EFFECTS OF PREBIOTICS, PROBIOTICS, AND ANTIBIOTICS ON THE HUMAN GUT MICROBIOME

by

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ABSTRACT

EFFECTS OF PREBIOTICS, PROBIOTICS, AND ANTIBIOTICS

ON THE HUMAN GUT

MICROBIOME

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The human gut microbiome is a community of microbes that are found in the gastrointestinal tract and serves many purposes to the human host, such as immune system regulation and food digestion. In adults, thousands of bacterial species may develop over time based on changes in diet, environment, and other factors. A dietary category of interest is that of prebiotics and probiotics, both of which are necessary for a healthy microbiome. Prebiotics aid microorganisms by acting as "food," while probiotics are microorganisms themselves. A dataset was extracted from the National Center for Biotechnology Information that included abundant bacterial genera in a study involving prebiotics, probiotics, and antibiotics. Phyla were analyzed in terms of numerical changes and unique properties. Major changes in the abundant phyla, *Firmicutes* and *Bacillota*, could be inferred as a product of a modified diet. In previous studies, it was shown how these phyla

could affect obesity-related metabolic diseases and gut health based on their quantities. This could provide implications on how the microbiome can influence a person's overall health and how the microbiome can be altered.

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CHAPTER 1

INTRODUCTION

It has been recognized that the human gut microbiome plays a vital role in human health. From protection against pathogens to maintenance of the gut mucosal barrier and nutrient metabolism in the body, it harbors many important functions (Jandhyala et al., 2017). In multiple instances, the bacteria residing in the gut can synthesize amino acids and vitamins. Additionally, the gut microbiota is becoming more and more known as a promising target for the prevention and management of various metabolic and inflammatory disorders (Magne et al., 2020). It is reasonable, then, to wonder how the human microbiome composition in each individual comes to be and if any factors may affect what bacterial communities occupy it over time.

From infancy through adulthood, the human microbiome often experiences numerous changes in microbial composition. Though factors such as mode of birth and genetics can affect the composition, it has also been observed that sanitary living conditions and long-term diet can cause change. In a review by Voreades in 2014, it was observed that though infants experience a large amount of instability with their gut microbiomes, the adult gut microbiome is far less suspectable to long-term change without consistency. Though alterations in diet can be seen with changes in the microbiome in as little as 24 hours, the community composition often returns to its initial state just as rapidly when the normal diet resumes.

Another review led by Singh in 2017, investigated how different diets influenced the microbiome. This review looked at dietary changes in many categories, some of which included proteins, sugars, fibers, as well as fermented foods. Fermented foods aid in intestine regulation and have associations with aiding against inflammatory bowel disease (Shen et al., 2014). An example of a significant fermented food is soybeans, which are a good source of bioactive peptides and uniquely contain both prebiotic and probiotic properties that are necessary for a healthy human microbiome. While probiotics are live microorganisms that can offer health benefits to their host, prebiotics are ingredients that stimulate growth for the microorganisms, acting as food

The combination of both traits makes fermented soybeans a syn-biotic. In addition, the fermentation of soybeans rather than roasting or frying them improves their bio functional properties (Sanjukta & Rai, 2016). It has been studied that the incorporation of soybeans into a diet can decrease the likelihood of degenerative bone diseases, menopausal hot flashes, among many other things (Dukariya & Singh, 2020). Though there have been studies that have explored different diets and their effect on the microbiome, a complete understanding of probiotics, prebiotics, and antibiotics on the microbiome is still being developed.

This research will examine a dataset from the National Center for Biotechnology Information (NCBI) for changes in bacterial composition according to various diets. The dataset used includes changes in diet according to categories of prebiotics, probiotics, and antibiotics. The significant changes in the top five genera will be observed, and implications for the changes will be explored. The objective of this paper is to analyze how the bacterial composition of the human gut microbiome changes when prebiotics,

probiotics, and antibiotics are implemented into a diet. The overall purpose is to provide a greater understanding of not only how the microbiome changes but also what each change might, resulting in a broader understanding of the importance of diet on human health.

