



**TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKES SCIENCE AND TECHNOLOGY
UNIVERSITY**

**GRADUATE SCHOOL
BIOENGINEERING DEPARTMENT**

**IDENTIFICATION OF ON INTESTINAL MICROBIOTA ASSOCIATED
WITH IRRITABLE BOWEL SYNDROME**

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M.Sc.



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DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Türkeş Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

08/01/2024

[Signature]

Merve ZORLUKOL

ÖZET

İrritabl Bağırsak Sendromu İle İlişkili Bağırsak Mikrobiyotasının Tanımlanması

Merve ZORLUKOL

Biyomühendislik

Danışman: Doç.Dr. Rozelin AYDIN

Ocak 2024, 95 sayfa

İnsan bağırsağında bulunan mikrobiyal gruplar arasındaki denge, sağlığın korunması için çok önemlidir. İrritabl bağırsak sendromu (IBS), kronik ve tekrarlayan karın ağrısı ve bağırsak alışkanlıklarında değişiklik gösteren yaygın bir sindirim bozukluğudur. Genetik, epigenetik ve cinsiyetle ilişkili faktörlerin sinir ve bağışıklık-endokrin üst sistemlerinin işleyişini etkilediği, ayrıca beyin-bağırsak fizyolojisinin düzenlenmesini, safra asidi üretimini ve emilimini etkilediği bilinmektedir. Bu çalışmanın amacı, IBS'li hastalarda bağırsak mikrobiyota çeşitliliğini araştırmaktır. Çalışma, mikrobiyal çeşitlilikteki değişikliklerin (düşük veya yüksek) IBS semptomları üzerindeki etkisinin yanı sıra hastaların sahip olduğu hastalıkların mikrobiyota ve IBS üzerindeki etkilerini belirlemeyi amaçlamaktadır. Balcalı Hastanesi'nde bir gastroenterolog tarafından kolonoskopi biyopsisi ile IBS hastalarından toplam 10 bağırsak örneği alınmış ve Illumina tabanlı yeni nesil dizileme yöntemleri kullanılarak bakteriyel biyoçeşitlilik analiz edilmiştir. IBS semptomları olan 10 hastadan alınan örnekler karşılaştırıldı ve semptomlar arasındaki ilişkileri yorumlamak için bakteri çeşitliliği analiz edildi.

Anahtar Kelimeler: Metagenomiks, İrritabl bağırsak sendromu, Mikrobiyota, Bağırsak Örneği, Kolonoskopi biyopsi

ABSTRACT

Identification of on Intestinal Microbiota Associated with Irritable Bowel Diseases

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Ocak 2024, 95 sayfa

The balance of microbial communities in the human gut is critical to maintaining good health. Irritable bowel syndrome (IBS) is a common digestive disorder characterized by chronic and recurrent abdominal pain and altered bowel habits. Genetic, epigenetic, and gender-related factors are known to influence the function of the higher nervous and immune-endocrine systems, as well as the regulation of brain-gut physiology, bile acid production, and absorption. The objective of this study is to investigate the diversity of gut microbiota in patients with IBS. The study aims to determine the impact of changes in microbial diversity (low or high) on IBS symptoms, as well as the effects of diseases that patients have on microbiota and IBS. A gastroenterologist at Balcalı Hospital collected samples by colonoscopy biopsy, obtaining a total of 10 gut samples from IBS patients. Bacterial biodiversity was analyzed using Illumina-based next-generation sequencing methods. Samples from 10 patients with IBS symptoms were compared, and bacterial communities were analyzed to interpret the relationships between symptoms.

Keywords: Metagenomics, Irritable bowel syndrome, Microbiota, Intestinal Sample, Colonoscopy biopsy

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NOMENCLATURE

AID	: Activation-Induced Deaminase
ANS	: Autonomic Nervous System
AGP	: American Gut Project
Bas	: Bile Acids
CVD	: Cardiovascular Diseases
CNS	: Central Nervous System
CSE	: Cigarette Smoke Extract
CAD	: Coronary Artery Disease
CD	: Crohn's Disease
ENS	: Enteric Nervous System
FODMAPS	: Fermentable Carbohydrates
FABD	: Functional Abdominal Bloating And Distension
FGID	: Functional gastrointestinal disorder
GI	: Gastrointestinal
HPA	: Hypothalamo-Pituitary-Adrenal
IBS-D	: IBS with diarrhea
IBS-C	: IBS with constipation
IBS	: Irritable Bowel Syndrome
IBD	: Inflammatory Bowel Disease
MMC	: Migrating Motor Complex
IBS-M	: Mixed IBS
PI-IBS	: Postinfectious IBS
PCR	: Polymerase Chain Reaction
SCFA	: Short-Chain Fatty Acids
Tlr-4	: Toll-Like Receptor 4
IBS-U	: Unspecified
VC	: Valve Calcification
VH	: Visceral Hypersensitivity

1. INTRODUCTION

The human gut is an ecosystem where nutrients and host cells are concentrated. It contains approximately 500-1000 different microbial species that remain in balance. It is estimated that bacteria make up 35-50% of the volume of the gut (Vanhoutte et al. 2004). These include *Bacteroides*, *Lactobacillus*, *Clostridium*, *Fusobacterium*, *Bifidobacterium*, *Eubacterium*, *Peptococcus*, *Peptostreptococcus*, *Escherichia* and *Veillonella* (Ley, Peterson, and Gordon 2006).

Establishing the microbial balance in the human intestine is very important for maintaining health. When this balance is disrupted, it can lead to increased disease. Dysbiosis can have negative effects on health. Additionally, many diseases have been associated with imbalances in the intestinal microbiota (McDonald et al. 2016). The microbiota has been linked to diseases such as inflammatory bowel disease, irritable bowel disease, and antibiotic-associated diarrhea. More research is needed to fully understand the relationship between the microbiota and these conditions (Juntunen et al. 2001). Therefore, maintaining the microbiota balance is important for maintaining and improving health.

Irritable bowel syndrome (IBS) is a common digestive disorder marked by chronic and recurrent abdominal pain and changes in bowel habits (Barbara et al. 2016). Genetic, epigenetic, and sex-related factors can affect the functioning of the nervous and immune-endocrine supersystems, as well as the regulation of brain-gut physiology and bile acid production and absorption. These factors are implicated in the onset of symptoms (Gazouli et al. 2016).

The role of bacterial, viral, and parasitic infections in triggering IBS is backed by robust evidence, and targeting the microbiota yields promising results with some patients responding positively to microbiota-related therapies. The absence of significant diagnostic markers and diversity of IBS patients proves arduous, resulting in unfavorable treatment outcomes for many cases and negatively impacting patients' quality of life whilst increasing the healthcare burden (Öhman and Simrén 2010).

There is increasing evidence to suggest that dysbiosis is an important feature of Irritable Bowel Syndrome (IBS). A significant body of evidence suggests that the microbiota plays a dominant role in the pathophysiology of IBS. Overall, the data indicate an increase in pro-inflammatory bacterial species, primarily *Enterobacteriaceae*, and a decrease in *Lactobacillus* and

Bifidobacterium (Balsari et al. 1982). *Lactobacillus* and *Bifidobacterium* genera were detected at a reduced rate in the microbiota of individuals with IBS. These genera can interact with other bacterial species or the host to regulate the microbiota as well as the immune system (Angelakis, Merhej, and Raoult 2013). Certain genera, including *Bifidobacterium*, the *Clostridiales* order, *Ruminococcaceae*, and *Erysipelotrichaceae* families, all of which produce short-chain fatty acids (SCFAs), have been identified in lower proportions amongst IBS patients (Załęski, Banaszkiewicz, and Walkowiak 2013). The *Firmicutes/Bacteroidetes* ratio, which roughly indicates bacterial population changes, has been reported as both higher and lower in IBS. To gain a deeper understanding of the alterations in microbiota, there is a requirement for novel metagenomic bioinformatic techniques that enable genus-species analysis (Tap et al. 2017).

The precise nature of small intestinal bacterial overgrowth (quantitative) and dysbiosis (qualitative) changes, and whether they are a cause or effect of disrupted local gut microenvironments, are unclear in individuals with IBS (Jalanka and Spiller 2017). The risk of developing IBS can increase due to gastrointestinal infections, depending on the pathogen involved (Klem et al. 2017). Randomized placebo-controlled trials have suggested that non-absorbable antibiotics such as rifaximin could benefit patients with irritable bowel syndrome (IBS) (Pimentel et al. 2011). Certain pro-/prebiotics may also alleviate IBS symptoms, although further evidence is needed (Juntunen et al. 2001). Meanwhile, randomized placebo-controlled trials have shown that dietary interventions that modify the intestinal microbiota can be an effective treatment for IBS (Ford et al. 2014). Preliminary observations have also indicated symptom improvement following fecal microbiota transplantation (Borody, Eslick, and Clancy 2019).

2. LITERATURE REVIEW

2.1 DEFINITION OF IBS

Irritable Bowel Syndrome (IBS) is a chronic and recurrent gastrointestinal condition that is both common and complex (Spiller et al. 2007). This syndrome is referred to as a functional bowel disorder, diagnosed by a typical cluster of symptoms, without detectable structural abnormalities (Anon n.d.). Current theories explaining the pathophysiology of IBS include changes in gastrointestinal motility, intestinal epithelium, and immune function. There is significant evidence supporting the role of psychosocial and physical stressors, such as gastroenteric infections, in the initial onset of symptoms or their persistence (Mayer and Collins 2002). IBS is one of the most common non-infectious diagnoses seen by gastroenterologists, accounting for approximately 1 in 20 cases in general practice (Ottillinger et al. 2013).

Neither structural abnormality nor biochemical causes such as inflammation or infection can explain the origin of IBS symptoms. Diagnosis of IBS poses a challenge due to the lack of pathophysiology (Bradesi et al. 2003).

2.2 HISTORY OF IBS

Irritable bowel syndrome (IBS) has confused researchers for over a century.

- Powell (1818), while describing intestinal disorders, referred to the three main symptoms of IBS: abdominal pain, indigestion, and bloating (Chen et al. 2013).
- Twelve years later, Howship (1830) defined “spasmodic stricture of the colon”, reflecting the conclusions that intestinal spasms contribute to functional bowel disorders (Thompson, 1989).
- In the middle of the 19th century, Cumming (1849) stated that “in the same person the intestines are sometimes constipated, sometimes diarrhea. "I can't explain how the disease has two such different symptoms," explained. (Thompson, 2006).
- The concept of irritable bowel first appeared in the Rocky Mountain Medical Journal in 1950 to describe patients who developed symptoms of diarrhea, abdominal pain, and constipation (Brown, 1950).
- The first findings to define functional bowel disorders were found in the book “The Irritable Gut” (Thompson, 1979).

After these studies, the research area in IBS has increased exponentially in publications on etiology, features, diagnostic criteria, epidemiology, and treatment. However, there is still no consensus on these issues that contribute to IBS being a confusing clinical syndrome.

2.3 DIAGNOSIS AND SUBTYPES OF IBS

IBS is a recognized disease that has been known for a long time. It has been referred to by several names, including mucous colitis and chronic nonspecific enteropathy. The Roman criteria provide the current standard for the diagnosis, classification, and treatment of IBS (Grad and Dumitrascu 2020). Diagnosing IBS remains a matter of debate in research, as there is no objective diagnostic test or physical finding that confirms its presence, despite several researched biomarkers (Barbara and Stanghellini 2009).

2.3.1 History of Criteria for Diagnosis of IBS

The diagnosis of Irritable Bowel Syndrome (IBS) is based on clinical symptoms alone. In 1978, Manning et al. attempted to establish criteria for IBS by examining 109 unselected patients who presented to gastroenterology clinics with abdominal pain or a change in bowel habits. The IBS criteria were subsequently developed from this study (Manning et al. 1978). Thirty-two participants were diagnosed with IBS. A survey of 15 questions about bowel symptoms revealed commonalities among IBS patients (refer to table 2.1). Only one of the Manning criteria, noticeable abdominal bloating/visible abdominal distention, differentiated IBS patients from those with inflammatory bowel disease (Thompson 1984).

In 1984, Kruis et al. developed a diagnostic score for symptoms that suggest a functional etiology. Although accurate, the score was cumbersome and never gained widespread use (Kruis et al. 1984).

In 1988, an international group of experts was assembled for the International Meeting of Gastroenterology in Rome. The team used the 'Delphi' decision-making method to address challenging clinical questions that could not be answered by scientific evidence at the time, to generate a consensus. Subsequently, the team published the initial diagnostic criteria for IBS based on this consensus (B. E. Lacy and Patel 2017).

Table 2.1 Diagnostic criteria for IBS (Grad and Dumitrascu 2020)

Manning	Rome I	Rome II	Rome III	Rome IV
Onset of pain linked to more frequent bowel movements	Abdominal pain and discomfort that is	Abdominal pain or discomfort for at least 12 weeks (which need not be consecutive) in the preceding 12 months associated with 2 or more of the following	Recurrent abdominal pain or discomfort at least 3 days/month in the last 3 months associated with 2 or more of the following	Recurrent abdominal pain, on average, at least 1 day/week in the last 3 months, associated with 2 or more of the following criteria
Looser stools associated with onset of pain	Relieved with defecation and/or	Relieved with defecation; and/or	Improvement with defecation	Related to defecation
Pain relief with defecation	Associated with a change in frequency of stool; and/or	Onset associated with a change in frequency of stool; and/or	Onset associated with a change in frequency of stool	Associated with a change in frequency of stool
Noticeable abdominal bloating/visible abdominal distention	Associated with a change in the consistency of stool and	Onset associated with a change in appearance of stool	Onset associated with a change in appearance of stool	Associated with a change in appearance of stool
Sensation of incomplete evacuation >25% of the time	Two/more of the following at least on one fourth of occasions/days: – Altered stool frequency – Altered stool form – Altered stool passage – Passage of mucus and/or Bloating or feeling of abdominal distension			
Diarrhea with mucus >25% of the time				
Patient must meet ≥3 criteria			Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis	Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis

In 1989, Torsoli and Corazziari initiated the development of diagnostic criteria and a classification system for all functional gastrointestinal disorders (FGIDs), which are divided into anatomical regions, including the esophagus, gastroduodenal area, bowel, biliary system, and anorectal region (B. Lacy and Patel 2017).

Rome I: 1994, A series of publications on each anatomical area were announced and published in Gastroenterology International over the next few years. These articles were compiled into a book titled 'Functional Gastrointestinal Disorders: Diagnosis, Pathophysiology and Treatment' and were retrospectively adopted as Rome I. The Rome I criteria are increasingly used for the classification and diagnosis of FGIDs (B. E. Lacy and Patel 2017).

Rome II: In 2000, developed the Rome II book following literature reviews by 52 researchers from 13 countries(Xin et al. 2014).

Rome III: 2006, Following the development of Rome II, there was a significant increase in publications. As a result, the Rome III criteria were established by reviewing the works of 87 authors from 18 countries since 2002. Unlike Rome I and II, Rome III places greater emphasis on evidence and explanation rather than consensus data (Xin et al. 2014).

Rome IV: 10 years later, in 2016, The Rome IV publication has made advancements in the understanding of gut content, gut-brain interactions, and the biopsychosocial model (Schmulson and Drossman 2017).

2.3.2 IBS Subtypes

In clinical practice and epidemiological studies, the Rome IV classification for IBS subtypes is based solely on stool frequency. The patient's type of stool consistency into account when determining IBS subtypes. (Figure 1) (Menees and Chey 2018).

IBS subtypes;

- IBS with diarrhea (IBS-D),
- IBS with constipation (IBS-C),
- mixed IBS (IBS-M) and
- unspecified (IBS-U)(Palsson, Baggish, and Whitehead 2014).

In Rome IV, the association was made with the proportion of symptomatic stools, specifically loose/watery and hard/lumpy stools, rather than all stools(Table 2.2) (Enck et al. 2016). This addition of the term 'predominant' in the classification of IBS subtypes has significantly reduced the number of unclassified cases (Whitehead, Palsson, and Simrén 2017).

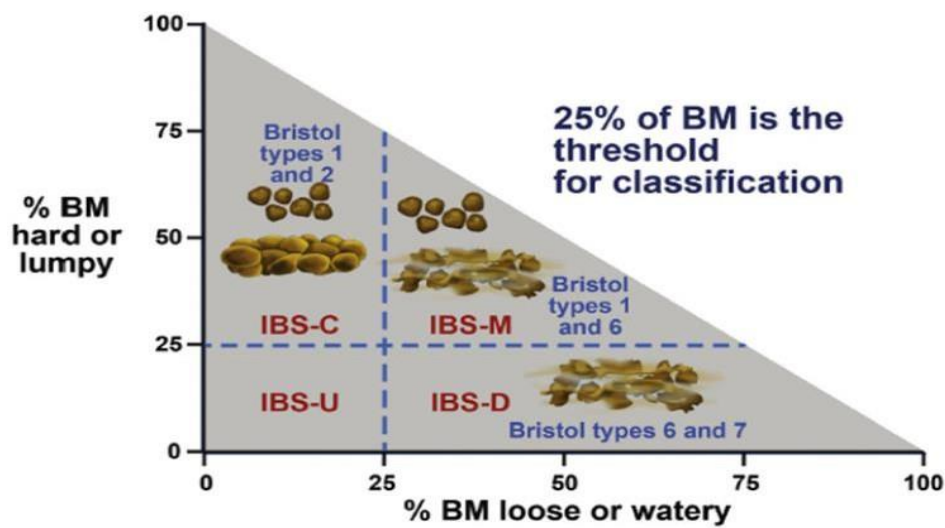


Figure 2.1 IBS subtypes (using the Bristol Stool Form Scale)

Table 2.2 In Rome IV the criteria for subtypes of IBS (Enck et al. 2016).

	Rome III		Rome IV
IBS – C (IBS with constipation)	Hard/lumpy stools >25% and loose/watery stools <25% of bowel movements	IBS – C (IBS with predominant constipation)	>25% of bowel movements with Bristol Stool form types 1–2 and <25% of bowel movements with Bristol Stool form types 6–7
IBS – D (IBS with diarrhea)	Loose/watery stools >25% and hard/lumpy stools <25% of bowel movements	IBS – D (IBS with predominant diarrhea)	>25% of bowel movements with Bristol Stool form types 6–7 and <25% of bowel movements with Bristol Stool form types 1–2
IBS – M (mixed IBS)	Hard/lumpy stools >25% and loose/watery stools >25% of bowel movements	IBS – M (IBS with mixed bowel habits)	>25% of bowel movements with Bristol Stool form types 1–2 and >25% of bowel movements with Bristol Stool form types 6–7
IBS – U (unsubtyped IBS)	Insufficient abnormality of stool consistency to meet criteria for IBS-C, D, M	IBS – U (IBS unclassified)	Patients meet diagnostic criteria for IBS but their bowel habits cannot be accurately categorized in any of the above subtypes
Patients should not be on any type of medication used to treat bowel habit abnormalities. IBS, irritable bowel syndrome.			

