overweight donor to treat *Clostridium difficile* infection, gained 34 pounds and became obese despite never having a history of being overweight (Alang and Kelly 2014). This phenomenon has been shown to be reproducible outside of an isolated case as well. A study by Ridaura et al. (2013) performed FMT on germ-free mice using fecal samples from four sets of human twin donors, with one twin being

obese and one not in each set. The mice receiving the “obese” microbiome developed obesity, experiencing a 10% increase in fat mass, while the ones receiving the “lean” microbiome did not develop obesity or the related metabolic phenotypes (Ridaura et al. 2013). The evidence provided by these reports indicate that donor microbiomes carry persistent metabolic effects on the recipient, and a comprehensive matching system may need to be implemented to reduce physical variability between donors and patients and avoid negative bacteria-host interactions. Such a screening model can be drawn from the Wang et al. study, in which donors were selected from family, friends, or from the universal stool bank fmtBank and carefully screened using exclusion criteria such as history of disease, metabolic syndrome, and presence of potential pathobionts in the stool sample (Wang et al. 2018). While this screening system would improve the safety of FMT, it would complicate the identification of proper donors and the administration of therapy, adding further challenge in using FMT in individual patients.

### *Probiotics*

Like FMT, probiotics involve the ingestion of live beneficial bacteria which are known to be residents in a healthy microbiome in an effort to promote their colonization in the gut. Unlike FMT, however, probiotics are artificially combined cocktails of single or multiple species of bacteria, providing a therapeutic in which the exact microbial content is known (Harnett et al. 2016). Although this provides promise for eliminating the risk of suffering the aforementioned effects of physical donor-host disparity, there is discouraging evidence regarding the efficacy of probiotics and the ability of probiotic bacteria to maintain residency in the gut. In a 2016 study by Harnett et al., subjects with celiac disease were given probiotics over a 12-week period containing multiple species of *Lactobacillus* and *Bifidobacterium*, two genera which are

regularly depleted in the gut of celiac patients (Harnett et al. 2016). However, no significant alterations of the microbiota or improvement in symptoms were observed over the 12-week period, indicating that the probiotic species were unable to survive the environment of the intestinal tract (Harnett et al. 2016).

Despite the doubt about the robustness of probiotic bacteria, data from other studies suggest that these bacteria may be able to successfully colonize the gut if there is an available metabolic or phylogenetic niche. A study by Maldonado-Gómez et al. (2016) reported that orally ingested *Bifidobacterium longum* AH1206 probiotics persisted in the gut for a minimum of six months in just 30% of the total human subjects (Maldonado-Gómez et al. 2016). It was found that those patients who experienced persistence of *B. longum* AH1206 hosted microbiomes that were lacking in other strains of *B. longum* and showed underrepresentation of carbohydrate utilization genes (Maldonado-Gómez et al. 2016). It was theorized that the lack of existing *B. longum* and resource availability stemming from decreased carbohydrate utilization developed a comfortable niche for the probiotic bacteria to fill, creating successful conditions for colonization (Maldonado-Gómez et al. 2016). The findings of this study expose an obstacle in that inter-individual variability in the microbiome composition may affect the success of therapeutics which introduce new bacteria to the microbiota. It is possible that this marked difference in success between probiotics and FMT stems from the fact that FMT bacteria already exist in a developed niche while probiotic bacteria must establish themselves in a pre-ordered community.

## **Indirect Microbiome Modification**

### *Metabolite Therapy*

As observed above, bacteria-based therapies introduce the risk of failed persistence of transplanted bacteria or negative host-microbe interactions. This leads to a novel question: What if we could modify the microbiome while avoiding direct microbial transplant? Metabolite-based interventions are unique in that they focus on providing the metabolic products that would be produced by a functional microbiome directly to the patient rather than using the microbiome as a central target, effectively aiming downstream of the microbiome to support the growth of beneficial bacteria.

Multiple studies have established the efficacy of metabolite-based therapies for microbiome-linked diseases. Research by Levy et al. (2015) reports that dysbiosis causes deficiency of the NLRP6 inflammasome in mice by creating distorted metabolite levels, including overabundance of spermine and histamine and reduced taurine levels (Levy et al. 2015). When mice colon tissue samples were supplemented with overabundant concentrations of spermine or histamine, interleukin 18 (IL-18) production, essential to inflammasome function, was cut to nearly an eighth of its original level, showing that overabundance of these metabolites directly inhibit IL-18 production (Levy et al. 2015). However, supplementing the mice with similar levels of taurine in an effort to correct its reduced concentration nearly doubled IL-18 levels both in cultured mouse colon samples and in mice (Levy et al. 2015). Taurine supplementation also restored normal immune system signaling, antimicrobial peptide balance, and microbiome composition by correcting the irregular metabolic pathways caused by dysbiosis (Levy et al. 2015). Therefore, the metabolites produced by the microbiota may be a key player in creating disease phenotypes in the host, and these phenotypes can be corrected by balancing metabolite levels. A report by Buffie et al. (2015) demonstrated that transplant of just one species, *Clostridium scindens*, into mice can provide resistance to *Clostridium difficile* infection