1.1 Healthy Microbiota Composition

Though each person's microbiome is distinct, there are still shared characteristics between most healthy microbiota compositions. This includes the different bacterial species that typically reside in the microbiome. For optimal function, there is a host-microorganism balance that must occur for peak performance in metabolic and immune function. A review led by Rinninella in 2019 explores this concept. Largely using DNA extraction and amplifying of the 16S ribosomal RNA gene (rRNA), researchers have been able to quantify the contents of the gut microbiota through nucleic acid analysis in stool samples.

Rinninella's review outlines that there are only a few phyla that account for the more than 160 species of bacteria that are observed in the gut microbiome. Most bacterial species fall into the phyla of *Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria,* and *Verrucomicrobia* (Figure 1.1). There have also been discussions of a candidate phylum, *TM7* (Shin et al., 2015). However, between the phyla, *Bacteroidetes* and *Firmicutes* comprise about 90% of the overall gut microbiota. Phylum *Firmicutes* includes over 200 genera in the gut microbiome and is mainly seen in the form of the *Clostridium* genus. The predominant genus in the phylum *Bacteroidetes* is the genus *Bacteroides* (Arumugam et al., 2011).

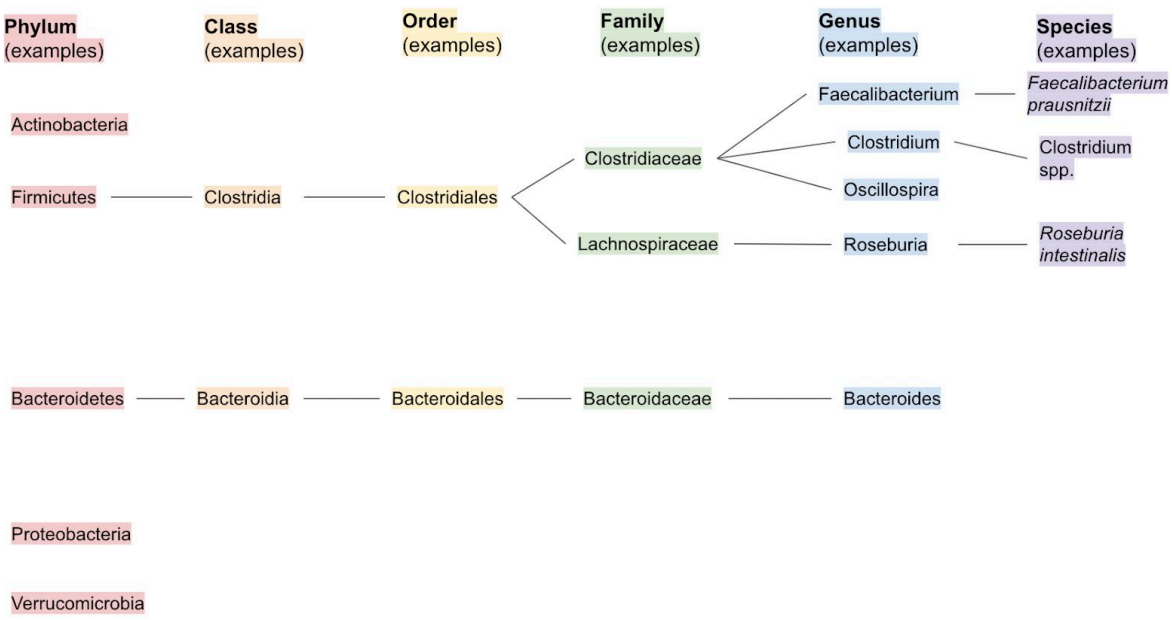

Figure 1.1: Examples of bacterial species that form the gut microbiome (Rinninella et al., 2019).

CHAPTER 2

METHODOLOGY

In this paper, a database was pulled from NCBI. The NCBI database used was provided from a study led by Ciaran in 2017, where the bacterial microbiome composition was collected under three categories of prebiotics, probiotics, and antibiotics, as well as baseline data. The control group was not given an intervention, and data was collected once by a fecal sample from each participant

For the other groups, stool samples were collected three times: before, during, and after product consumption. Each group started with eight participants. The time frame of the study was from Day 0- Day 56. The samples before treatment were collected on averages of Day -7 and Day 0. During the treatment, samples were collected on average on Day 10 and Day 14. Then after treatment, the average day for sample collection happened on Days 21, 28, and 56 (Ciaran, 2017).