2.4 EPIDEMIOLOGY

2.4.1 Prevalence

IBS is a functional bowel disorder that affects a significant proportion of the population, with prevalence rates ranging from 3% to 25% (Chang 2004). Various criteria are used to assess the prevalence of IBS. Counseling behavior is another factor affecting prevalence rates. It is estimated that 33% of IBS patients do not consult or receive a diagnosis from their doctor (Spiller et al., 2007). Although IBS is more common among those aged 20-30, it can affect individuals of all ages. A study conducted in the USA found that the prevalence of IBS was higher in patients aged 30-64 (17%) than in those aged 65-93 (10.9%) (Camilleri, Heading, and Thompson 2002).

2.4.2 Gender differences

In most populations, women show more IBS symptoms than men. Rates are approximately 1.5 to 3 times higher in women than in men (Talley, Boyce, and Jones 1998). In Southern Asia, South America, and Africa, the rates of IBS symptoms in men are similar to those in women, and in some cases, even higher. Therefore, when classifying prevalence by geographic region, there is no significant difference in gender (Lovell and Ford 2012). Various factors have been suggested to contribute to this diversity, including hormonal differences, cultural pressures, response tendencies towards potentially harmful events, and differences in brain serotonin synthesis (Camilleri et al. 2002). One could argue that the high prevalence of IBS symptoms in the population without access to health services is an indication of unmet needs (Quigley et al. 2006).

2.4.3 Incidence

The precise incidence of new cases of Irritable Bowel Syndrome (IBS) remains unclear due to limited research on the topic. In a recent study, Locke et al. (2004) found that, after adjusting for age and sex, there are 2 new cases of IBS per 1000 people per year based on a 10-year medical record period. The data indicate that only a small percentage of patients seek medical attention, and the overall incidence of IBS in the population is still unknown (Camilleri et al. 2002).

2.4.4. Age

IBS can affect individuals of all ages, including children and the elderly, with no significant difference based on age. However, it is worth noting that half of all IBS patients experience their first symptoms before the age of 35 (Hongo 2011). Those over 50 years of age are 25% lower than those younger. Patients over the age of 50 also report milder pain, but have a worse overall quality of life (Tang et al. 2012).

2.4.5 Socioeconomic status

Studies show that IBS is associated with low socioeconomic status. Low income is associated with poorer health care, resulting in lower overall quality of life and increased life stress (Marmot et al. 2012). Similarly, people working in professional and managerial roles have higher rates of IBS. It is claimed that this situation causes higher stress levels. This supports that IBS is a disease of industrialization and urbanization (Cremonini and Talley 2005). Higher-income groups may experience increased rates of IBS due to different dietary preferences or greater internalization of stress. It is important to note that this statement is based on objective evidence and not subjective evaluations (Mohammed et al. 2005).

2.5 AETIOLOGY OF IBS

The aetiology of IBS remains elusive to date, but recent advancements in our knowledge have facilitated a better comprehension of it. Numerous mechanisms are proposed to contribute to the development of IBS, such as disease-related factors in early life (genetic or environmental), irregular bowel motility, heightened visceral sensitivity, brain-gut interactions, or psychological problems (Spiller et al. 2007).

2.5.1 A biopsychosocial model of IBS

Engel (1980) biopsychosocial model; A new IBS model, influenced by psychosomatics and psychoneuroimmunology, began to be proposed. (Mayer 1999) proposed a model in which the interaction between cognitive, behavioral, emotional, and physiological components explain IBS symptoms. In the same year, Drossman et al. (1999) proposed a biopsychosocial interpretation of IBS, currently considered one of the most relevant models for this disease (Figure 2.2)

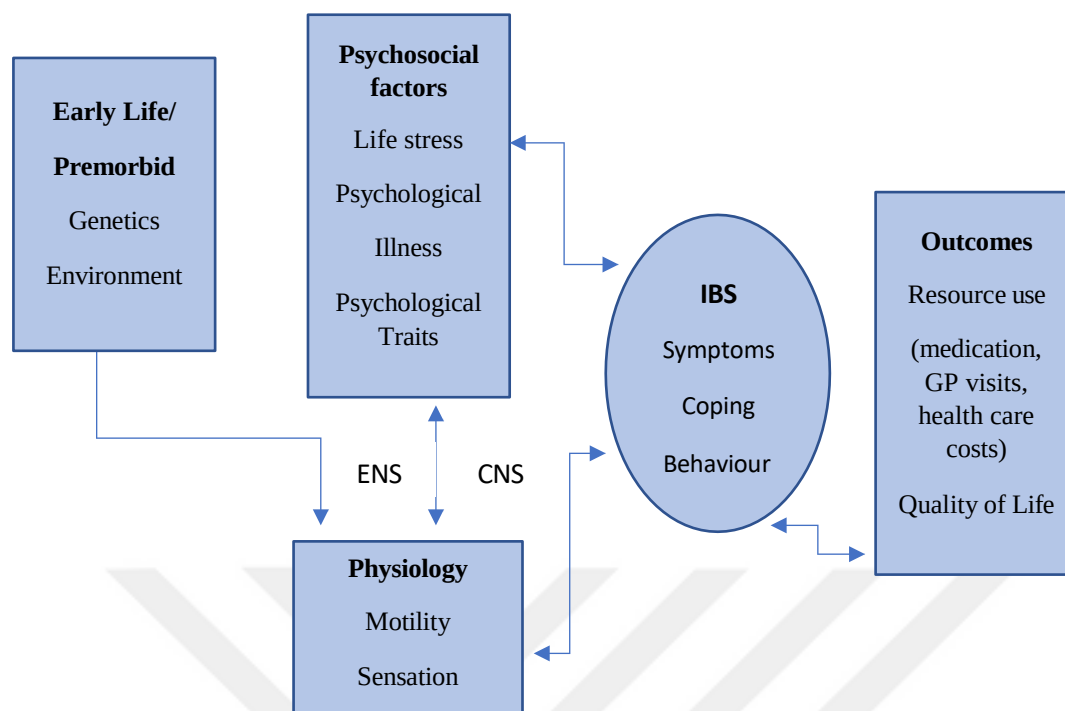


Figure 2.2. Biopsychosocial conceptualization of IBS (Drossman et al., 1999)

In this model, we can see how changes in early life/premorbid genetic and environmental factors may play a role in the development of both psychosocial and physiological factors that lead to the expression of IBS symptoms. Furthermore, interactions between the Central Nervous System (CNS) and the Enteric Nervous System (ENS) have been highlighted as an influence on IBS expression.

The remainder of this chapter will examine early life/pre-disease factors, physiological factors, and brain-gut interactions.

2.5.2 Early life/Premorbid factors

The development of irritable bowel syndrome (IBS) is a controversial topic, with debates surrounding the role of distal early life factors such as genetics, parenting, and trauma, as well as proximal premorbid factors like infection.

2.5.2.1 Genetics

Findings from studies suggest that there may be a genetic component to IBS (Camilleri 2009). These studies have led to several investigations into possible associations between candidate genes (e.g. serotonin transporter 5-HTT, α -adrenergic receptor, interleukin IL-10, tumor

necrosis factor α) and IBS (Spiller and Garsed 2009). Although many associations between candidate genes and IBS have been reported, these studies have generally been inconclusive (Camilleri 2009).

2.5.2.2 Traumatic events

Traumatic events in infancy or childhood may contribute to the development of IBS later in life. Bengtson et al. (2006) found that restricted fetal growth and low birth weight due to nutritional problems significantly affected IBS symptoms. Anxiety and stress from these events also increase the symptoms of IBS (Bengtson et al. 2006).

2.5.2.3 Infection

Irritable bowel syndrome (IBS) is a clinical condition characterized by abdominal pain, changes in bowel habits, and structural abnormalities. Research has shown that IBS can develop after acute infectious gastroenteritis, a condition known as postinfectious IBS (PI-IBS). PI-IBS cases have been reported following episodes of bacterial, viral, or parasitic acute gastroenteritis. The predominant form of diarrhea is more common in PI-IBS (Campbell 2006). Between 5% and 32% of patients with IBS experience symptoms within six months of an acute episode of gastroenteritis (Spiller et al. 2007). Markers of this condition include diarrhea, abdominal cramps, increased stool frequency, bloody or mucousy stools, positive stool culture, and weight loss. (Locke et al. 2004). Abdominal cramps increase the risk of the disease fourfold. Bacterial pathogens such as *Campylobacter*, *Shigella*, *Salmonella*, and *Escherichia coli* (*E. coli*) 0157:H7 have been development of PI-IBS, but it is unclear whether all organisms are involved (Wang, Fang, and Pan 2004). Anxiety disorders have also been observed to increase the risk of PI-IBS. Additionally, depression, neuroticism, somatization, stress, and negative perception of illness have all been associated with PI-IBS (Gwee et al. 1999). The mechanisms responsible for Post-Infectious IBS (PI-IBS) are not yet fully understood. However, it is believed that it may cause inflammation due to alterations in intestinal permeability. Several studies have observed changes in intestinal permeability in patients with PI-IBS (Thabane and Marshall 2009). Intestinal permeability can increase inflammation by exposing the submucosa to luminal antigens, which can disturb enteric sensation and motility (Spiller et al. 2000).

2.5.3 Physiological Factors

Other symptoms such as abdominal pain/discomfort, abnormal bowel function, and bloating have been implicated as physiological factors in the pathophysiology of IBS. Evidence has supported that primarily disturbances in gastrointestinal motility and gastrointestinal sensation (visceral hypersensitivity) contribute to IBS symptoms.

2.5.3.1 *Disturbance of gastrointestinal motility*

Gastrointestinal motility is responsible for crucial processes in digestion, including pushing food contents from one area of the intestine to another, mixing them with digestive secretions, exposing them to absorbent surfaces, and ultimately excreting remaining residues. According to Litleskare et al. (2015), patients with IBS are believed to experience motility issues in the esophagus, stomach, small intestine, and colon. IBS patients have been shown to experience small bowel immobility due to periodic alteration of the migrating motor complex (MMC) (Litleskare et al. 2015). IBS patients have been shown to experience small bowel immobility due to periodic alteration of the migrating motor complex (MMC) (Johnsen et al. 2018). MMCs are waves of activity that sweep the intestines in a regular cycle, facilitating the transport of indigestible substances such as fibers. According to Kellow, Eckersley, and Jones (1992), patients with irritable bowel syndrome (IBS) who have predominant diarrhea have a shorter MMC periodicity, whereas those with predominant constipation have a longer periodicity (Kellow, Eckersley, and Jones 1992).

Abnormal bowel contractions can cause abdominal pain and discomfort, which may lead to symptoms of IBS. Research has shown that individuals with IBS often experience gastrointestinal (GI) motor disturbances that are responsive to stimuli such as diet and stress (Dickhaus et al. 2003). Epidemiological studies have shown that the prevalence of IBS is increasing due to rapid socioeconomic development and changes in diet (Lu et al. 2000). Antispasmodics and promotility drugs can be used to control abdominal pain, discomfort, and abnormal gastrointestinal transit and related symptoms in patients with IBS (Chen et al. 2018). A study conducted in Taiwan reported that antispasmodic drugs have therapeutic benefits for patients with diarrhea-predominant IBS. Chen et al. (2018) suggest that controlling abnormal motility is crucial for treating certain IBS symptoms (Chen et al. 2018).

2.5.3.2 Visceral Hypersensitivity

In non-pathological conditions, gastrointestinal contractions and internal organ functions are typically painless. Visceral hypersensitivity (VH) is defined as altered sensation in response to physiological stimuli. (Camilleri 2002). VH has two main components: allodynia and hyperalgesia. Hyperalgesia is the sensation of pain in response to pain-causing stimuli, while allodynia is an increased nociceptive sensation in response to normal stimuli. Previous studies have shown that VH is produced by disturbances in the central nervous system (CNS) (Bouin et al. 2001). Epidemiological studies have reported varying estimates of VH prevalence in patients with IBS, ranging from 33% to 90%. VH is typically observed in IBS-D patients with increased intestinal permeability (Farzaei et al. 2016).

Research suggests that pain may contribute to infection by sensitizing the gut. Infection is believed to affect the excitability and sensitivity of sensory receptors in the gut, leading to an increase in discomfort and pain (Bd et al. 1997). Additionally, emotional distress and stress have been linked to changes in brain activity that cause visceral pain (Drossman et al. 2003).

In summary, gastroenterologists consider IBS to be the most common disease. The major contributors to IBS include intestinal motility disorder, inflammation, immune dysfunction, and psychological factors, in addition to VH. It has been suggested that IBS is a heterogeneous disease with VH (Ludidi et al. 2012). VH is a key pathophysiological mechanism in nociceptive sensation in large patient groups with functional gastrointestinal disorders, particularly IBS. It refers to reduced thresholds of perception of stimuli produced from the gastrointestinal tract (Barbara and Stanghellini 2009). The exact etiology of VH is unknown; however, both peripheral and central neural mechanisms are hypothesized to be involved in pain sensitivity (Greenwood-van Meerveld 2007). Visceral hypersensitivity in the colon is considered a crucial factor in the development of pain and discomfort symptoms observed in IBS. This hypersensitivity appears to be influenced by both internal and external factors. It is important to note that any subjective evaluations have been excluded from this analysis.

2.5.4 Brain-gut interactions

The gut microbiota can interact with the host by releasing bioactive molecules such as lipids, peptides, gas, and neurotransmitters that act on the nervous system (Rastelli, Cani, and Knauf 2019). Modifying the composition and function of the gut microbiota can have a direct and indirect impact on the activity of the central nervous system (CNS). The gut microbiota can indirectly interact with the brain through the enteric nervous system (ENS), which is directly

connected to the central nervous system (CNS) (Cani and Knauf 2016). The Enteric Nervous System (ENS) is a subdivision of the peripheral nervous system that controls the gastrointestinal tract. It communicates with the Central Nervous System (CNS) via the parasympathetic (eg vagus nerve) and sympathetic nervous systems (Burns and Thapar 2006). Several researchers have hypothesized that the close connection between the enteric nervous system (ENS) and the central nervous system (CNS) may be due to the correlation between gut and brain events (Drossman 1998).

According to Drossman's brain-gut-axis (BGA) model, the primary symptoms of IBS, including altered motility and visceral hypersensitivity, result from dysregulation of communication pathways between the ENS and CNS. Research on the impact of stress on IBS provides evidence of the brain-gut connection. Stress affects both the mobility and sensitivity of the colon (Drossman et al. 2003). IBS symptoms are believed to be a response to stressors through the integration of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (Spiller et al. 2007). The autonomic nervous system (ANS) and hypothalamus-pituitary-adrenal (HPA) axis are generally considered the centers of the stress response system. Changes in this complex system have been associated with psychiatric disorders and stress-sensitive pain syndromes. Psychological factors, including psychiatric illness, anxiety, and low mood, have been associated with the activation of IBS, particularly the post-infectious variety (PI-IBS)(Spiller and Garsed 2009). The HPA response is also thought to be associated with immune activation of the intestinal mucosa in IBS patients (Spiller et al. 2000).

In summary, there is evidence to suggest that the brain gut axis plays a significant role in the development of IBS and IBS-like symptoms. Future research should aim to fully define the CNS-ENS systems over the gut and determine the appropriate level of pharmacological therapy that may benefit patients with IBS.

2.5.5 Psychosocial factors

The topic of psychosocial factors in IBS has gained scientific attention over the last 20 years. Symptoms in IBS patients may be caused by the presence and impact of stressful life events, psychological and some psychological characteristics (Blanchard et al. 2008).

Chaudhary and Truelove (1968) found that over 80% of patients with functional gastrointestinal (GI) complaints also experienced psychological distress. This was determined through a psychiatric interview, but no control group was used (Connell 1968). Most studies have found

that between 54% and 94% of patients with irritable bowel syndrome (IBS) have at least one psychiatric disorder (Mayer et al. 2001). Psychological illnesses such as depression, generalized anxiety disorder, panic disorder, and post-traumatic stress have been associated with IBS. These patients are more likely to experience IBS symptoms than healthy individuals (Garakani et al. 2003). Depression and general anxiety disorders are prevalent among IBS patients seeking treatment. According to Guthrie et al. (2003), who conducted structured interviews with a sample size of 107, 44% of patients with IBS symptoms had a psychiatric diagnosis, with anxiety disorders (30%) being the most common, followed by depression (26%) (Guthrie et al. 2003).

2.6 CURRENT APPROACHES TO THE TREATMENT OF IBS

Although previous studies have identified biological, psychological, and social factors that contribute to the different symptoms of IBS, the precise pathophysiology of this complex disease is still unknown. Therefore, the primary goal of treatments for IBS is symptom management and improving quality of life (Grundmann and Yoon 2010).

2.6.1 Explanation/Addressing patient's concerns

Recognition of IBS patients' disease is an important factor that should be addressed by the physician. A lack of information or an unclear diagnosis can sometimes lead to worse outcomes. Difficulties may be encountered in administering the treatment (Dickhaus et al. 2003). Therefore, it is necessary for the physician to clearly explain the diagnostic process. Future treatment or research procedures must address their explanatory model to match their perceptions and expectations of illness (Casiday et al. 2009).

Education and doctor consultation about the diagnostic procedure, patients' concerns are addressed in more depth. A positive physician-patient relationship is therefore essential for a good outcome.

2.6.2 Lifestyle and Dietary Management

Before considering pharmacological treatment, physicians consider an initial treatment based on relatively simple lifestyle and/or dietary changes (Grundmann and Yoon 2010).

2.7 MICROBIAL SIGNATURE OF IBS

The gut microbiota of an adult human contains approximately 100 trillion microorganisms, which is approximately ten times the total number of somatic and germ cells in the body. This complex microbial community develops through a colonization process that begins at birth and continues into adulthood, transforming an environment that is initially devoid of bacteria. Intestinal microorganisms have several metabolic functions, including the synthesis of short-chain fatty acids, and vitamins, and the breakdown of carbohydrates. They also play a role in modulating the immune system and protecting against pathogens by secreting hydrogen peroxide and nitric oxide with antimicrobial properties (Jalanka and Spiller 2017). The phyla most abundant in the GI tract are *Firmicutes* and *Bacteroidetes*, but *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, and less represented *Fusobacteria*, *Tenericutes*, *Spirochaetes* and *Cyanobacteria* are also present (Thursby and Juge 2017). Difficult to define the health-relevant microbial content of the GI tract, as the microbiota may harbor functional and distinctive compositional variants that reflect early life events such as mode of birth, diet type, and sex. In general, a "healthy" microbial signature is characterized by a prevalence of *Firmicutes* and *Bacteroidetes* (Martin et al. 2016).