by restoring a *C. difficile*-inhibitory secondary bile acid biosynthesis pathway up to 100% of the pre-infection gene family abundance (Buffie et al. 2015). In culture media, addition of as little as a 0.01% suspension of these secondary bile acids limit *C. difficile* growth between 90% and 100%, suggesting that direct supplementation of secondary bile acids can act as a very effective metabolite-based substitute for FMT in patients of *C. difficile* infection (Buffie et al. 2015). These findings indicate that metabolite-based therapy can target downstream from the microbiome and work retroactively to create conditions that combat the pathogenic microbes creating the negative metabolic environment.

Metabolite therapy is not without limitations, though. Although it avoids the risk of inadvertent bacteria-host interactions, it is unknown whether existing members of the microbiota will produce molecules that interact with these metabolites to form inactive or even toxic compounds (Suez and Elinav 2017). Furthermore, serum metabolites have been shown to be greatly affected by microbiota-produced gut metabolites, meaning that supplemented metabolites may have far-reaching effects outside the gut (Suez and Elinav 2017). For these reasons, the safety of metabolite-based therapy is still in question. This gap in knowledge calls for more advanced characterization of inter-metabolite interaction and the reach of gut-centered metabolites to other areas of the body.

### **An Integrative Approach to Microbiome-Based Therapy**

This paper reviewed the viability of three different microbiome-centered therapies for disease, emphasizing the safety and efficacy of each technique. It appears that being able to avoid donor-matching, colonization resistance, and negative host-microbe interactions make metabolite therapy the best option for treating microbiome-based diseases. Metabolite therapy

was also shown to be able to inhibit the growth of pathogenic bacteria to a great extent in culture (90-100%), although the percentage of clinical recovery was not quantified in human subjects in the study by Buffie et al. Clinical remission was achieved to a lesser extent through FMT (76.7%) and was not achieved at all through probiotics, so these may be less effective treatment options than metabolite therapy. However, for accurate comparison of the effectiveness of these therapies, the clinical effects of metabolite therapy need to be defined *in vivo* in human subjects. A table comparing the clinical effects and risk considerations for each therapy is shown in Figure 2. While dysbiosis is often considered the forefront of therapeutic targeting for these potential techniques, it is very often the case that the way the dysbiotic community affects host metabolism is the direct cause of a given disease phenotype. Therefore, targeting these underlying effects of dysbiosis rather than the bacterial composition itself still allows reconstruction of microbiome-stabilizing conditions while directly working to normalize host metabolic pathways.

Method	Clinical Effect Reports	Risk Considerations and Limitations
FMT	<p>86.7% clinical response, 76.7% clinical remission (Cui et al. 2015)</p> <p>75.6% clinical response, 63% clinical remission (Wang et al. 2018)</p> <p>45% clinical response, 20% clinical remission in patients with severe symptoms (Wang et al. 2018)</p>	<p>Inter-individual symptoms and microbiome variability affect the success of FMT (Wang et al. 2018)</p> <p>Donor-patient matching and screening required to avoid negative metabolic effects (Alang and Kelly 2014; Ridaura et al. 2013)</p>
Probiotics	<p>No clinical effect in celiac disease (Harnett et al. 2016)</p> <p>Persistence of <i>Bifidobacterium longum</i> for 6 weeks in 30% of patients;</p>	<p>Niche required for successful persistence of the bacteria in the gut (Maldonado-Gómez et al. 2016)</p>

	these patients exhibited an available niche (Maldonado-Gómez et al. 2016)	
Metabolite Therapy	<p>Taurine supplementation doubled recovery of IL-18 levels in colon explants and in mice as well as improving symptoms (Levy et al. 2015)</p> <p>90-100% clearing of <i>C. difficile</i> in culture with small volumes of bile acids (Buffie et al. 2015)</p>	<p>Unknown interactions with other bacterial compounds (Suez and Elinav 2017)</p> <p>Whole-body effects (Suez and Elinav 2017)</p>

**Figure 2:** Quantitative comparison of clinical and *in vitro* effects of three microbiome-based therapies and description of safety concerns and limitations.