Using this data, the different genera were sorted into their appropriate phyla. Each of the groups were compared, and changes in composition were analyzed. An investigation of each genus and phylum were conducted, and information from varying studies and reviews on each group were collected and recorded. The recorded information for each included unique properties and associations with various diseases.

2.1 Prebiotics, Probiotics, Antibiotics

The prebiotic group took a 1,200 mg dietary supplement of *Trametes Versicolor* extract three times daily on an empty stomach for two weeks (Ciaran, 2017). *Trametes*

Versicolor, also called Turkey Tail, is a medicinal mushroom from fungal mycelium on which a fermented substrate may grow (Benson et al., 2019). Aside from nutritional benefits, mushrooms are known for their antioxidant, anti-cancerous, and anti-diabetic properties. *Trametes Versicolor,* especially, have been shown to have effective anti-inflammatory properties (Bains and Chawla, 2020). The prebiotic properties of *Trametes Versicolor* can alter the microbiota and pH and are thought to help explain the health benefits provided to its host (Yu et al., 2013).

The probiotic group took a 250 mg dietary supplement of *Saccharomyces boulardii* three times daily on an empty stomach for two weeks (Ciaran, 2017). *Saccharomyces boulardii* is a probiotic yeast that has been observed to have numerous benefits, such as the production of peptides and improving the function of the gut barrier (Pais et al., 2020). It has been shown to have high efficiency in the therapy and prevention of several human gastro-digestive disorders as a probiotic (Kelesidis, 2012).

The antibiotic used was 250 mg of amoxicillin, taken thrice daily an hour before meals for a week (Ciaran, 2017). Amoxicillin is a penicillin antibiotic and is commonly used to treat different types of infections, which includes chest and ear. Typically, infection results start to show within days of usage (NHS, 2021).

CHAPTER 3

RESULTS

Though there were changes in nearly all the genera of microbial bacteria throughout treatments, the most notable changes observed and investigated were in the genera *Clostridium, Bacteroides, Faecalibacterium, Roseburia,* and *Oscillospira* (Table 3.1). The other genera not acknowledged either made a minor part of the gut composition to begin with or the changes were not drastic enough to consider a significant change. Only the most abundant bacterial genera in the gut and the largest changes were investigated.

The prebiotic treatment group showed notable decreases in *Clostridium* and *Bacteroides* as well as an increase was seen in the genera of *Faecalibacterium* from before treatment to during the treatment (Figure 3.1). *Faecalibacterium* increased from 12.7% to 22.6% bacterial composition found in stool. *Clostridium* decreased from 11.3% to 8.0%, and *Bacteroides* decreased from 23.6% to 17.6%. After treatment, many of the genera returned to numbers similar to baseline values. However, a notable change occurred in genera *Roseburia* that showed an increase in composition throughout the study, from 8.6% to 11.4% and ending with 15.1% after treatment had ended.

The probiotic treatment group showed a decrease in *Bacteroides* and a decrease in *Faecalibacterium* from before to during (Figure 3.2). The *Bacteroides* went from 12.6% to 10.8%, while the *Faecalibacterium* genera increased from 19.3% to 25.2%. Once

again, many genera returned to baseline, but an increase in *Bacteroides* was seen after the treatment, to16.6% of the total bacterial composition found in the stool.

The antibiotic treatment group displayed decreases in genera *Roseburia* and *Oscillospira* with an increase seen in *Bacteroides* from before to during (Figure 3.3). *Roseburia* and *Oscillospira* went from 12.6% to 6.1% and 10.4% and 6.3%, respectively. *Bacteroides* increased 13.9% to 17.9%.

Table 3.1: Bacterial Genera Composition in the Treatment Groups.