2.7.1 The 'healthy' gut microbiota

The intestinal microbiota of healthy individuals; provides various benefits such as pathogen protection, metabolism, and immune modulation. Analyzes examined types of bacteria common in the intestines of a healthy adult (Turnbaugh et al. 2007). Based on 16S rRNA-based studies, it is clear that overall in the adult gut, two bacterial phyla, *Firmicutes* and *Bacteroidetes*, predominate, and other phyla, including *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, and *Fusobacteria*, are present in lower proportions (Eckburg et al. 2005).

Sequence data from 22 fecal metagenomes from individuals in four countries provided information on the type and functional composition of the intestine. This research shows that the human gut microbiome consists of three enterotypes. These clusters were named to reflect their dominant members, namely *Bacteroides*, *Prevotella* and *Ruminococcus* (Arumugam et al. 2011).

However, the study found that individuals in clean environments had high levels of *Bacteroidetes* in their microbiota, while those living in the community had high levels of

Firmicutes. Additionally, individuals in long-term healthy/care environments had significantly less microbiota diversity. Loss of community-associated microbiota was associated with increased susceptibility (Claesson et al. 2009). Developing an understanding of what constitutes a healthy microbiota, regardless of age, enables the detection of microbiota in individuals with various diseases.

2.7.2 The ‘IBS’ gut microbiota

IBS is defined according to symptom-based diagnostic criteria only (Brint et al. 2011). Recent research into the pathophysiology of IBS suggests that alterations in the normal gut microbiota may contribute to the low-grade intestinal inflammation associated with the syndrome (Ponnusamy et al. 2011). Studies have identified differences in the microbiota of stool samples between healthy individuals and people with IBS. Specifically, there were higher or lower *Firmicutes: Bacteroides* ratio and differences observed at the phylum level in individuals with IBS (Labus et al. 2017). At the genus level, IBS patients generally have increased *Ruminococcus*, *Clostridium*, *Coprococcus*, and *Blautia*; and reduced *Faecalibacterium* relative abundance (Rajilić-Stojanović et al. 2011).

IBS has been associated with changes in gut microbiota composition, including an increase in pathobionts such as *Veillonella* and *Enterobacteriaceae*, *Bacteroides*, or a decrease in *Prevotella* and *Desulfovibrionaceae* (Gobert et al. 2016). *Desulfovibrionaceae* comprises bacteria that reduce sulfur and compete with methanogens to remove hydrogen in the human colon (Strocchi et al. 1994). In general, the differential relative abundance of taxa from the phylum *Bacteroidetes* and the families *Ruminococcaceae* or *Lachnospiraceae* have been reported for IBS symptoms (Jeffery et al. 2012).

Several studies have reported differences in stool microbiota among different subtypes of IBS. While some studies report no difference in the microbial community between IBS-C and IBS-D (Table 2.3), others have associated specific microbial signatures with different IBS subtypes (Pittayanon et al. 2019).

Table 2.3 Differences in microbiota composition between IBS subtypes (Duan et al. 2019).

	IBS-C	IBS-D	IBS-M
Phylum	↑ Firmicutes ↑ Actinobacteria	↑ F/B ratio ↑ Proteobacteria ↓ Bacteroidetes ↓ Actinobacteria	↑ F/B ratio
Class	↑ Clostridia		
Order	↑ Clostridiales ↑ Coriobacteriales		
Family	↑ Incertae Sedis XIII ↑ Lachnospiraceae ↑ Ruminococcaceae ↑ Rhodospirillaceae ↑ Coriobacteriaceae	↓ Erysipelotrichaceae ↓ Ruminococcaceae ↓ Porphyromonadaceae ↓ Ruminococcaceae ↓ Unknown Clostridiales ↓ Methanobacteriaceae ↓ Incertae sedis XIII	↓ Erysipelotrichaceae ↓ Ruminococcaceae ↓ Incertae sedis XIII ↓ Eubacteriaceae
Genus	↓ Roseburia ↓ Bifidobacterium	↓ Bifidobacterium ↓ Lactobacillus	
Species	↓ Eubacterium rectale ↓ Eubacterium hallii ↓ Anaerostipes caccae ↑ Methanobrevibacter smithii		

IBS-C typically involves higher levels of *Firmicutes* and a reduction in certain lactate-producing and lactate-utilizing bacteria, such as *Bifidobacterium* and *Eubacterium hallii*/*Anaerostipes caccae*, respectively (Chassard et al. 2012).

IBS-D is characterized by a reduction in overall microbial diversity and an increase in harmful bacteria, such as Proteobacteria, as well as lower numbers of *Actinobacteria* and *Bacteroidetes* compared to IBS-C (Malinen et al. 2005). Both fecal and mucosal samples have shown decreased relative abundances of *Bifidobacterium* and *Lactobacillus* in IBS-D (Shankar et al. 2015). The reduction of *Bifidobacterium* and *Lactobacillus* differ in their activity against pathogens. These bacteria are noteworthy for their ability to enhance immune tolerance by producing metabolites, such as organic acids, including short-chain fatty acids (SCFAs) (Ma et al. 2018). Altered levels of short-chain fatty acids (SCFAs) in feces have been reported to be associated with a different distribution of Clostridiales in individuals with IBS-C and IBS-D, as well as with stool consistency (Gargari et al. 2018). Increased relative abundance of SCFA producers such as the order *Clostridiales*, the genus *Bifidobacterium*, and the families

Ruminococcaceae and *Erysipelotrichaceae* have been reported (Rajilić-Stojanović et al. 2011).



3. MATERIAL AND METHODS

3.1 SAMPLE COLLECTION

The diagnosis was made by a gastroenterologist at Balcalı Hospital based on a standardised clinical presentation and further investigations if deemed necessary. The ethics committee decision was taken on 15.10.2021. All participants were informed about the study both verbally and in writing. Bowel samples were collected by colonoscopy biopsy and a total of 10 sterile containers were used to collect bowel samples from IBS patients. The samples were kept on ice and transported to Adana Alparslan Türkeş Science and Technology University Bioengineering Laboratory. Tissue DNA was extracted using commercially available extraction kits by the given protocol. Subsequently, microbiota analysis was performed using next-generation sequencing methods (16S rRNA), and a microbiota map was created. The results of the analysis will be interpreted to develop innovative therapeutic approaches.

Intestinal samples from our IBS patients were obtained through colonoscopy biopsy. A colonoscopy is a diagnostic and therapeutic procedure used to assess both the large intestine and the distal part of the small intestine. It involves employing a handheld, flexible tube-like instrument known as a colonoscope. A high-definition camera is fitted to the colonoscope's tip, which transmits visual data to a screen to aid in identifying any abnormalities or excessive growth in the wall of the colon. Utilizing various biopsy instruments via additional channels, it enables us to evaluate, biopsy, and eliminate mucosal lesions. Due to its vast capabilities, colonoscopy has become a key tool for preventing and detecting colorectal cancer in recent decades. This procedure's extensive utility warrants its prominent position in the field (Telford 2012).

3.2 MICROBIOLOGICAL ANALYSIS

Microbiological analysis of samples Metagenomic DNA was extracted using the FastDNA®SPIN kit for soil according to the manufacturer's instructions (MP Biomedicals, Solon, OH). To amplify the variable V3-V4 regions of the 16S rRNA gene, the primers 341 F (5'-CCTACGGGNGGCWG-CAG-3') and 805 R (5'-GACTACHVGGG-TATCTAATCC-3') were used. MiSeq sequencing adaptor sequences were added to the 5' ends of forward and reverse primers. Approximately 12.5 ng of purified DNA from each sample was used as a template for PCR amplification in a 25 µl reaction mixture by using 2 × KAPA HiFi Hot Start Ready Mix (Kapa Biosystems, MA, USA). For PCR amplification, the following conditions were followed: denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 sec., annealing at 55 °C for 30 sec., and extension at 72 °C for 30sec., with a final extension at 72 °C for 5 min. Amplified PCR products were purified with Agencourt AMPure XP purification system (Beckman Coulter).

4. RESULTS

In total, 10 samples were analyzed. Numerous phyla and species were identified (Table 2.5). The samples' manifestation of IBS symptoms is indicated in Table 2.4. This study aims to investigate the diversity of gut microbiota by sampling from patients. Furthermore, seeks to determine which variations in microbial diversity, whether low or high, might contribute to supporting IBS symptoms

Table 4.1 In the table, patients' information and symptoms are specified in this study.

	SYMPTOM	INFORMATION
EXAMPLE 1	Weakness Abdominal Pain	*No smoking *No regular medication
EXAMPLE 2	Abdominal pain	*No smoking *No regular medication *Treated for cancer
EXAMPLE 3	Abdominal pain Bloating	*No smoking *No regular medication
EXAMPLE 4	Abdominal pain	*No smoking *No regular medication
EXAMPLE 5	Abdominal pain	*No smoking *No regular medication
EXAMPLE 6	Stomach and abdominal pain	*Smoking *No regular medication
EXAMPLE 7	Abdominal pain	*No smoking *Regular medication (heart medication)
EXAMPLE 8	Abdominal pain	*No smoking *Regular medication (high blood pressure and heart medication)
EXAMPLE 9	Abdominal pain and bloating	* No smoking *No regular medication
EXAMPLE 10	Abdominal pain and bloating	*No Smoking *No regular medication

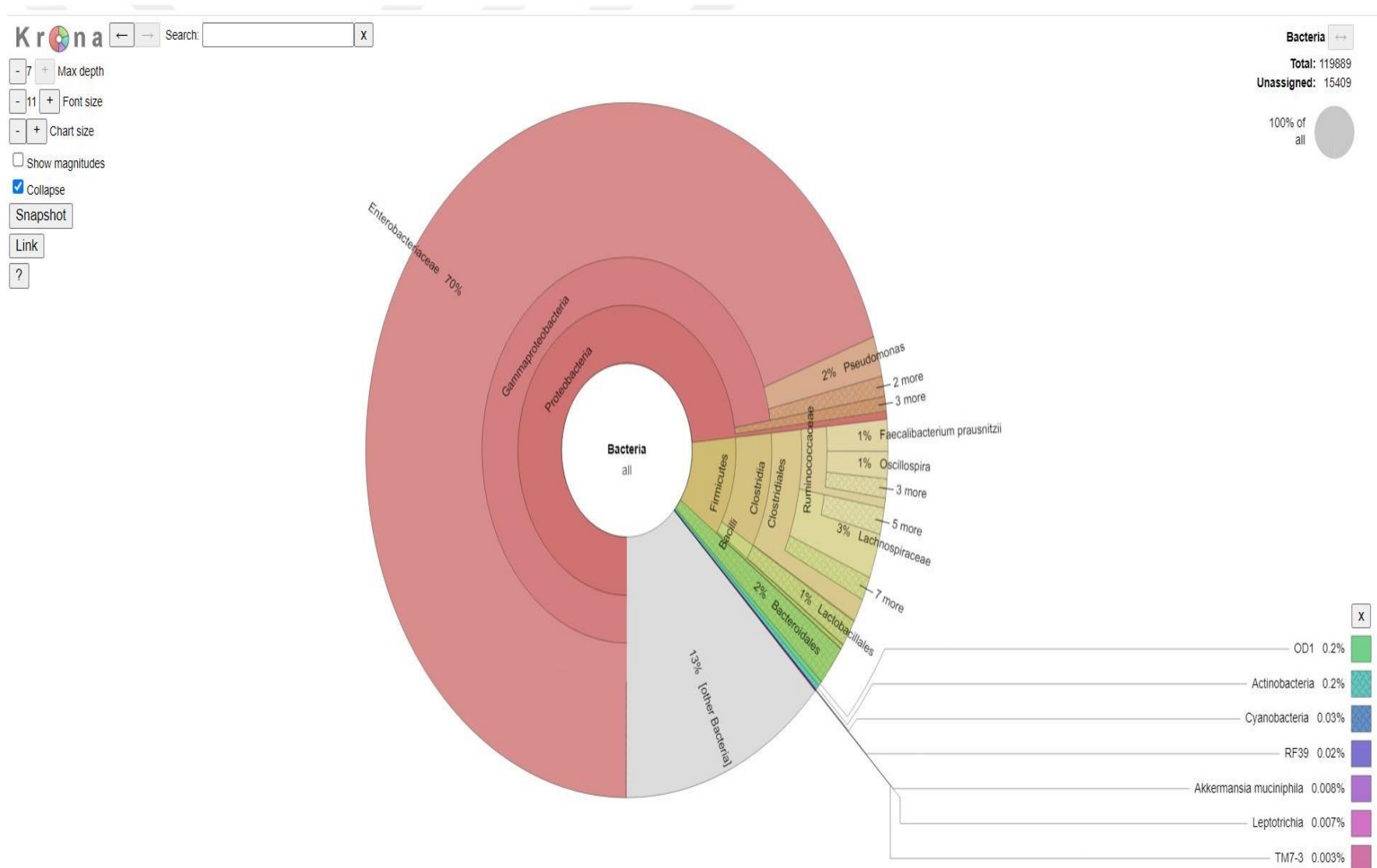


Figure 4.1 Example 1 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Excluded are species that represent less than 1% of the sequences. Supplementary tables contain more detailed information

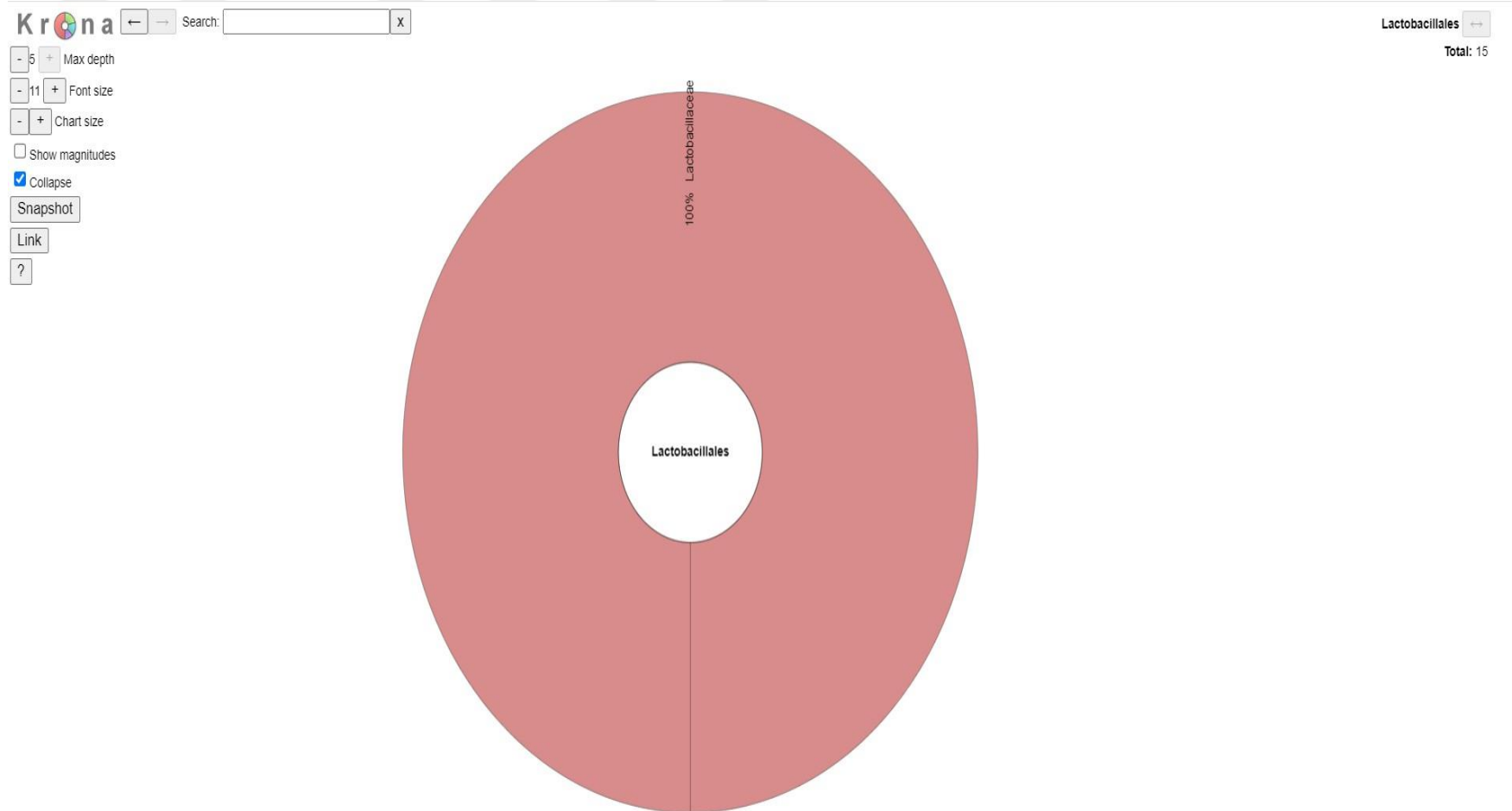


Figure 4.2 Example 2 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Supplementary tables contain more detailed information.