While FMT and probiotics each carry concerns regarding donor matching and colonization resistance, metabolite therapy removes the need for proper donor matching and screening stool samples for pathogens, and there is no worry about failure to engraft successfully into the microbiome because there are no bacteria involved for which success of the therapy is dependent on survival. Furthermore, since the process of metabolite therapy involves identification of the metabolic factors behind an individual’s given disease phenotype, it is not necessary to account for inter-individual variability in the microbiome which can reduce the effect of bacteria-based therapies.

Although metabolite therapy evades many of the challenges put forth by FMT and probiotics, the limitations of the technique described earlier in the review cast doubt on its ability to work alone as a therapeutic. Therefore, an integrated approach is recommended combining direct bacterial modification and metabolite therapy. For example, metabolite supplementation can solve the major limitation of probiotics in that it can create a stable metabolic niche for the probiotic bacteria to overcome colonization resistance and perform successful engraftment into

the microbiome. Since probiotics overcome the risk of pathobiont transfer and the need for donor-recipient matching that are present in FMT, the indirect modification of the microbiome to accommodate such probiotics alleviates concerns about the effect of inter-individual variability on treatment success and provides great promise to the field of microbiome-based disease treatment.

### **Challenges and Future Directions**

While a combined approach of metabolic therapy and probiotics may support probiotic colonization in the gut, it is still yet to be determined whether this would fully alleviate the transience of probiotic bacteria. There is a need for more research regarding the ability of these microbes to maintain their position in the gut and prevent a reversion to a dysbiotic disease state. Suez and Elinav (2017) propose the use of prebiotics, or dietary interventions affecting resource availability in the microbiome, to provide continuous support to the newly introduced bacteria (Suez and Elinav 2017). The effect of these therapies and precautionary practices in combination should be tested to determine whether their integration improves microbiome regulation over any single practice.

It is also important to recognize that the relationship between dysbiotic states and the host metabolome is not fully characterized. Therefore, it may not be apparent which metabolite or combination of metabolites would provide the most potent effect on microbiome stabilization. Further research is required regarding the modulation of molecular and metabolic products in common dysbiotic states in order to build an extensive metabolome map from which to draw information for accurate metabolite therapy.

## References

- Buffie, C. G., Bucci, V., Stein, R. R., McKenney, P. T., Ling, L., Gobourne, A., ... & Littmann, E. (2015). Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature*, *517*(7533), 205.
- Cui, B., Feng, Q., Wang, H., Wang, M., Peng, Z., Li, P., ... & Ji, G. (2015). Fecal microbiota transplantation through mid-gut for refractory Crohn's disease: Safety, feasibility, and efficacy trial results. *Journal of gastroenterology and hepatology*, *30*(1), 51-58.
- Durack, J., & Lynch, S. V. (2019). The gut microbiome: Relationships with disease and opportunities for therapy. *Journal of Experimental Medicine*, *216*(1), 20-40.
- Harnett, J., Myers, S. P., & Rolfe, M. (2016). Probiotics and the microbiome in celiac disease: a randomised controlled trial. *Evidence-Based Complementary and Alternative Medicine*, *2016*.
- Lan, N., Ashburn, J., & Shen, B. (2017). Fecal microbiota transplantation for *Clostridium difficile* infection in patients with ileal pouches. *Gastroenterology report*, *5*(3), 200-207.
- Levy, M., Thaiss, C. A., Zeevi, D., Dohnalova, L., Zilberman-Schapira, G., Mahdi, J. A., ... & Pevsner-Fischer, M. (2015). Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell*, *163*(6), 1428-1443.
- Maldonado-Gómez, M. X., Martínez, I., Bottacini, F., O'Callaghan, A., Ventura, M., van Sinderen, D., ... & Walter, J. (2016). Stable engraftment of *Bifidobacterium longum* AH1206 in the human gut depends on individualized features of the resident microbiome. *Cell host & microbe*, *20*(4), 515-526.
- Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., ... & Muehlbauer, M. J. (2013). Cultured gut microbiota from twins discordant for obesity modulate adiposity and metabolic phenotypes in mice. *Science (New York, NY)*, *341*(6150).
- Scher, J. U. (2016). The microbiome in celiac disease: Beyond diet-genetic interactions. *Cleveland Clinic journal of medicine*, *83*(3), 228-230.
- Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. *Current opinion in gastroenterology*, *31*(1), 69.
- Suez, J., & Elinav, E. (2017). The path towards microbiome-based metabolite treatment. *Nature microbiology*, *2*(6), 17075.
- Wang, H., Cui, B., Li, Q., Ding, X., Li, P., Zhang, T., ... & Zhang, F. (2018). The Safety of Fecal Microbiota Transplantation for Crohn's Disease: Findings from A Long-Term Study. *Advances in therapy*, *35*(11), 1935-1944.