	Antibiotic - Before	Antibiotic - During	Antibiotic - After	Probiotic - Before	Probiotic - During	Probiotic - After	Prebiotic - Before	Prebiotic - During	Prebiotic - After	Control
Unit of Measure: percentage of bacterial genera in stool										
Faecalibacterium	22.3	20.9	18	19.3	25.2	21.2	12.7	22.6	12.6	25.5
Bacteroides	13.9	17.9	17.3	12.6	10.8	16.6	23.6	17.6	21.8	22.3
Roseburia	12.6	6.1	10.2	7.3	6.2	7.3	8.6	11.4	15.1	9.1
Clostridium	7.9	9.9	9.4	10.6	10.9	10.5	11.3	8	12.1	6.4
Oscillospira	10.4	6.3	7.2	2	2.1	2.2	7.1	7	7.8	5.8
Dialister	8.2	2.5	8.8	12.2	14.3	11.4	6.6	7.3	5.4	9
Ruminococcus	5.1	3.2	4	3.7	5.9	5	6.4	6.6	4.8	3.8
Coprococcus	3.1	1.8	4	1.9	2.2	2.9	2.8	1.8	1.8	1.8
Prevotella	1.8	3.7	4.8	2.7	1	1.3	0.9	0.3	0.4	2.7
Blautia	2.3	1.3	1.5	1.6	1.9	2.4	2.6	2.5	4.4	1.9
Escherichia/shigella	1.2	14.8	3	0.9	0.3	0.3	0.5	0.2	1	1.5
Other	4.4	3.9	5.6	7.1	4.3	6	6.7	5.8	6.4	5.8

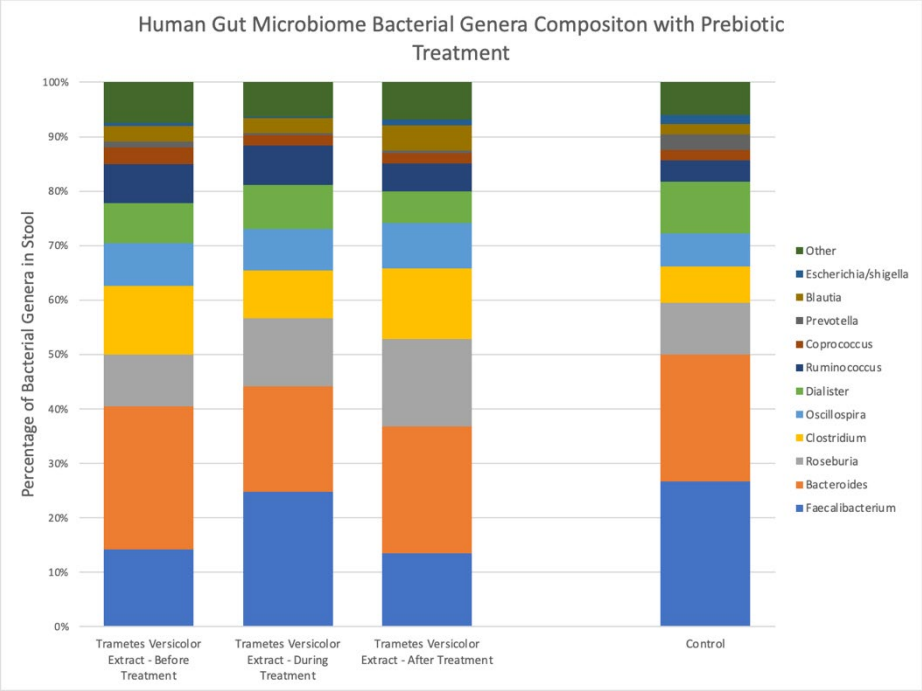

Figure 3.1: Mean of bacterial genera composition in gut microbiome with prebiotic treatment (*Trametes Versicolor*) found in fecal samples.