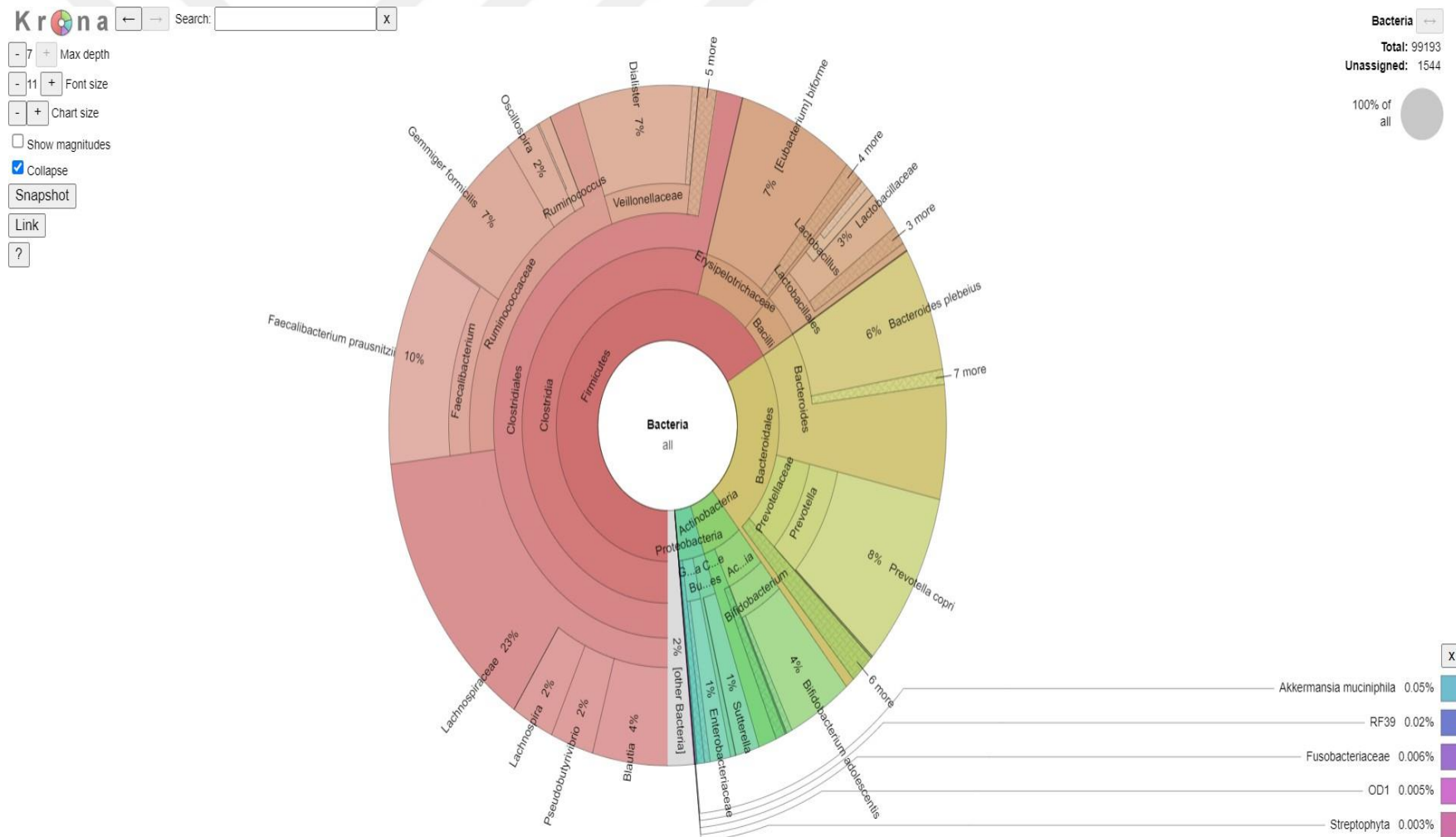


Figure 4.3 Example 3 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Excluded are species that represent less than 1% of the sequences. Supplementary tables contain more detailed information

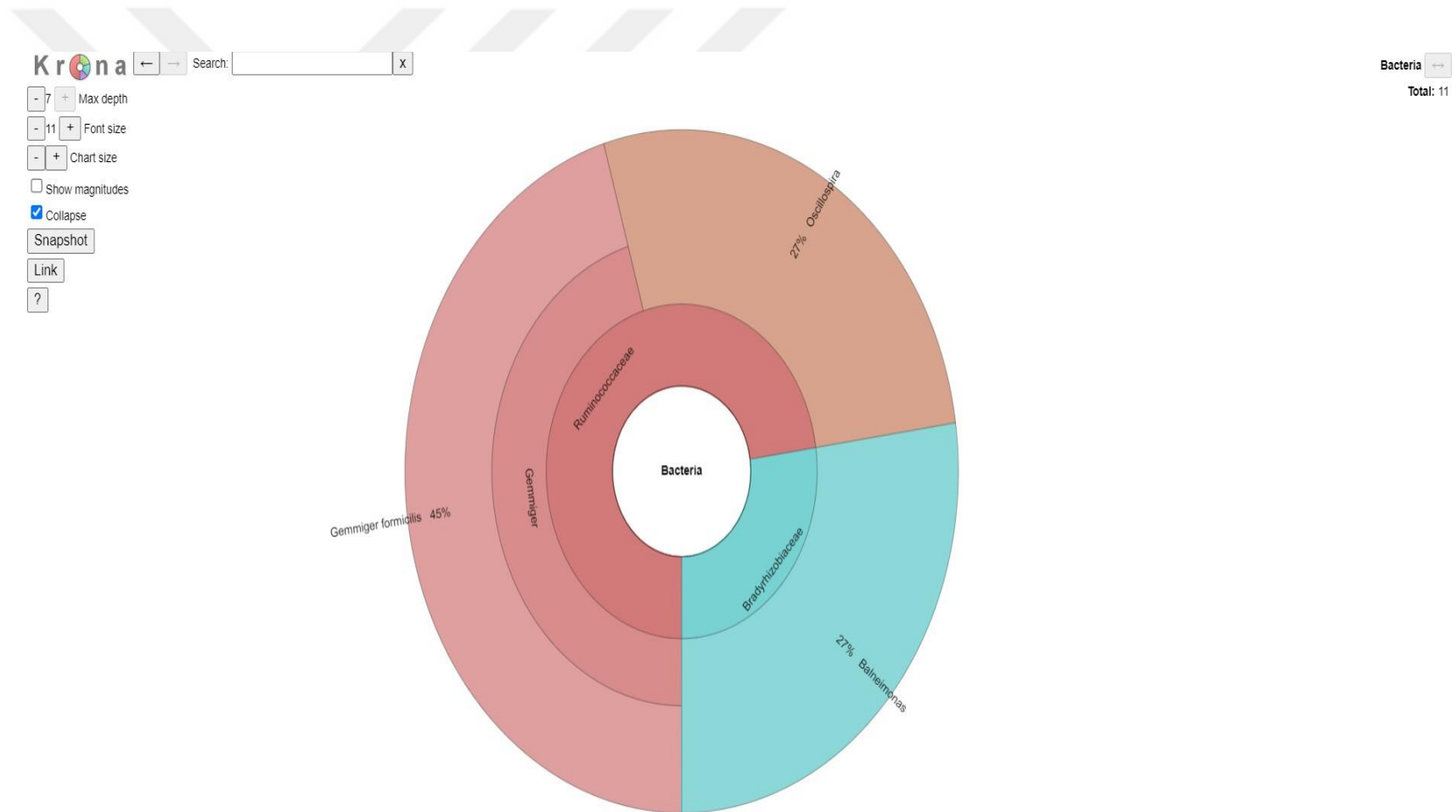


Figure 4.4 Example 4 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Excluded are species that represent less than 1% of the sequences. Supplementary tables contain more detailed information.

27

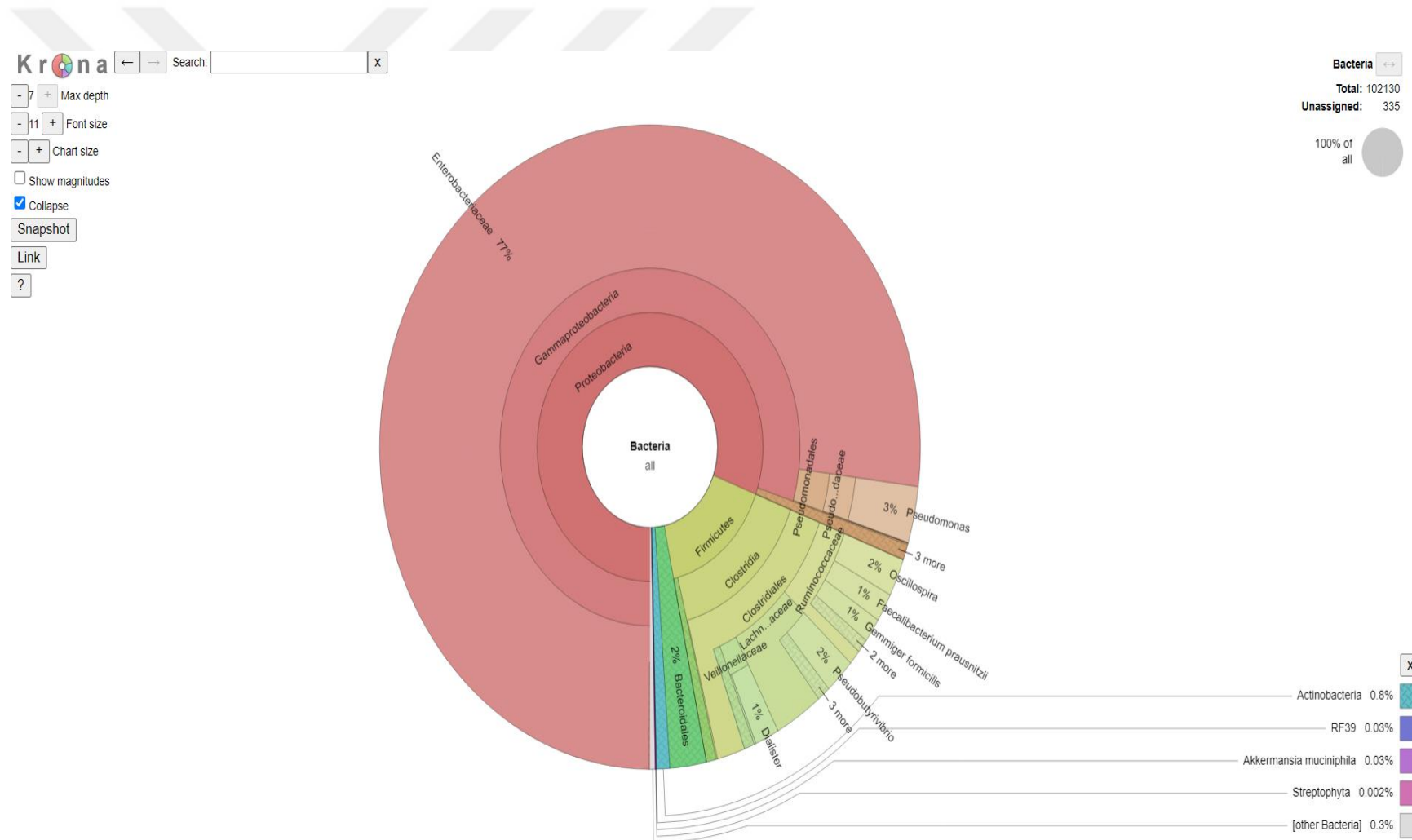


Figure 4.6 Example 6 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Excluded are species that represent less than 1% of the sequences. Supplementary tables contain more detailed information.

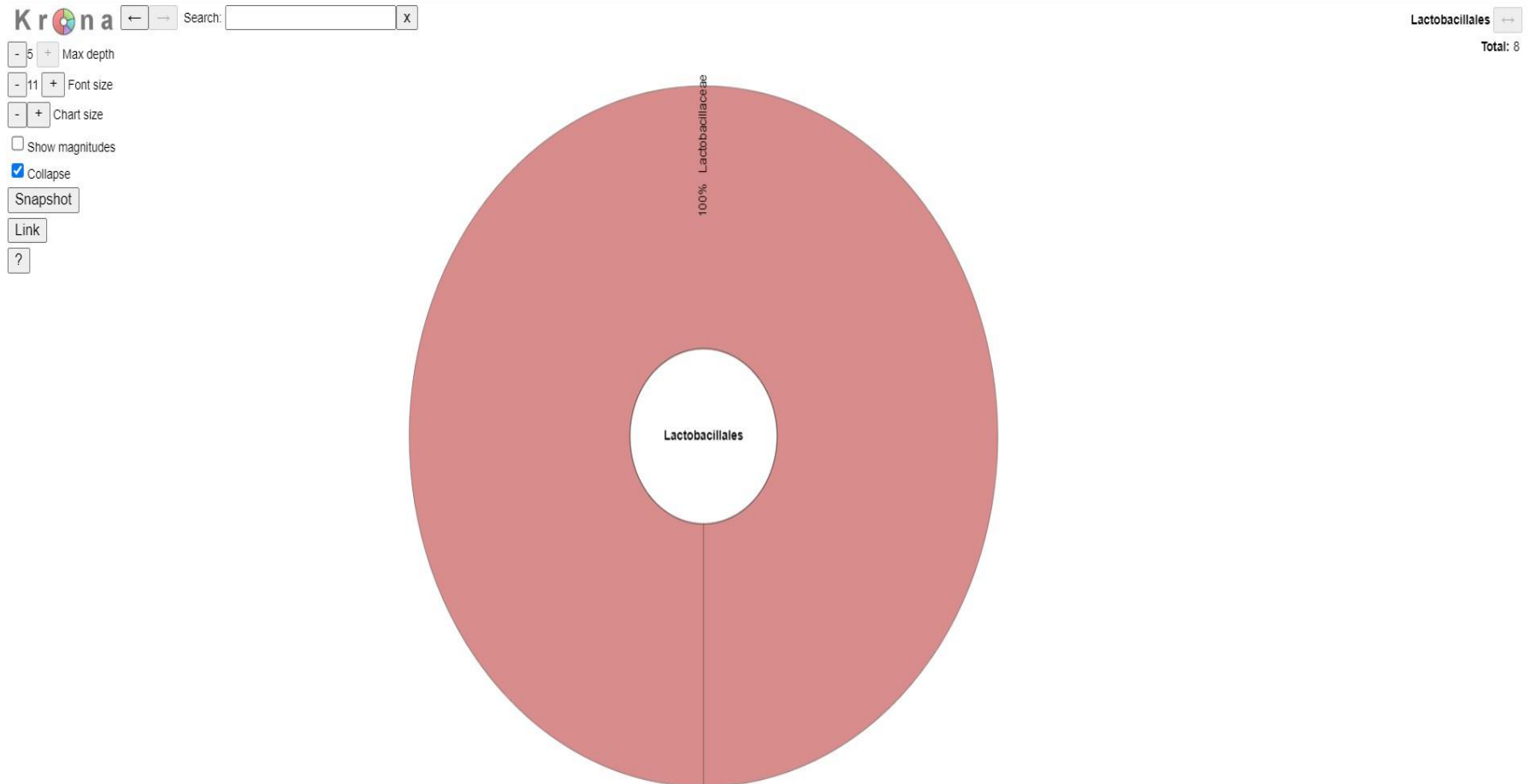


Figure 4.7 Example 7 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Supplementary tables contain more detailed information.

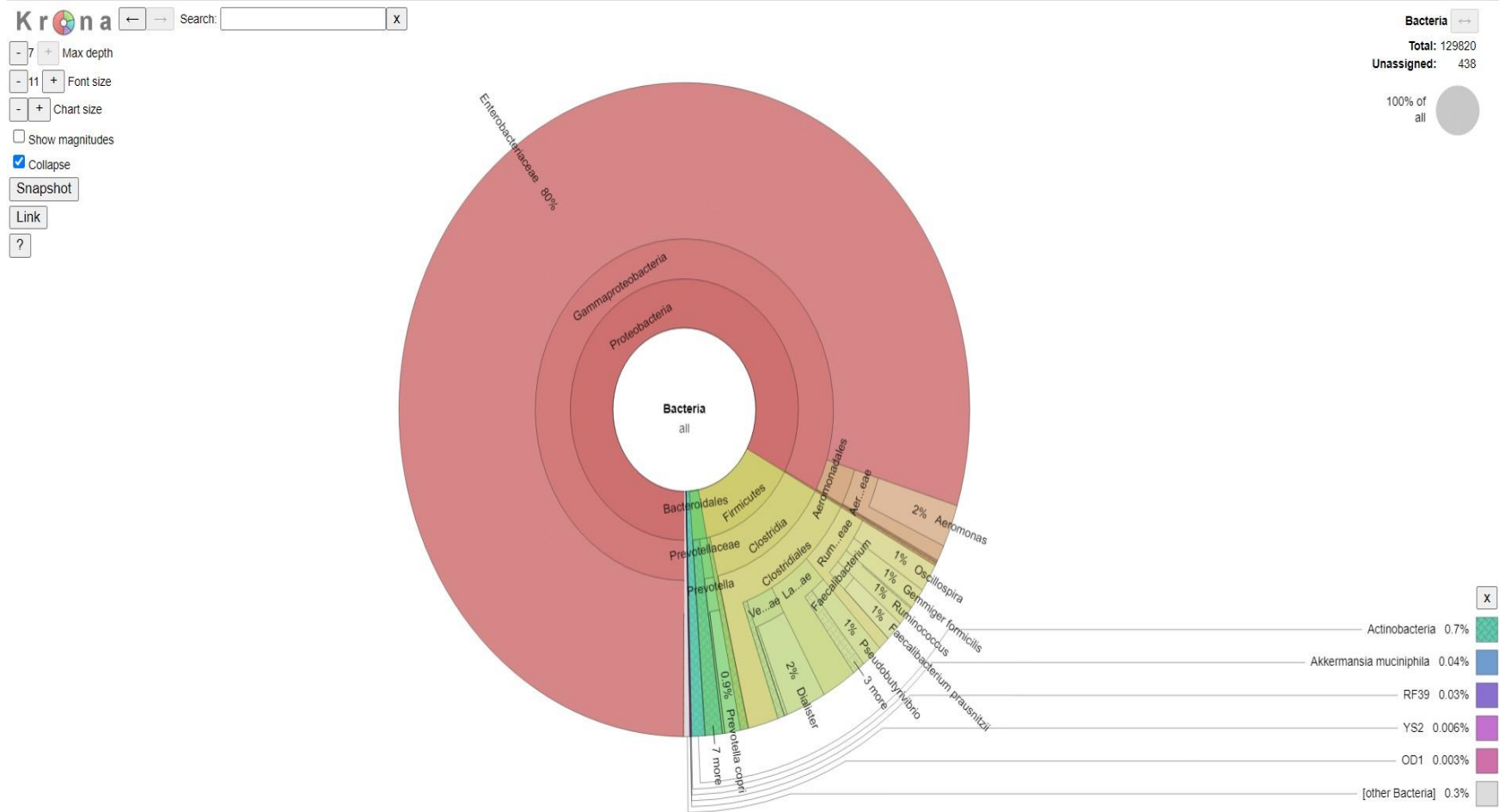


Figure 4.8 Example 8 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Excluded are species that represent less than 1% of the sequences. Supplementary tables contain more detailed information.