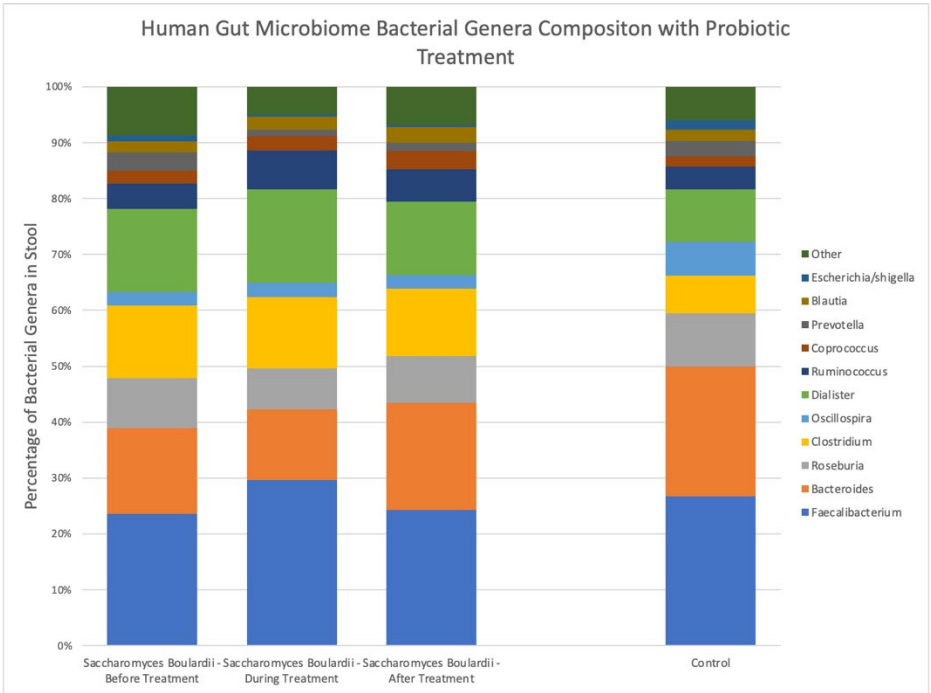

Figure 3.2: Mean of bacterial genera composition in gut microbiome with probiotic treatment (*Saccharomyces boulardii*) found in fecal samples.

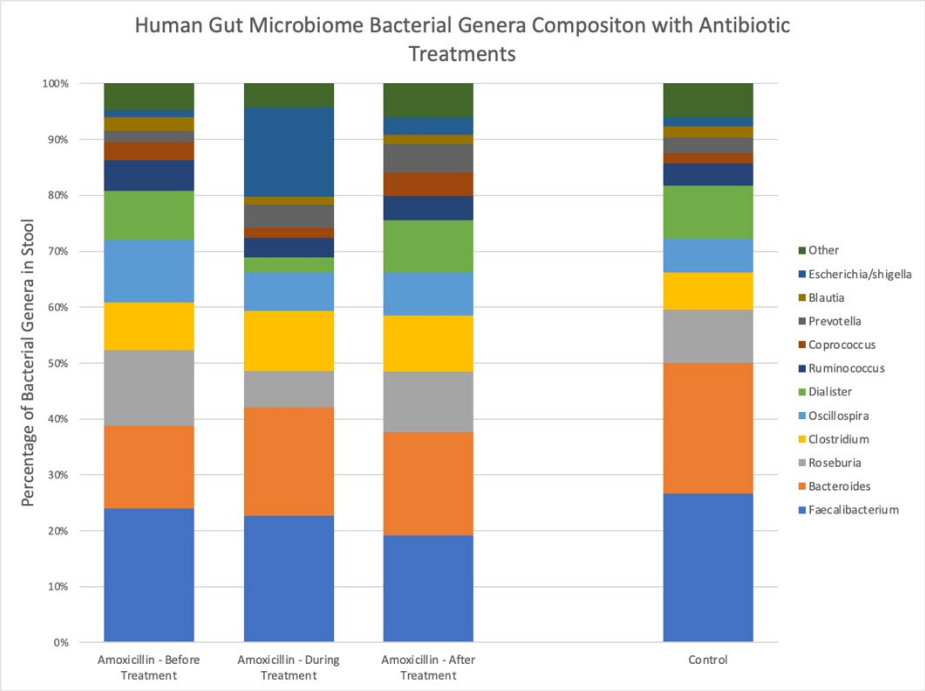

Figure 3.3: Mean of bacterial genera composition in gut microbiome with antibiotic treatment (Amoxicillin) found in fecal samples.

CHAPTER 4

DISCUSSION

A few important things from the results can be gathered. First, prebiotics may stimulate growth in certain bacterial species more than others and a favorability may occur according to the prebiotic being used. Further supported in 2017 by Markowiak and Śliżewska, antibiotics to allow for a change in the gut composition on a species level. While this change in composition may be true for the *Trametes Versicolor* extract*,* it may vary for a different prebiotic.

A similar idea can be argued for probiotics. With the changes seen, perhaps this could be due to the interactions between species themselves. In mammalian gut microbiota, there have been contractions from scientists of positive cross-feeding interactions between microbes as well as competition and interference with other microbes (Coyte & Rakoff-Nahoum, 2019). However, interactions between bacterial species are still being heavily studied by researchers. Though specifics on this topic are currently unclear with scientists, both positive and negative interactions may very well happen in the human gut at once between different groups of bacteria.

The antibiotic supports the notion that bacterial groups have varying levels of resistance. While some groups decreased due to the antibiotic, others showed greater resistance and increased in quantity. Decreasing numbers of the less resistant group can allow for the resistance group to grow in large quantities (Reygaert, 2018).

4.1 Phylum *Bacteroidota*

As one of the major phyla in the human microbiome, *Bacteroidota* has been observed to help maintain the interbacterial bonds in the gut as well as being involved in bile acid metabolism (Gryaznova et al., 2022). Another advantage displayed by *Bacteroidota* is the ability to transform toxic compounds (Kim and Milner, 2007). This phylum is also known to produce butyrate, a short-chain fatty acid with preventive cancerous properties, argued to have high potential in medicine (Canani et al., 2011).

4.1.1 Genus Bacteroides

In the genus, *Bacteroides* are species of bacteria that act to metabolize and provide vitamins and nutrients. This genus of gram-negative anaerobes make up a large part of the gut microbiome (Kim et al., 2017).

While they are beneficial to the host when concentrated in the gut, when relocated to other parts of the human body, they become opportunistic pathogens. This can result in infection and disease. This genus has seen isolated from meningitis patients, in brain, neck, and lung abscesses, and in oral infections. There has also been a connection between *Bacteroides* species in Crohn's disease, among other bowel diseases. (Zafar & Saier, 2021).

Bacteroides have a strong ability to adapt to their environment to ensure survival in the GI tract, making them strong competitors to other bacteria. Something that contributes to this is their ability to utilize available nutrient sources for growth (Hooper & Gordon, 2002). Dietary patterns have the potential to alter quantities. If there is a lack of competition in the gut, the overgrowth of this phylum can occur, which can increase mucus degradation.

Although this reduces inflammation, it also can compromise the intestinal barrier, making it more vulnerable to potential pathogens (Desi et al., 2016). This can be especially dangerous since species within *Bacteroides* possess the highest resistance rates of all the anaerobic pathogens due to obtaining the most antibiotic resistance mechanisms—all the while showing increasing resistance to many clinical antibiotics (Wexler, 2007).

In prebiotics and probiotics, a decrease in this genus while an increase was seen with the antibiotic treatment. This is likely due to its resistant nature to antibiotics. Since many of the other genera are less resistant, *Bacteroides* may outcompete their neighbors and increase in quantity.

4.2 Phylum *Firmicute*

The phylum *Firmicute* contains gram-positive bacteria with cell walls that range from semi-rigid to rigid. Members of this phylum typically have important jobs in metabolism and nutrient for the human host. They have been seen to indirectly related to other organs and may regulate satiety and hunger. In fact, there have been studies arguing about the linkage between *Bacteroidetes* and *Firmicutes* ratios and the development of obesity and irritable bowel disease (Stojanov et al., 2020). The favorability of *Firmicutes* in the gut microbiome has been debated. Some refer to them as “bad gut microbes” due to their negative influence on fat metabolism and glucose (Hassan et al., 2022).

Prominent genera in this phylum include *Lactobacillus, Bacillus, Clostridium, Enterococcus, Faecalibacterium, Roseburia,* and *Ruminococcus* (Martinez et al., 2022).

4.2.1 Genus Clostridium

Clostridium is a rod-shaped group of bacteria that, while it can be beneficial to its host by aiding in metabolic pathways in the gut, also runs the risk of being pathogenic and the cause of a variety of diseases by the release of toxins (Bien et al., 2013). They have been observed to reduce inflammation and can assume a probiotic role by strengthening the intestinal barrier and aiding the immune system. Their position in intestinal homeostasis makes them efficient regulators and, therefore, good candidates for the relief of intestinal disorders (Guo et al., 2020).

This genus also can ferment different nutrients, such as proteins and carbohydrates, while producing various acids and solvents. In humans, the genus *Clostridium* can use indigestible polysaccharides to produce beneficial metabolites (Guo et al., 2020). A decrease in this genus was seen in prebiotics.