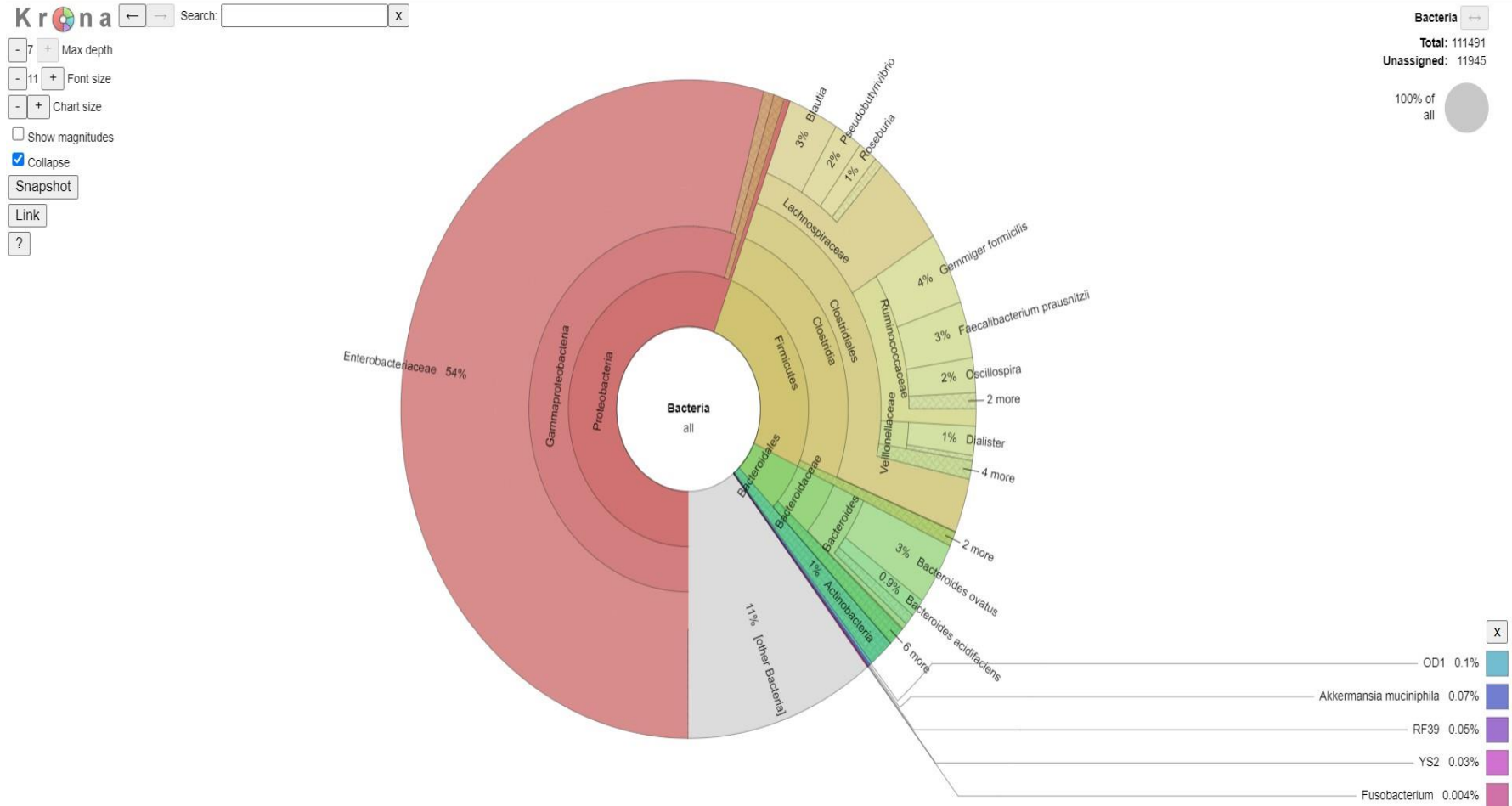
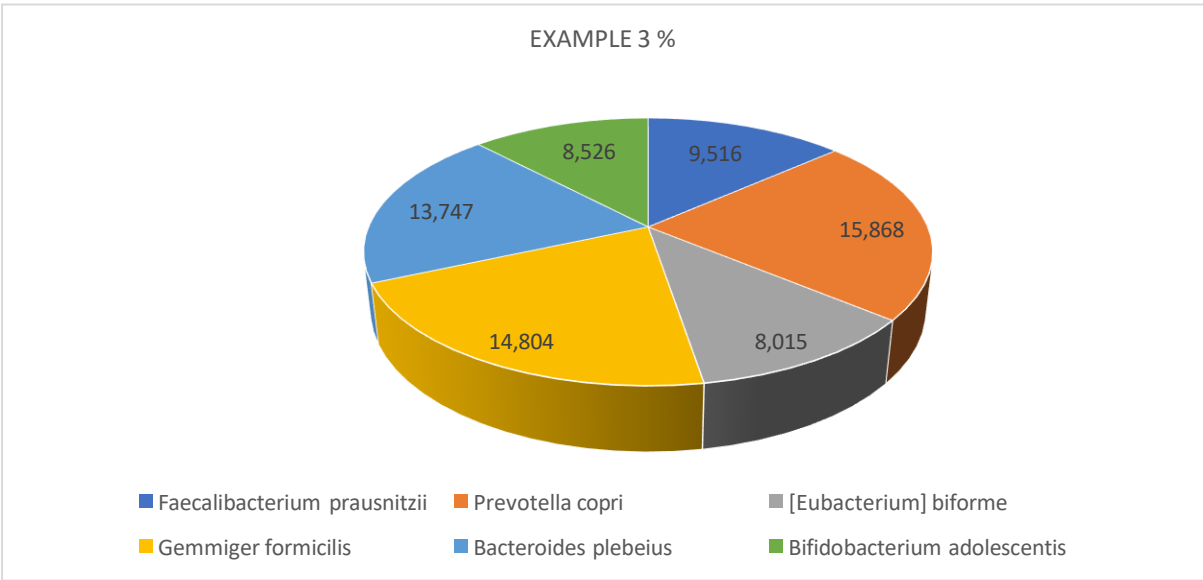
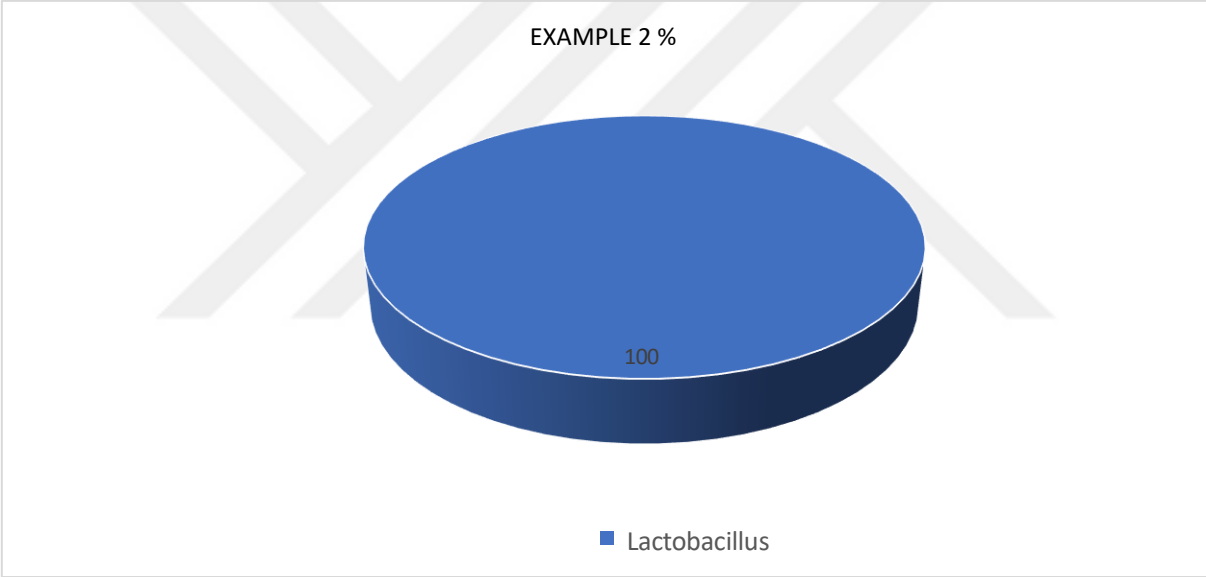
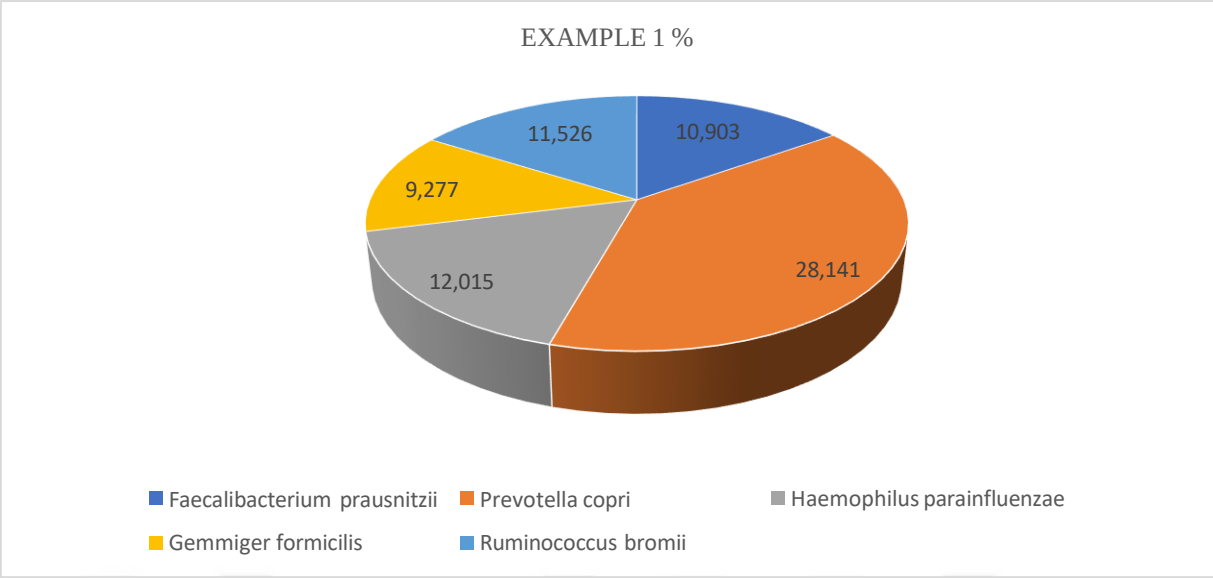


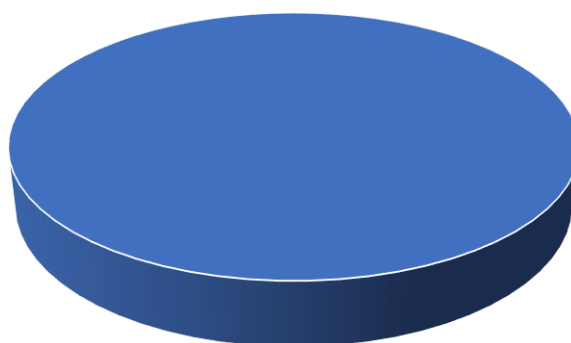
Figure 4.9 Example 9 bacterial map. The figure presents the relative abundance of the observed bacterial classes. Excluded are species that represent less than 1% of the sequences. Supplementary tables contain more detailed information.

Table 4.2 In the table, the prevalent species of the patients have been specified.

EXAMPLE 1		EXAMPLE 2	
Species	%	Species	%
<i>Faecalibacterium prausnitzii</i>	10.903	<i>Lactobacillus</i>	100
<i>Prevotella copri</i>	28.141		
<i>Haemophilus parainfluenzae</i>	12.015		
<i>Gemmiger formicilis</i>	9.277		
<i>Ruminococcus bromii</i>	11.526		
EXAMPLE 3		EXAMPLE 4	
Species	%	Species	%
<i>Faecalibacterium prausnitzii</i>	9.516	<i>Gemmiger formicilis</i>	100
<i>Prevotella copri</i>	15.868		
<i>[Eubacterium] biforme</i>	8.015		
<i>Gemmiger formicilis</i>	14.804		
<i>Bacteroides plebeius</i>	13.747		
<i>Bifidobacterium adolescentis</i>	8.526		
EXAMPLE 5		EXAMPLE 6	
Species	%	Species	%
<i>Faecalibacterium prausnitzii</i>	27.516	<i>Faecalibacterium prausnitzii</i>	8.516
<i>Prevotella copri</i>	3.868	<i>Collinsella aerofaciens</i>	4.045
<i>Gemmiger formicilis</i>	44.804	<i>Prevotella copri</i>	25.868
<i>Bifidobacterium adolescentis</i>	3.747		
EXAMPLE 7		EXAMPLE 8	
Species	%	Species	%
<i>Lactobacillus</i>	100	<i>Faecalibacterium prausnitzii</i>	20.536
		<i>Collinsella aerofaciens</i>	7.045
		<i>Prevotella copri</i>	22.466
		<i>Gemmiger formicilis</i>	26.104
EXAMPLE 9		EXAMPLE 10	
Species	%	Species	%
<i>Faecalibacterium prausnitzii</i>	20.136	<i>Faecalibacterium prausnitzii</i>	10.136
<i>Bacteroides ovatus</i>	19.125	<i>Bifidobacterium longum</i>	5.125
<i>Bacteroides acidifaciens</i>	6.466	<i>Haemophilus parainfluenzae</i>	24.466
<i>Gemmiger formicilis</i>	26.604	<i>Gemmiger formicilis</i>	11.604



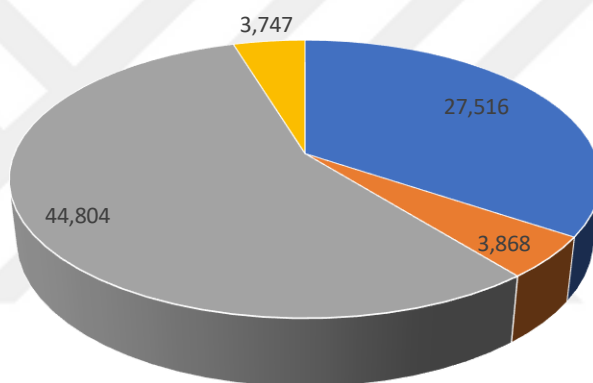
EXAMPLE 4 %



100

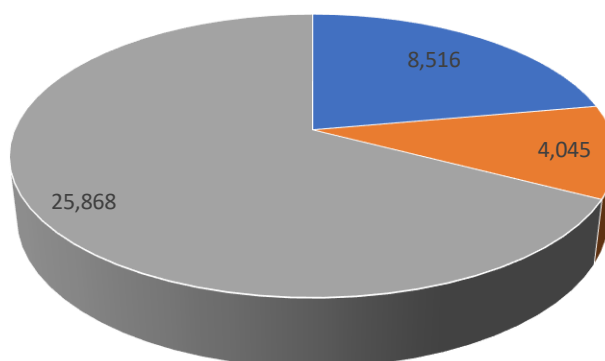
■ Gemmiger formicilis

EXAMPLE 5 %



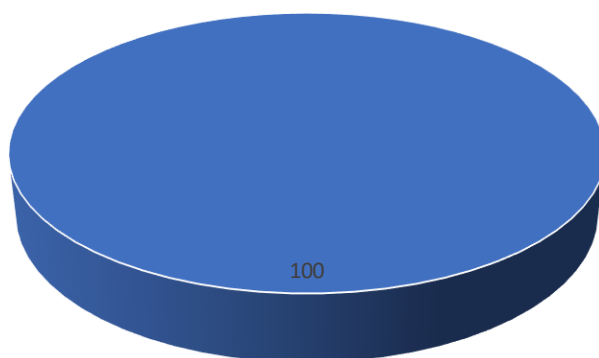
■ Faecalibacterium prausnitzii ■ Prevotella copri ■ Gemmiger formicilis ■ Bifidobacterium adolescentis

EXAMPLE 6 %



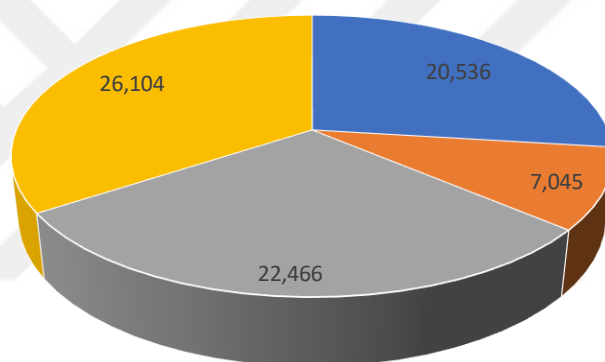
■ Faecalibacterium prausnitzii ■ Collinsella aerofaciens ■ Prevotella copri

EXAMPLE 7 %



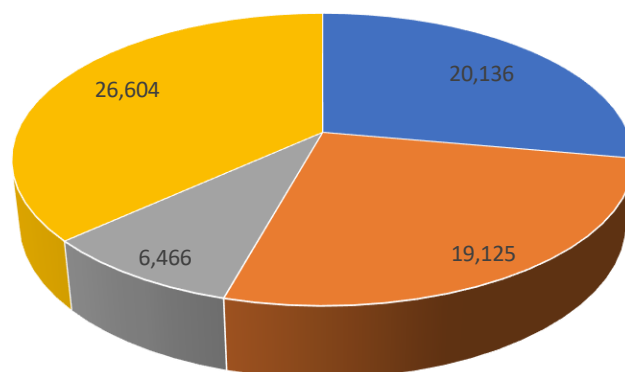
■ Lactobacillus

EXAMPLE 8 %

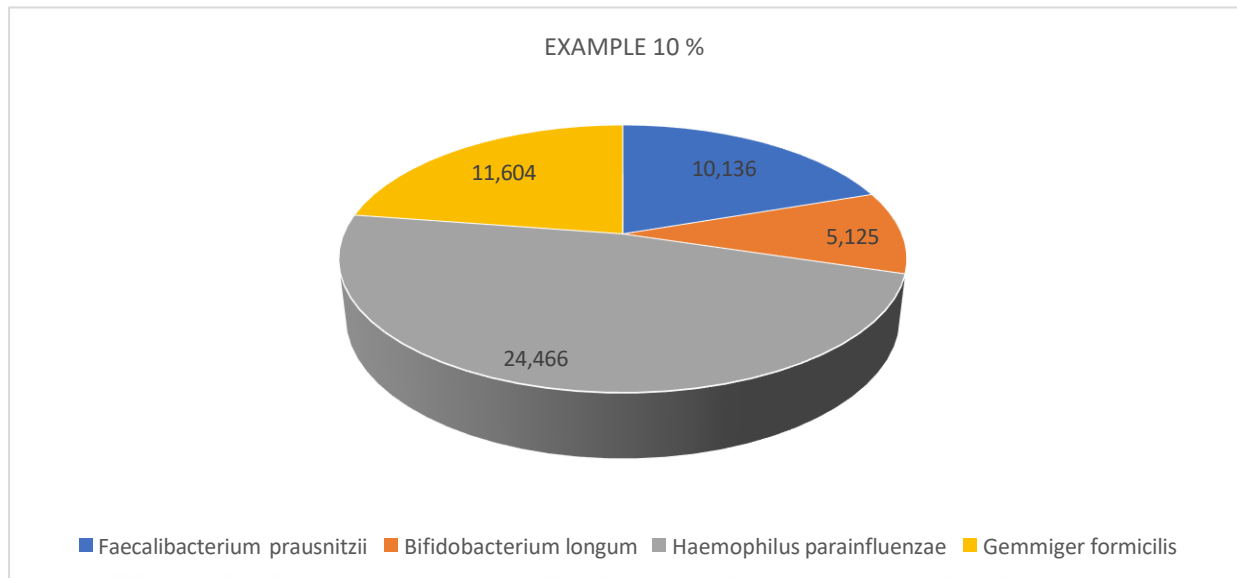


■ Faecalibacterium prausnitzii ■ Collinsella aerofaciens ■ Prevotella copri ■ Gemmiger formicilis

EXAMPLE 9 %



■ Faecalibacterium prausnitzii ■ Bacteroides ovatus ■ Bacteroides acidifaciens ■ Gemmiger formicilis



Based on 16S rRNA-based studies, it is clear that overall in the adult gut, two bacterial phyla, *Firmicutes* and *Bacteroidetes*, predominate, and other phyla, including *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, and *Fusobacteria*, are present in lower proportions (Eckburg et al. 2005).