4.2.2 Genus Faecalibacterium

Currently, the commensal bacterium, *Faecalibacterium prausnitzii,* is the only identified and successfully isolated species within the genus *Faecalibacterium* though there have been studies that propose the existence of another species (Benevides et al., 2017). This bacterium appears to produce butyrate, which activates a receptor and, in turn, facilitates downstream control of alterations in the gut in relation to diabetes and obesity (Brown et al., 2003). Butyrate also acts to improve adiposity and glucose sensitivity (Fernandes et al., 2014).

This species has also been proposed as a biomarker for gut pathologies between irritable bowel disorder (IBD) patients and healthy individuals. It has been observed to

produce anti-inflammatory metabolites. The decline of this species in the gut has been associated with chronic inflammation (Maioli et al., 2021). Its reduced numbers are also prevalent in IBD and obese individuals, making it a potential tool in patient prognosis and diagnosis (Lopez-Siles et al., 2016). Increases in this genus were seen in prebiotics and probiotics.

4.2.3 Genus Oscillospira

The genus *Oscilliopsira* contains bacteria with low G + C content. They are associated with obesity, leanness, gallstones, and chronic constipation. Like *Faecalibacterium*, they are thought of as being able to produce butyrate, which makes it a good candidate as a "next-generation probiotic." *Oscilliopsira* has been seen to exhibit positive regulatory functions in relation to inflammation and obesity (Yang et al., 2021). In addition, the abundance of this genus has been seen to show a positive correlation with high-density lipoprotein, sleep time, and microbial diversity (Chen et al., 2020). A decrease in this genus was seen in antibiotics.

4.2.4 Genus Roseburia

Roseburia is a genus of rod-shaped bacteria with subterminal flagella. The *Roseburia* species that reside in the gut metabolize and stimulate metabolic activities. They, like many other species within this phylum, are known for producing short-chain fatty acids such as butyrate. Changes in quantities may indicate diseases such as obesity, diabetes, IBS, allergies, gallstone formation, and conditions with the nervous system (Tamanai-Shacoori et al., 2017). A decrease in this genus was seen in antibiotics.

Interestingly, both *Roseburia* and *Oscilliopsira* have associations with obesity. It has been observed that the usage of antibiotics may link to obesity through the

modification it does on the human microbiome (Vallianou et al., 2021). This could perhaps be related to the decrease in these two bacterial genera with the usage of Amoxicillin, especially since *Oscilliopsira* has been seen to have regulatory functions related to obesity.

CHAPTER 5

CONCLUSION

The way that prebiotic, probiotic, and antibiotic-modified diets change the human gut microbiome's composition are all different. The benefits and risks of each treatment group vary. By altering the bacterial composition during and even long after treatment, it shows how important not only diet is but also the maintenance of the diet.

Prebiotics and probiotics seem to display beneficial roles in inflammatory bowel diseases such as Crohn's. Antibiotics, although they have positive roles in fighting bacterial infections, may show higher risks of obesity and IBS.

This investigation was based on a dataset found on NCBI. However, there were limitations in the amount of data posted for usage. Because of that, "significance" of each genus for investigation had to be made based on individual judgment and not with the usage of a statistical test of significance. However, this is only a small piece of a much larger topic of human gut microbiota.

There is still much to explore with the human microbiota. But what has been studied thus far seems promising for future medicine and therapies as both a means of diagnosis and disease remission. Understanding how to maintain a healthy microbiome is important not only in a greater scope but also to individuals. By continuing to learn more about the gut microbiome, perhaps we can all begin to take steps towards healthier and happier lifestyles.

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BIOGRAPHICAL INFORMATION

Born in Plano, Ai-Vy is a Texas native. She is anticipated to graduate from The University of Texas at Arlington in the Spring of 2023 with an Honors Bachelor of Science in Biology and a minor in Creative Writing.

During her time at UTA, she has had numerous unique experiences, including serving as a reporter and editor for *The Shorthorn*, where she won the Charles LeMaistre Award for excellence in newspaper reporting. She also had the pleasure of working for the Academic Success Center for many semesters as a Supplemental Instruction Leader and SI mentor.

Outside of school, Ai-Vy enjoys reading, writing, listening to music, taking photos, and volunteering at the library. She has also taken up a new interest in birdwatching. Her favorite bird is the pigeon.

After graduation, she plans to take a year off before applying to dental school. Though the future is still uncertain, she hopes no matter what happens, she will continue to learn and grow in new ways every day.