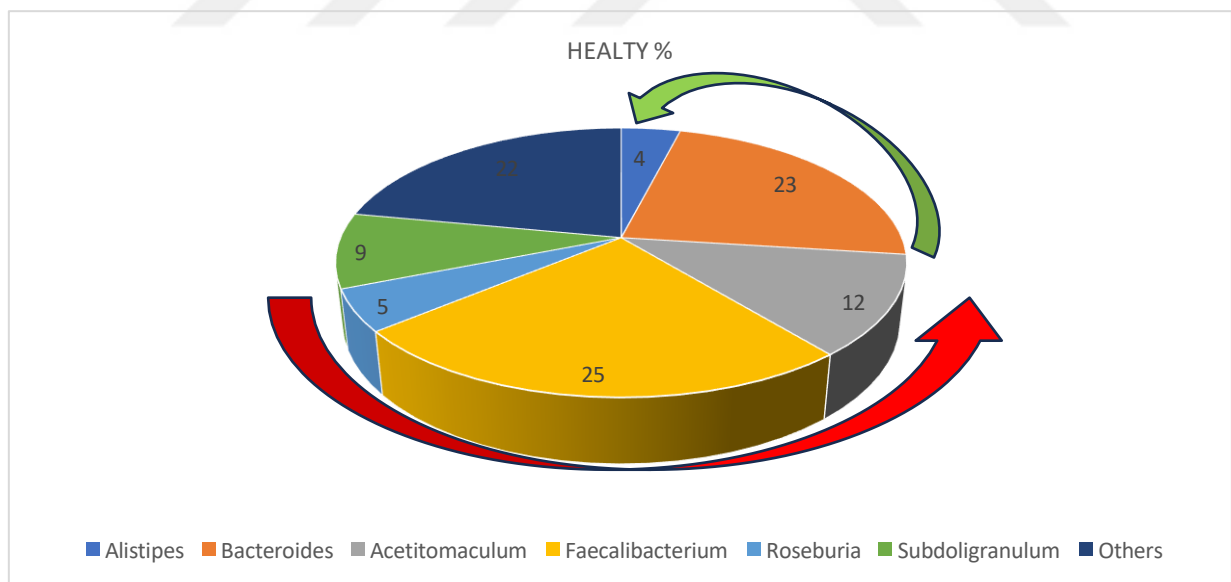


Figure 4.11 Healthy Gut Map. The colors indicate differential distribution of classes of bacteria, *Firmicutes* (red) and *Bacteroidetes* (green)(Hou et al. 2022).

When selecting patients, we have considered the following criteria:

- Is there smoking?
- What was the reason for his visit to the doctor?
- Is there any medication used regularly?
- Do you have a chronic disease?

Table 2.5 illustrates the dominant species in the patient's intestines. Table 2.4 provides information about the patients' conditions. Below is a detailed explanation of the patient's conditions.

Information about our first patient is as follows (Example 1): The patient sought medical help with complaints of fatigue and abdominal and stomach pain. The patient does not smoke. The patient does not take any medication regularly. There are no chronic diseases and the colonoscopy performed as part of the treatment process revealed the presence of intestinal leakage. The bacterial profile of our patient is as follows: 73% *Proteobacteria*, 11% *Firmicutes*, 2% *Bacteroidetes*, 2% *Pseudomonas*, and other bacteria make up the remaining 13%. Specific bacterial species include *Faecalibacterium prausnitzii* with 10.903%, *Prevotella copri* with 28.141%, *Haemophilus parainfluenzae* with 12.015%, *Gemmiger formicilis* with 9.277%, and *Ruminococcus bromii* with 11.526%. Species representing less than 1% of the sequences were eliminated.

Information for our second patient is as follows (Example 2): The patient sought medical attention for complaints of abdominal and stomach pain. The patient does not smoke. The patient is not regularly taking any medication. The patient has previously been treated for breast cancer and the treatment has been completed. The bacterial profile of our patient is as follows: %100 *Lactobacillales*.

Information on our third patient is as follows (Example 3): The patient sought medical help for abdominal and stomach pain. The patient does not smoke. The patient is not regularly taking any medication. Recently had gallbladder removed due to gallstones. The gallbladder was removed because of gallstones. The patient no longer has a gallbladder. The bacterial profile of our patient is as follows: 4% *Proteobacteria*, 11% *Firmicutes*, 6% *Antinobacteria*, 22% *Bacteriodales*, and %4 *Bacilli*. Specific bacterial species include *Faecalibacterium prausnitzii* with 9.516%, *Prevotella copri* with 15.868%, *[Eubacterium] bifforme* with 8.015%, *Gemmiger*

formicilis with 14.804%, *Bacteroides plebeius* with 13.747% and *Bifidobacterium adolescentis* with 8.526%. Species representing less than 1% of the sequences were eliminated.

Information on our fourth patient is as follows (Example 4): The patient sought medical help because of severe abdominal pain. The patient does not smoke and does not take medication regularly. Our patient's bacterial profile is as follows: %27 *Proteobacteria*, %73 *Firmicutes*. Among the specific bacterial species, *Gemmiger formicilis* accounts for %100, and at the genus level, %45.455 *Gemmiger*, %27.273 *Balneimonas* and %27.273 *Oscillospira* are present. Species representing less than 1% were excluded.

Information on our fifth patient is as follows (Example 5): The patient presented with abdominal pain and sought medical attention. Not report smoking or regular medication usage. The bacterial profile of the patient comprised 13% *Proteobacteria*, 75% *Firmicutes*, 5% *Actinobacteria*, and 6% *Bacteroidales*. The specific bacterial species identified comprise *Faecalibacterium prausnitzii* at 27.516%, *Prevotella copri* at 3.868%, *Gemmiger formicilis* at 44.804%, and *Bifidobacterium adolescentis* at 3.747%. Excluded from this analysis are species that account for less than 1% of the sequences.

Information on our sixth patient is as follows (Example 6): The patient sought medical attention due to abdominal pain. The patient had a history of smoking but quit voluntarily. The patient underwent angiectasia due to heart disease. They do not use any regular medication. Our patient's bacterial profile is as follows: 81% *Proteobacteria*, 16% *Firmicutes*, 2% *Pseudomonas*, and 2% *Bacteroidales*. Specific bacterial species include *Faecalibacterium prausnitzii* at 8.516%, *Prevotella copri* at 25.868%, and *Collinsella aerofaciens* at 4.045%. Species representing less than 1% of the sequences were excluded.

Information on our seventh patient is as follows (Example 7): The patient sought medical help for abdominal pain. The patient does not smoke and is on regular heart medication. Due to gastric inflammation, the patient is cautious about their diet. The bacterial profile of our patient is as follows: 100% *Lactobacillales*.

Information on our eighth patient is as follows (Example 8): The eighth patient presented with abdominal bloating that persisted for 7/8 months, prompting medical consultation. The patient has no history of smoking but takes prescribed medication for hypertension. They have no known chronic conditions. Our patient's bacterial profile is as follows: 83% *Proteobacteria*, 14% *Firmicutes*, 3% *Aeromonadales*, and 2% *Bacteroidales*. Specific bacterial species include

Faecalibacterium prausnitzii at 20.536%, *Collinsella aerofaciens* at %7.045 *Prevotella copri* at 22.466%, and *Gemmiger formicilis* at 26.104%. Species representing less than 1% of the sequences were excluded.

Information on our ninth patient is as follows (Example 9): The patient presented with abdominal pain and bloating and has a previous history of consulting with a dietitian, and receiving advice on maintaining a nutritious diet. They are a non-smoker and do not regularly take any medication or have any chronic illnesses. Our patient's bacterial profile is as follows: 56% *Proteobacteria*, 26% *Firmicutes*, 1% *Aeromonadales*, 6% *Bacteroidales*, and other bacteria 11%. Specific bacterial species include *Faecalibacterium prausnitzii* at 20.136%, *Bacteroides ovatus* %19.125, *Bacteroides acidifaciens* at 6.466%, and *Gemmiger formicilis* at 26.604%. Species representing less than 1% of the sequences were excluded.

Information on our tenth patient is as follows (Example 10): The patient sought medical attention due to abdominal pain and bloating. They have a history of breast cancer treatment, which has been completed, and they currently do not have any chronic illnesses. They consult a dietitian for regular dietary guidance. They do not take any medication regularly. Our patient's bacterial profile is as follows: 4% *Proteobacteria*, 66% *Firmicutes*, 6% *Antinobacteri*, 22% *Bacteroidales* and %2 other bacteria. Specific bacterial species include *Faecalibacterium prausnitzii* at 10.136%, *Bifidobacterium longum* %5.125, *Haemophilus parainfluenzae* at 24.466%, and *Gemmiger formicilis* at 11.604%. Species representing less than 1% of the sequences were excluded.

5. DISCUSSION

This research aims to identify how variations in the community of microbes living in the intestines of IBS patients (gut microbiota diversity) might be linked to the presence or severity of IBS symptoms. We took their specimens and analyzed samples from participants to investigate this potential connection. The percentages of species are given in Table 2.5. The most frequently observed species in the samples is *Faecalibacterium prausnitzii* (Exp: 1,3,5,6,8,9,10). The interpretation of the samples will be based on the patient's medical history and symptoms of irritable bowel syndrome.

5.1 *Faecalibacterium prausnitzii*

Faecalibacterium prausnitzii, a member of the *Ruminococcaceae* family in the phylum *Firmicutes*, is a highly prevalent bacterium in the human gut (Albenberg et al. 2014). Further research is required to understand the impact of this bacterium on the gut microbiome and human health. Studies have shown that *F. prausnitzii* is less abundant in various disease conditions, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), metabolic syndrome, diabetes, colorectal cancer, obesity, and frailty. (Cao, Shen, and Ran 2014). Functionally, *Faecalibacterium prausnitzii* contributes to immune system modulation and inflammation reduction through various mechanisms.

Functionally, *F. prausnitzii* contributes to modulating the immune system and attenuating inflammation through multiple mechanisms that can work independently or synergistically (Martín, Bermúdez-Humarán, and Langella 2018). Furthermore, *F. prausnitzii* regulates inflammatory signals via the release of immunosuppressive molecules like salicylic acid and anti-inflammatory signals such as microbial anti-inflammatory molecules (MAM) (Quévrain et al. 2016). In addition to evidence from associations observed in clinical studies, scientists support the idea of using *F. prausnitzii* as a probiotic (Martín et al. 2018). The abundance of *F. prausnitzii* in the human intestine can be affected by antibiotic use and dietary factors. Therefore, prebiotic approaches aimed at improving health by increasing the abundance of the commensal *F. prausnitzii* may be effective (David et al. 2014). The healthy eating index can be used to associate dietary health with the relative abundance of *F. prausnitzii* in the human intestines (Ruiz-Saavedra et al. 2020). The consumption of prebiotics and probiotics has been

demonstrated to increase *F. prausnitzii* in both healthy individuals and patients with irritable bowel syndrome (IBS) (Hustoft et al. 2017). Changes to the abundance of *Faecalibacterium prausnitzii* have been associated with various illnesses, such as metabolic syndrome and diabetes. (Rautio et al. 2003). A reduced quantity of *Faecalibacterium prausnitzii* has been observed in numerous health conditions, such as inflammatory bowel disease, chronic fatigue, *Clostridioides difficile*, and COVID-19 infections (Martinez et al. 2022). Moreover, it has been associated with cognitive scores and reduced in Alzheimer's type dementia (Lopez-Siles et al. 2017). *Faecalibacterium* levels in the stool of patients with IBS are lower than in those of healthy individuals. Decreasing the relative abundance of *Faecalibacterium prausnitzii* was observed among patients with IBS and chronic fatigue, which was strongly correlated with higher levels of fatigue and pain (Guo et al. 2023).

Patient 1, fatigue and intestinal leakage have additionally been observed alongside the symptoms of IBS. Guo et al. have reported the *Faecalibacterium* abundance in the stools of IBS patients to be lower than the healthy range (Guo et al. 2023). Moreover, individuals afflicted with IBS and chronic fatigue have exhibited a decrease in the abundance of *Faecalibacterium prausnitzii*, and this decrease has been linked to higher levels of fatigue and pain. Bermúdez-Humarán and colleagues suggest that *F. prausnitzii* contributes to the modulation of the immune system and attenuation of inflammation through several mechanisms that may act independently or synergistically (Miquel et al. 2013). Specifically, the butyrate created by *F. prausnitzii* and other butyrate-producing bacteria serves to lessen intestinal mucosal inflammation by preventing NF- κ B activation, increasing expression of peroxisome proliferator-activated receptor- γ , and inhibiting interferon- γ expression. This scenario might elucidate our patient's condition of leaky gut. A reduction in the prevalence of *Faecalibacterium prausnitzii* has the potential to amplify intestinal permeability.

Patient 3, sought medical help due to abdominal discomfort and pain. The patient does not smoke. No regular medications are reported. The gallbladder has been removed due to gallstones. Gallstones form when the chemical composition of bile in the gallbladder becomes imbalanced. Bile, a fluid produced by the liver to aid digestion, is essential (Sticova and Jirsa 2013). Bilirubin is a substance that forms when red blood cells break down in the body (Tenhunen, Marver, and Schmid 1968). Liver cirrhosis, biliary tract infections, and certain blood disorders can cause the liver to produce excess bilirubin, which can contribute to the formation of gallstones (Schmid 1957). The gut-liver axis plays a key role in maintaining the homeostasis of molecules such as bilirubin, cholesterol, and bile acids. This interface between

the gut, gut microorganisms, and liver serves as one of the primary pathways for nutrient absorption and waste excretion in the body. Bilirubin metabolism is a crucial aspect of heme metabolism and the interplay between the gut and liver. Therefore, it is essential to understand the microbes and enzymes involved (Kang et al. 2008).

Bilirubin is present in both long and short forms across multiple phyla, but the short form appears to be more specific to *Firmicutes*. *Firmicutes* are responsible for a significant percentage of bilirubin degradation in the human intestine, including common bacterial species such as *Roseburia intestinalis*, *Roseburia inulinivorans*, and *Faecalibacterium prausnitzii*. The increased occurrence of calcium bilirubin gallstones in patients with IBS/IBD is thought to be caused by a combination of factors, including a decrease in the prevalence of bilirubin-reducing microorganisms, an excess of unconjugated primary bile acids, and unconjugated bilirubin. Studies have reported a reduction in the prevalence of bilirubin reductase in these patients, which may contribute to the formation of gallstones. (Lapidus, Åkerlund, and Einarsson 2006). The reduced abundance of *Faecalibacterium prausnitzii* in *patient 3* may have contributed to the development of bile problems. Furthermore, the absence of the gallbladder could have led to a decline in bile-inducing bacteria. Besides, the scarcity of *Faecalibacterium prausnitzii* may be a potential cause of IBS symptoms.

The proportion of *Faecalibacterium prausnitzii* in **Patient 5** is significant in comparison to the other patients. The sample for *patient 5* exhibited *Faecalibacterium prausnitzii* at a rate of 27%, in comparison to other patients. According to David et al, numerous factors can impact the relative abundance of *F. prausnitzii*, including the use of antibiotics and a healthy diet. Specifically, the prevalence of *F. prausnitzii* can be amplified by taking certain prebiotic/probiotic ingredients. A potential strategy for improving health is the use of prebiotics/probiotics, which aims to increase the levels of the commensal *F. prausnitzii* (David et al. 2014). To assess the correlation between dietary health and the relative abundance of *F. prausnitzii* in the human gut, the healthy eating index can be utilized (Ruiz-Saavedra et al. 2020). The reason for the relative abundance in our *patient 5* may be related to a healthy diet to reduce the symptoms of IBS. In addition, the lack of long-term medication may have led to a significant increase in *F. prausnitzii*.

Patient 6 presented to the doctor with abdominal pain. The patient had a past smoking habit but had quit voluntarily. Smoking is a widely researched environmental factor linked to numerous intestinal diseases. Furthermore, the intestinal microbiota has a key role in the pathogenesis of

irritable bowel syndrome (IBS). J. Z. H. von Martels et al. aimed to investigate the impact of *Faecalibacterium prausnitzii*, a potentially advantageous bacterium, on the intestinal epithelium. To do so, they evaluated the growth of *F. prausnitzii* in a newly established co-culture system for anaerobic bacteria together with human intestinal epithelial cells requiring oxygen. They subsequently examined the effects of cigarette smoke extract (CSE) on the growth of *F. prausnitzii*. *F. prausnitzii* was found to decrease in abundance when associated with smoking (von Martels et al. 2016). Additionally, our patient underwent angioectasia due to heart disease. The composition of gut microbiota is considered a factor in host metabolism and the development of cardiovascular diseases (CVD) and intestinal immunity. Ufnal et al, have shown that the diversity and composition of intestinal microbiota differ among cardiac patients. The abundance of the *Bacteroidetes* phylum is lower in cardiac patients while the *Firmicutes* and *Proteobacteria* phyla are higher. Patients exhibited a notable decrease in the population of both beneficial and commensal bacteria such as *Faecalibacterium prausnitzii* and *Bacteroides fragilis*. By contrast, there was a marked increase in the presence of opportunistic pathogens (Ufnal, Zadlo, and Ostaszewski 2015). *Patient 6* has a history of smoking, which may have led to a decrease in *Faecalibacterium prausnitzii*. Also, having a heart condition, as mentioned above, causes a decrease in *Faecalibacterium prausnitzii*. Such a decrease in our patient's beneficial bacteria causes an increase in IBS symptoms.

Patient 8, presented with abdominal bloating that persisted for 7/8 months and required medical consultation. The patient has no history of smoking but uses prescription medication for hypertension. The pathophysiology of functional abdominal bloating and distension (FABD) remains unclear according to Choong-Kyun Noh et al (2020), there is a significant relationship between the diversity of fecal microbiota and small intestinal bacterial overgrowth (SIBO) and dysbiosis in FABD patients and healthy individuals. (Noh and Lee 2020). Abdominal distension is a common symptom that causes a feeling of pressure in the abdomen, regardless of whether there is actual distension present. It is important to note that this is a subjective symptom and should not be confused with objective distension (Houghton et al. 2006). Functional gastrointestinal disorders such as irritable bowel syndrome (IBS), functional dyspepsia, and functional constipation are often associated with this condition (Kwan et al. 2003). The composition of the fecal microbiota in the FABD group differed significantly from that of the control group. Microbial diversity was notably lower in the FABD group compared to the control group. Furthermore, FABD patients showed a considerably higher proportion of *Proteobacteria* and a significantly lower proportion of *Actinobacteria*, as opposed to healthy

controls. Compared to healthy controls, a significantly higher proportion of *Faecalibacterium* was found in FABD patients, while SIBO patients with FABD showed significantly higher proportions of *Prevotella* and *Faecalibacterium*. Additionally, patients with FABD had significantly more *Faecalibacterium prausnitzii*, but significantly less *Bacteroides uniformis* and *Bifidobacterium adolescentis* than healthy controls. A higher prevalence of *Prevotella copri* and *F. prausnitzii*, and a lower prevalence of *B. uniformis* and *B. adolescentis*, were observed in SIBO patients as compared to healthy controls. *Patient 8's* presentation of abdominal bloating may be due to significantly increased *Faecalibacterium*. In IBS, *Faecalibacterium prausnitzii* is not only significantly reduced but also increased, causing IBS symptoms. The bacterial balance must be maintained.

The patient has no history of smoking but uses prescription medication for hypertension. Yan et al. (2017) showed that increased MLGs of *Klebsiella*, *Streptococcus*, and *Parabacteroides merdae* in patients with hypertension, consistent with genus-level. Patients in the control group had elevated metagenomic linkage groups (MLGs) of *Roseburia* and *Faecalibacterium prausnitzii* (Yan et al. 2017). Additionally, patients in the control group had elevated MLGs of *Roseburia* and *Faecalibacterium prausnitzii*. The variation of *Faecalibacterium prausnitzii*, as supported by previous research, plays an important role in the identification of irritable bowel syndrome (IBS) symptoms. *Patient 8's* hypertension increases *Faecalibacterium prausnitzii*. Disruption of the intestinal balance creates IBS symptoms.

Patient 9 reported symptoms of abdominal pain and bloating and had previously sought advice from a registered dietitian regarding the importance of maintaining a healthy diet. The individual's dietary habits can provide insight into their understanding of bowel contents. *Faecalibacterium prausnitzii* is a bacterial species present in the human gut that possesses anti-inflammatory properties and is negatively associated with chronic inflammatory conditions. Although *F. prausnitzii* may play a crucial role in the benefits of healthy eating habits, there is limited knowledge available on the nutrients that promote its growth, apart from simple sugars and fibers. Shaillay Kumar Dogra and colleagues (2022) analyzed diet and microbiome data from the American Gut Project (AGP) to identify nutrients that may correlate with the relative abundance of *F. Prausnitzii* (Dogra et al. 2022). *F. prausnitzii* is a prevalent anaerobic bacterium in the human gastrointestinal tract. The study implemented a machine learning algorithm for the microbiome composition and a food frequency survey dataset gathered from 3816 American Gut Project (AGP) members. The study identified nutrients that could affect *F. prausnitzii's* abundance. Inositol, sorbitol, and lycopene were identified as potentially

increasing the bacterial strain's growth based on experiments conducted on the *F. prausnitzii* strain. Conversely, it was noted that the in vitro growth of *F. prausnitzii* remained unaffected by xylitol, erythritol, and betaine (Lopez-Siles et al. 2012). Xylitol, erythritol, and betaine are many sugar alcohols found in fruits; Inositol or myoinositol is commonly found in vegetables and meat (Jiang et al. 2020). When the bacterial ratios in Table 2.5 for *patient 9* were analyzed, the amount of *F. prausnitzii* showed a positive correlation. Positive results were obtained in terms of the presence of *F. prausnitzii* and the nutritional status of our patient. It was discovered that a change in diet gave more favorable results than other patients. The fact that our patient paid attention to what she ate and had a diet plan from a dietician explains her healthy diet. Considering the abundance of *F. prausnitzii*, we can interpret that the patient was fed with fibrous vegetables and healthy proteins during the diet process.

Patient 10 consulted a doctor due to abdominal pain and bloating. They have a history of completed breast cancer treatment and do not have any chronic diseases. Breast cancer is a type of cancer that starts in the cells of the breast. It is a serious health concern for women. Detecting and diagnosing the disease early is important for effective treatment and better outcomes (Greenlee et al. 2017). The diagnosis and differential diagnosis of breast cancer should be based on the patient's medical history, clinical manifestations, and results of physical, imaging, histopathological, and cytopathological examinations. The treatment of breast cancer has evolved with a better understanding of its biological behavior and the development of new treatment concepts. Comprehensive treatment, including both local and systemic approaches, is now the standard of care (Greenlee et al. 2017). The 16S rDNA sequence is a valuable tool for identifying biological species and is widely regarded as the best indicator for bacterial phylogeny and classification (Goto et al. 2002). To facilitate the identification of additional biomarkers and therapeutic targets for breast cancer, Ma et al. (2020) employed 16S rDNA sequencing and LC-MS metabolomics to assess the mutual influence between gut bacteria and blood metabolites in breast cancer patients, thus laying the groundwork for future research. The authors also presented noteworthy findings on the inhibitory properties of *Faecalibacterium prausnitzii* against the proliferation of breast cancer cells via the IL-6/STAT3 signaling pathway (Ma et al. 2020). Although the mechanism of *Faecalibacterium prausnitzii* in breast cancer has not been investigated before, this is the first report of growth inhibition of breast cancer cells by *Faecalibacterium prausnitzii*. The data show a reduced abundance of *Faecalibacterium* compared to the group of women without breast cancer (Ma et al. 2020). It is observed that the abundance of *Faecalibacterium* has decreased despite the completion of *patient 10* breast cancer

treatments by the patient. This decrease is responsible for triggering IBS symptoms. The treatment process can lead to a reduction in beneficial gut bacteria. In this case, it can be stated that the levels of beneficial bacteria in patients who have undergone cancer treatment have decreased. Furthermore, this imbalance of bacteria can trigger symptoms of IBS.

5.2 *Prevotella copri*

The percentages of species are given in Table 2.5. The second most frequently observed species in the samples is *Prevotella copri*. (Example: 1,3,5,6,8). Interpretation of samples will be based on the patient's medical history and irritable bowel syndrome symptoms.

Prevotella copri represents the primary constituent of the *Prevotella* genus found in the human gastrointestinal tract. Research findings indicate a positive correlation between the prevalence of *Prevotella* and certain inflammatory conditions, thus proposing a possible pathobiont character (Hayashi et al. 2007).

Prevotella copri is the most prevalent member of the genus *Prevotella* present in the human intestinal tract. Research indicates that *Prevotella* is connected to high-fiber diets, particularly non-Western diets (Tett et al. 2021). An increase in the prevalence of *Prevotella* is correlated with improved glucose metabolism, indicating a potential advantage to human health (Kovatcheva-Datchary et al. 2015). However, an increase in *Prevotella* abundance is associated with inflammatory conditions such as periodontitis, bacterial vaginosis, rheumatoid arthritis, ankylosing spondylitis, metabolic disorders, and low-grade systemic inflammation. This suggests that certain strains have pathobiotic characteristics (Larsen 2017). The pro-inflammatory impact of *Prevotella* has been confirmed through the activation of Toll-like receptor 4 (TLR-4) via lipopolysaccharide (LPS) production and a reduction in colonic interleukin-18 expression (IL-18) (Iljazovic et al. 2021). Furthermore, the rise in *Prevotella* has been demonstrated to increase intestinal permeability by producing mucin-degrading enzymes (Wright, Rosendale, and Robertson 2000).

The pathogenesis of irritable bowel syndrome (IBS) has been suggested to be linked to changes in the intestinal microbiota (Su et al. 2018). In recent research on Irritable Bowel Syndrome (IBS), scientists have identified three distinct enterotypes featuring dominant genera (*Bacteroides*, *Prevotella*, and *Ruminococcus*) (Arumugam et al. 2011). Julien et al. (2017) found that individuals with irritable bowel syndrome (IBS) have a prevalence of the

Bacteroides-Prevotella dominant enterotype. This was part of their investigation into the alteration of intestinal microbiota and its potential correlation with visceral hypersensitivity in IBS. To further investigate this correlation, they developed a PI-IBS model that shares similarities with IBS (Tap et al. 2017). This PI-IBS model demonstrates long-lasting impediments in intestinal motility and visceral hypersensitivity (Bercík et al. 2004). It should be noted that PI-IBS mice exhibited an elevated level of *Prevotella*. As a result, these mice experienced a decrease in both *Prevotella* levels and visceral hypersensitivity. When mice are co-housed from different sets, their gut microbiota is transferred as they consume each other's excrement. (Sivan et al. 2015). Therefore, we united PI-IBS mice with control mice. The persistent elevation of *Prevotella* levels in IBS patients and the corresponding increase observed in PI-IBS mice indicate a potential positive correlation between the *Prevotella*-enriched enterotype and the risk of IBS (Man et al. 2015).

Patient 1, presented to a medical facility with complaints of fatigue and abdominal pain. The presence of intestinal leakage was discovered. *Prevotella copri* possesses various enzymes and gene clusters necessary for fermenting and utilizing complex polysaccharides (Dodd, Mackie, and Cann 2011). Carbohydrate fermentation produces short-chain fatty acids (SCFAs), which are essential by-products and can cause dose-dependent visceral hypersensitivity (Bourdu et al. 2005). A study revealed that the enterotype dominated by *Prevotella* produced higher levels of SCFA compared to the enterotype dominated by *Bacteroides*. Furthermore, the fermentation of carbohydrates raises luminal H₂ and CH₄ production, resulting in luminal distension and pain among individuals with visceral hypersensitivity. Thus, *Prevotella* could potentially aggravate IBS symptoms by interacting with other microbiome members and promoting carbohydrate fermentation. In addition, Wright et al. reported that *Prevotella* possesses enzymes that play a crucial role in mucin degradation, which could cause increased intestinal permeability (Staudacher et al. 2014). The substantial quantity of *Prevotella copri* in our patient-initiated and exacerbated symptoms of Irritable Bowel Syndrome (IBS); in addition, it extends the duration of IBS complaints by inducing intestinal permeability.

Patient 3, sought medical help for abdominal and stomach pain. The gallbladder was removed because of gallstones. The formation of gallstones is closely linked to both the bile ducts and intestinal flora. The research aims to demonstrate the diversity and amount of intestinal flora present in patients who suffer from gallstones. In addition, it intends to analyze the correlation linking the formation structure of gallstones and the flora, while also carrying out initial investigations into metabolic pathways with gene function annotations (Xie et al. 2022). Min

Xie and colleagues found a noteworthy rise in *Prevotella copri* in the guts of gallstone patients, suggesting that it could be used as a potential biomarker for bacterial dysregulation in such patients. Furthermore, *Prevotella copri* demonstrated a linear discriminant analysis (LDA) value >4 in the stool of gallstone patients, indicating that it may serve as a useful biomarker for bacterial dysregulation in this patient population. In this context, the analysis of 1791 patients revealed that *Prevotella copri* is strongly linked to the formation of gallstones (Di Ciaula and Portincasa 2018). *Patient 3* presented with complaints of gallstones. The presence of gallstones may have disrupted the balance of intestinal flora and triggered symptoms of IBS. Although gallstone removal was expected to have a positive effect on bacterial diversity, when combined with IBS symptoms, it resulted in negative diversity.

Patient 5 complained of abdominal pain and sought medical help. Do not report smoking or regular drug use. The value of *P. copri* in our patient is comparatively low when contrasted with that of other patients. We can attribute this to its impact on nutrition. Differences in the values of *P. copri* may arise due to the lack of continuous medication usage. The Western diet is a diet high in fat, high in sugar, and low in fiber (Rakhra et al. 2020). The diet described is characterized by a high proportion of processed food. A reduction in the abundance of *P. copri* was observed when the amount of advanced glycation end products, commonly found in Western diets, was significantly reduced. It is worth noting that a high relative abundance of *P. copri* was associated with diets that were high in protein and Western in nature. (Yacoub et al. 2017). The metabolic mechanism of *P. copri* allows it to easily adapt to the available intestinal nutrients. Some strains can break down carbohydrates and fiber, while others can biosynthesize branched-chain amino acids (BCAAs) from meat-based diets (Attia et al. 2023). Accordingly, the lower *P. copri* rate in *patient 5* compared to other patients may be related to eating habits.

Patient 6, sought medical attention due to abdominal pain. The patient had a history of smoking but quit voluntarily. The patient underwent angiectasia due to heart disease. There is evidence suggesting variations in the intestinal microbiota of patients with coronary heart disease (Karlsson et al. 2012). Additionally, studies show that the gut microbiota is associated with obesity, diabetes, dyslipidemia, and hypertension, which have been identified as risk factors for coronary heart disease. The gut microbiota, through its metabolites, regulates important metabolic processes such as oxidative stress and inflammatory reactions that can result in the progression of coronary heart disease (Emoto et al. 2017). Current areas of active research include altering the composition of the gut microbiota, introducing probiotics, and fecal transfer with the ultimate aim of preventing and treating coronary heart disease (Emoto et al. 2016).

Zuheng Liu and colleagues identified higher levels of *Enterococcus*, *Megamonas*, *Collinsella aerofaciens*, and *Fusobacterium* in individuals with coronary artery disease (CAD) compared to control individuals. Several of these bacteria have been linked to human diseases (Jie et al. 2017). Moreover, *Ruminococcaceae* and *Clostridiaceae*, which are considered beneficial, were less abundant in CAD patients. Furthermore, cardiac valve calcification (VC) patients had higher levels of opportunistic pathogens, including *Prevotella copri* and *Fusobacterium* (Cui et al. 2017). Interestingly, our findings suggest a positive correlation between *Prevotella copri* and low-density lipoprotein (LDL), which supports its potential role in pro-inflammatory processes. Therefore, *Prevotella copri* may potentially play a critical role in the development of cardiovascular disease (CVD) due to its involvement in both immunity and inflammation. It is important to consider this pathogen as a possible contributing factor (Gózd-Barszczewska et al. 2017).

The gastrointestinal microbiome is a complex ecosystem that forms a mutually beneficial relationship with the host. It remains relatively stable in terms of health but is affected by factors such as age, medication use, diet, alcohol consumption, and smoking. More research is needed to determine the exact cause-effect relationship between smoking and changes in the gastrointestinal microbiome. Recent research suggests that there may be a link between changes in microbiota composition and metabolic disorders such as obesity, diabetes, and metabolic syndrome (Tilg and Kaser 2011). Both *Prevotella* and *Bacteroides* have been shown to have wide-ranging effects on both health and disease, and several *Bacteroides* species have been recognized as having probiotic benefits. (Bäckhed et al. 2005). One study identified an increased abundance of *Prevotella* in the gut microbiome of smokers and reported an association with a heightened risk of heart disease. Kelly et al also discovered that *Prevotella* was linked to an increased risk of cardiovascular disease (CVD) (Kelly et al. 2016).

Upon observation of the *patient 6*'s current condition, it has been determined that they are suffering from angiectasia as a result of heart disease. It is known that gut microbiota plays a crucial role in regulating metabolic processes such as oxidative stress and inflammatory reactions, which can lead to the progression of atherosclerosis and coronary heart disease. It is possible that *patient 6* developed heart disease due to a dysregulated gut flora. Furthermore, the high prevalence of *Prevotella copri* may indicate its involvement in inflammation. The symptoms of IBS are a consequence of this dysregulation. Considering that *patient 6* has a history of smoking, the process is interconnected. Studies have observed an increased abundance of *Prevotella* in the gut microbiome of smokers and have been associated with an

increased risk of heart disease. This automatically destabilizes the intestinal flora and leads to IBS symptoms.

Patient 8, complained of abdominal bloating that had persisted for 7/8 months and required medical consultation. The patient has no history of smoking but uses prescription medication for hypertension. They do not have any known chronic conditions. Abdominal bloating is a common symptom characterized by a feeling of pressure in the abdomen (Houghton et al. 2006). Patients suffering from irritable bowel syndrome (IBS), and functional gastrointestinal disorders, often experience abdominal bloating (Kanazawa et al. 2016). Functional abdominal bloating and distension (FABD) can be diagnosed when IBS, constipation, diarrhea, and postprandial distress syndrome do not have sufficient criteria for diagnosis (Drossman 2016). Possible mechanisms include increased gas in the gut, small intestinal bacterial overgrowth (SIBO), decreased abdominal capacity due to imbalanced gut microbiota, food intolerance, or anatomical changes (Accarino et al. 2009). Research conducted by Choong-Kyun Noh and Kwang Jae Lee (2020), suggests that patients with FABD have lower fecal microbiota diversity compared to the healthy control group. Additionally, the relative abundance of bacteria in FABD patients significantly differs from that of the control group. Specifically, FABD patients exhibited a higher proportion of *Proteobacteria* and a lower proportion of *Actinobacteria* at the phylum level. At the genus level, a greater proportion of *Prevotella* and *Faecalibacterium* was observed in the patient group, in comparison to the healthy control group. These differences were statistically significant. At the species level, the patient group exhibited a higher level of *P. copri* and *F. prausnitzii*, whilst a lower level of *B. uniformis* and *B. adolescentis*, when compared to the healthy control group (Noh and Lee 2020). Abdominal distension is a common symptom in functional gastrointestinal disorders. The pathophysiology of FABD is considered multifactorial and can also trigger IBS activity. Hypertension is a significant contributor to cardiovascular diseases. Liu and colleagues have demonstrated that, in addition to the more established causes of hypertension, the gut microbiota has a close connection with it. A clinical analysis was conducted on the fecal flora of hypertensive patients, revealing similar findings. Specifically, rates of *Prevotella spp.* and *Klebsiella spp.* were found to have significantly increased. To further investigate, gut microbiota from both healthy control and hypertensive groups were transplanted into germ-free mice. The group of mice treated with fecal bacterial transplants from patients with hypertension showed a significant increase in blood pressure (Li et al. 2017). This text confirms the link between gut microbiota and host blood pressure. An imbalanced gut microbiota is a crucial pathogenic factor for hypertension. Studies have shown

that edible probiotics have the potential to lower blood pressure (Khalesi et al. 2014).

In patient 8, we observed persistent abdominal distention for 7/8 months and incompatibility in the existing microbiota. According to the studies, the relative bacterial abundance of microbiota diversity in patients with abdominal distension also differed significantly compared to the control group. At the genus level, a higher proportion of *Prevotella* and *Faecalibacterium* was observed in the patient group than in the healthy control group. The abundance of both bacteria was significantly higher in the bacterial profile of our patient. This condition triggers abdominal bloating, one of the symptoms of IBS. The association of patient 8 with hypertension confirms that an imbalanced gut microbiota is a very important pathogenic factor for hypertension. Significantly increased rates of *Prevotella* spp. and *Klebsiella* spp. play a role in triggering IBS symptoms.

5.3 *Gemmiger formicilis*

The percentages of species are given in Table 2.5. The third species most frequently observed in the samples is *Gemmiger formicilis*. (Example: 1,3,4,5,9,10). Interpretation of samples will be based on the patient's medical history and irritable bowel syndrome symptoms.

There are a few articles on IBS for the *Gemmiger formicilis* species. Therefore, the general IBS status of the patients will be taken into account. *Gemmiger formicilis* is a bacterium that belongs to the *Bacteroidetes* phylum and has been detected in the human gut microbiota. The gut microbiota, a multifaceted society of microorganisms that colonizes the digestive tracts of humans and other animals, performs fundamental functions such as digestion, metabolism, and regulation of the immune system (Gossling and Moore 1975). Although *Gemmiger formicilis* is present in the gut microbiota, its specific role and impact on human health have not been extensively studied as that of some other bacterial species. The gut microbiota's overall composition and the presence of various bacterial species are believed to influence health and may contribute to conditions like inflammatory bowel diseases, obesity, and metabolic disorders. Research into the specific roles and impacts of *Gemmiger formicilis* in the gut microbiota is ongoing and new findings may contribute to a better understanding of its impact on human health (Zhou et al. 2021). Studies have identified the presence of several opportunistic pathogens in the COVID-19 cohort. The abundance of *Actinomyces oris* has been linked to increased COVID-19 severity. Certain strains of *Escherichia coli* have been identified as significant pathogens responsible for different levels of intestinal and parenteral infections.

Streptococcus is enriched in stool samples with high COVID-19 characteristics (Liu et al. 2020). Furthermore, there is a link between IBS and certain bacteria found at higher levels in the COVID-19 group, such as *Subdoligranulum spp.*, *Burkholderiales sp.*, and *Gemmiger formicilis*. This clarifies why the opportunistic *Gemmiger formicilis* bacterial group, which has recently emerged in the COVID-19 cohort, triggers IBS symptoms (Zhou et al. 2021).

5.4 *Haemophilus parainfluenzae*.

Haemophilus parainfluenzae was found in two of our patients. Patient **1** and **patient 10**

There are very few articles related to IBS and *Haemophilus parainfluenzae* bacteria. *Haemophilus parainfluenza* is a prevalent bacterium within the human respiratory tract microbiota. Although typically considered a benign commensal, *Haemophilus parainfluenzae* has also been shown to have pathogenic properties. Specifically, infections caused by *Haemophilus parainfluenzae* include meningitis, epiglottitis, pneumonia, bacteremia, endocarditis, and numerous other maladies (Watts et al. 2020). One study found that *Haemophilus parainfluenza*, a respiratory pathogen, also functions as an intestinal pathogen by inducing inflammation in Crohn's Disease. Some researchers have reported a considerable positive correlation between high levels of *Haemophilus parainfluenzae* and obesity and cardiometabolic disease. The research demonstrates that this bacterium may be significantly associated with or even pathogenic in the intestines of people with risk factors for metabolic disease, particularly those belonging to ethnic minorities. Consequently, it can cause symptoms of bowel disease (Sohn et al. 2023). Previous studies have suggested that intestinal colonization by *Haemophilus parainfluenza*, a gram-negative oral commensal, is associated with IBS activity (Shapiro et al. 2021). One study found that *H. parainfluenzae* was the most common bacterial species in children with IBS. This bacterium was also found in the jejunal fluid of children in a Danish study, indicating that it may be a normal intestinal commensal bacterium in childhood that can vary in quantity during disease states (Saulnier et al. 2011).

Although *Haemophilus parainfluenzae* is commonly found in the respiratory tract microbiota, some studies have identified it as an intestinal pathogen. There is a limited amount of literature on the relationship between IBS and *Haemophilus parainfluenzae* bacteria. Several articles have reported a significant association between *Haemophilus parainfluenzae* and IBS. However, no data were found regarding other symptoms in our patients. Further investigation is required to study this area and its relationship with IBS in more detail.



5.5 *Eubacterium bifforme*

Among the samples, *Eubacterium bifforme* was only present in **patient 3**

Eubacterium species are an important member of the human gut microbiota and are present in the human digestive tract in various populations in Africa, Australia, Europe, India, South America, Asia, and North America (Wade 2006). Numerous studies of the human intestine have identified a significant number of metagenome-assembled *Eubacterium* genomes, regardless of geographic location, age, lifestyle, and clinical status (Almeida et al. 2019). *Eubacterium* species are frequently present in the gastrointestinal tract of animals, but they have been observed to be absent in the gastrointestinal tract of primates. The fact that they are widely distributed in the human gastrointestinal tract suggests a high degree of specificity and adaptation to the human host (Karcher et al. 2020). The gut microbiota's composition and diversity are significantly influenced by diet. Increased dietary fiber intake is associated with the presence of *Eubacterium spp.* in the gut, while a higher percentage of protein/fat in the diet is linked to their decrease (Feng et al. 2018). *Eubacterium spp.* contribute to gut and hepatic health by modulating bile acid metabolism. Bile acids (BAs) are metabolites produced by the host and are derived from cholesterol in liver pericentral hepatocytes. The liver produces two primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA), which then conjugate with taurine or glycine before being temporarily stored in the gallbladder. Postprandial secretion subsequently transports these BAs to the intestines (Staels and Fonseca 2009). Around 95% of the total bile acids in the intestines are reabsorbed and recycled to the liver via the portal vein, constituting enterohepatic circulation. The remaining 5% is used for bacterial metabolism in the stomach and helps to eliminate cholesterol from the body (Mukherjee et al. 2020). Bacteria within the gut convert primary bile acids (BAs), specifically cholic acid (CA) and chenodeoxycholic acid (CDCA), into deoxycholic acid (DCA) and lithocholic acid (LCA) via 7 α -dehydroxylation (Ridlon, Kang, and Hylemon 2006). This reaction is important quantitatively, but only a select few members of the gut microbiota (such as the *Eubacterium* and *Clostridium* clusters) have been observed to carry it out (Ridlon et al. 2014). Studies of *Eubacterium* strain VPI 12708 have identified enzymes encoded by the bile acid-inducible (bai) operon, which catalyze a multi-step pathway for primary bile acid 7 α -dehydroxylation (Björkhem et al. 1989). Butyrate inhibits the development of colorectal cancer and reduces the presence of butyrate producers in the gastrointestinal tract, including *Eubacterium spp.* Certain *Clostridium* cluster XIVa members, including *Eubacterium spp.*, can also generate secondary bile acids by 7- α hydroxylation of primary bile acids (Ridlon et al. 2006). Lower levels of

secondary bile acids were significantly reported among IBS participants with chronic gut inflammation, alongside elevated levels of fecal-conjugated bile acids, and a marked reduction in *Clostridium* cluster XIVa and *Eubacterium* spp (Lefebvre et al. 2009).

Studies have reported the importance of the *Eubacterium* strain in the intestine. This strain expresses bile acid-inducible enzymes that catalyze bile acid production in a multi-stage pathway. The lack of bile in the patient may have disrupted the balanced colony in the intestine, reducing the *Eubacterium* bacterial community and exacerbating the symptoms of IBS. It is possible that this condition, which cannot be treated regularly, may cause further exacerbation of severe IBS symptoms in the future.

5.6 *Bacteroides ovatus*

Among the samples, *Bacteroides ovatus* was only present in **patient 9**.

Bacteroidetes are the predominant bacteria found in mammalian faeces, and several *Bacteroides* species have been shown to influence various aspects of host physiology. However, despite its beneficial effects, *Bacteroides ovatus* has received little attention in recent years. The *Bacteroidales* order, situated within the *Bacteroidetes* phylum, is the most prevalent gram-negative bacterial species found in the human gastrointestinal tract (Wexler and Goodman 2017). *Bacteroides* and *Prevotella* are the predominant genera within the *Bacteroidales* order. *Bacteroides* has garnered particular attention as an intestinal commensal microbe. *Bacteroides* inhabit the gastrointestinal tracts of various mammals, including humans, and have the ability to establish stable synergistic relationships with their hosts, leading to a multitude of health advantages (Mazmanian et al. 2005). As *Bacteroides* are essential components of the human gut microbiota and play a foundational role in the gastrointestinal microbial food web, they provide an ideal model group for researching microbial colonization (Tan et al. 2019). *Bacteroides* spp. implement a range of colonization mechanisms, including the capacity to use different dietary polysaccharides and intestinal mucins (Sonnenburg et al. 2005). *Bacteroides* possess multiple glycosyl hydrolases that are necessary for breaking down dietary and host-derived glycans, in addition to the ability to utilize amino acids. For instance, *B. thetaiotaomicron* has the capability of metabolizing more than a dozen polysaccharides obtained from plants and hosts (Sonnenburg et al. 2010). To date, most research on *Bacteroides* has been centered on *B. thetaiotaomicron*, with fewer studies investigating the dietary use of other *Bacteroides* species such as *B. fragilis*, *B. vulgatus*, and *B. Ovatus* (Foley et al. 2019). *B.*

Ovatus exhibits versatile traits about the utilization of nutrients and the regulation of environmental stressors within the gut. Offering an extensive choice of dietary sources, *B. ovatus* can thrive in the ever-changing environment of the gastrointestinal tract (Fultz et al. 2021).

The intestinal microbiota ensures the maintenance of host health. Commensal gut microbes regulate the immune system, strengthen the epithelial barrier, prevent pathogen colonization, and provide essential nutrients (Engevik and Versalovic 2017). The importance of these microorganisms is emphasized by the connection between dysbiosis, or changes in the structure of the microbial community, and various conditions, including inflammatory bowel disease, cardiovascular disease, metabolic disorders, and neurological diseases (Collado et al. 2009). Controlling the intestinal microbiota is necessary to protect human health. Studies involving human subjects show that diet significantly affects the microbiota. It has been shown that even small dietary changes can cause significant microbial changes within 24 hours (Singh et al. 2017). *Bacteroides* is a dominant bacterial group in the microbiota of healthy adults. *B. ovatus*, a member of the *Bacteroides* genus, has gained attention due to its potential health benefits and anti-inflammatory properties (Hudcovic et al. 2009). Further research is needed to explore the effect of dietary nutrients on the composition of *Bacteroides*, particularly *B. ovatus*, in the human gastrointestinal tract. *B. ovatus* can use different sugars as growth substrates. However, most dietary monosaccharides are absorbed in the small intestine and are therefore not abundant in the colon, which is where *B. ovatus* primarily colonizes. *B. ovatus* encounters complex polysaccharides in the colon that are resistant to digestion by the host. These may include dietary fiber, animal polysaccharides, plant and animal glycoproteins, natural and synthetic polysaccharides used as food rheology modulators, and oligosaccharides (Sonnenburg et al. 2010). In addition, the data demonstrate that *B. ovatus* can metabolize a wide range of trisaccharides, polysaccharides, and polymers, thereby increasing its fitness (Birkeland et al. 2020). The density of *B. ovatus* in patient 9 has increased, possibly due to the dietary intake.

Patient 9 exhibited an increase in *B. ovatus* density, likely attributed to dietary intake. Further research is required to explore the potential influence of dietary nutrients on *B. ovatus* composition in the human gastrointestinal tract. *B. ovatus* can utilize various sugars as growth substrates. Within the colon, *B. ovatus* encounters and digests complex polysaccharides that are resistant to host digestion. The patient's healthy diet may have contributed to an increase in the beneficial bacteria, *Bacteroides ovatus*. However, there is limited research on the relationship between this bacterium and IBS, and further studies are needed to provide more detailed

information.

5.7 *Bifidobacterium adolescentis*

Among the samples, *Bifidobacterium adolescentis* was present in **3 and 5 patients**.

Bifidobacterium adolescentis is prevalent among bifidobacteria species observed in the large intestine of humans. With evolutionary adaptation for the fermentation of glycans derived from plants, it possesses various sugar transporters and degradation enzymes (Duranti et al. 2017). Therefore, dietary carbohydrates have a strong influence on this species, which is capable of utilizing a broad spectrum of prebiotic molecules (Duncan, Louis, and Flint 2004). Several strains of *B. adolescents* have demonstrated numerous health benefits, including the improvement of intestinal barrier function, anti-inflammatory and immunomodulatory effects, as well as the production of neurotransmitters (GABA) and vitamins (Duranti et al. 2016).

While the causal relationships have not yet been established, scientific studies have linked the reduced presence of *B. adolescentis* to a range of conditions, including inflammatory bowel diseases, irritable bowel syndrome, celiac disease, cystic fibrosis, *Helicobacter pylori* infection, type 1 and type 2 diabetes, metabolic syndrome, nonalcoholic fatty liver disease, and some allergies (Fan et al. 2020). Therefore, it is reasonable to regard *B. adolescentis* as a bacterial species linked to, or even supportive of, human health. *Bifidobacterium adolescentis* is a common species of bacteria found in the large intestine of humans. It is present in 60-80% of healthy adults and can reach cell densities of 10^9 - 10^{10} cells/g of faeces (Turroni et al. 2009). Inflammatory bowel disorders are characterized by long-term inflammation of intestinal tissues. Dysbiotic faecal microbiomes, found in various configurations linked to these conditions, usually display a lower frequency of *B. adolescentis* in studies using appropriate taxonomic resolution (Fan et al. 2020). A pivotal cohort study found decreased *B.adolescentis* in stool samples from patients diagnosed with irritable bowel syndrome (IBS) compared to the general population (Bolte et al. 2021). Other studies have indicated a significantly lower occurrence of *B. adolescentis* in the dysbiotic microbiota of adult CD patients than in healthy controls (Hedin et al. 2021). A tenfold decrease in the amount of *B. adolescentis* found in the faecal matter was identified in recently diagnosed IBS patients, compared to healthy controls (Krumbeck et al. 2018). Nevertheless, a diet low in fermentable carbohydrates (FODMAPs) proved effective in alleviating bowel symptoms and significantly reduced *B. adolescentis* and *F. prausnitzii* in the fecal microbiota, as well as fecal SCFA levels, in an interventional therapy trial in IBS

remission patients (Noh and Lee 2020). The table displays the prevalence of *B. adolescentis* in our third and fifth patients (Table 2.5). Notably, our third patient's IBS symptoms were provoked by low levels of *F. prausnitzii*.

The severity of IBS symptoms in *patient 3* may be due to decreased levels of *F. prausnitzii* and *B. adolescentis*, as studies have shown that the amount of *B. adolescentis* in IBS patients is reduced by 10 times compared to healthy controls. Similarly, in *patient 5*, the severity of IBS symptoms resulted from the decrease in *B. adolescentis* levels.

5.8 *Collinsella aerofaciens*

Among the samples, *Collinsella aerofaciens* was present in **6 and 8 patients**.

The bacterium *Collinsella aerofaciens* ferments a variety of carbohydrates derived from both plants and animals. It exhibits the capacity to generate H₂, ethanol, short-chain fatty acids, and lactate in the human colon. In particular, it is lactose that is predominantly used by *C. aerofaciens* in the human colon (Longstreth et al. 2006). Several studies have shown that *Collinsella* and *Bifidobacterium* can affect the ability of intestinal pathogens to adapt to virulence and pathogenicity (Hillilä, Siivola, and Färkkilä 2007). The *C. aerofaciens*-like phylotype demonstrated intriguing correlations with patient characteristics. In stool samples of IBS patients, the amount of *C. aerofaciens* decreased when compared to the healthy control group (Santacruz et al. 2009). Significant differences in *C. aerofaciens* numbers were detected in qPCR analyses across studies within the *Actinobacteria* phylum. This microbe was present in 96% of all control samples collected from participants not afflicted with gastrointestinal disorders. The abundance of *C. aerofaciens* has been linked to a reduced risk of colon cancer (Kassinen et al. 2007). A different study investigating a cohort of IBS patients discovered a decline in the number of *C. aerofaciens* compared to the healthy control group (Malinen et al. 2010). Individuals with heart disease showed significantly lower abundances of *Enterococcus*, *Megamonas*, *Collinsella aerofaciens*, and *Fusobacterium*, which have been linked to the development of their condition (Jie et al. 2017). Cigarette smoking impacts alterations in the intestinal microbiota and microbe-derived metabolites. Additionally, *Fusicatenibacter saccharivorans* and *Collinsella aerofaciens* are amongst the potentially advantageous bacteria that are decreased in the smoking community. It has been discovered that both *F. saccharivorans* and *Collinsella aerofaciens* possess anti-inflammatory proprieties that can mitigate the effects of ulcerative colitis (UC) in murine models (Qin et al. 2019). *Collinsella*

aerofaciens was discovered to be present in lesser quantities in the intestinal microbiota of patients with IBS compared to those who are healthy, according to a report (Garrett 2019). Prevention or treatment of conditions associated with a low intestinal population of *Collinsella aerofaciens* focuses on alleviating intestinal discomfort, including bloating, and reducing the risk of developing colon cancer (Sullivan 2012). Roseburia faecis and Collinsella aerofaciens have demonstrated a positive correlation with mean systolic blood pressure (Comanys et al. 2021).

When examining the results of our *patient 6* with IBS symptoms, it was found that the abundance of *Collinsella aerofaciens* was low. This low level of bacteria has been shown to trigger IBS symptoms in patients. Additionally, when comparing heart disease patients, the rate of *Collinsella aerofaciens* was low in our patients, which contradicts previous research that suggests it should be high. When examining the link between smoking and heart disease, it was found that *patient 6's* smoking led to a decrease in *Collinsella aerofaciens*, a bacterium associated with heart disease. Additionally, *patient 8's* IBS symptoms suggest a low population of bacteria. However, few articles explore the relationship between hypertension and Collinsella aerofaciens.

5.9 Bacteroides acidifaciens

Among the samples, *Bacteroides acidifaciens* was only present in *patient 9*.

Bacteroides acidifaciens, a member of the *Bacteroidetes* phylum, is regarded as one of the prevalent species in intestinal commensal bacteria. The presence of *Bacteroides acidifaciens* in the gut is promoted by consuming a high-soluble fibre diet, as evidenced by an increased relative abundance of *Bacteroides acidifaciens* (Nakajima et al. 2020). The study by Then et al. investigated the relationship between the gut microbiota and irritable bowel syndrome (IBS) and confirmed the beneficial effect of *Bacteroides acidifaciens* on IBS. Additionally, they discovered that *B. acidifaciens* may be responsible for alleviating IBS symptoms. The study demonstrated that *B. acidifaciens* could alleviate IBS symptoms and penetrate brain tissue to potentially act as a regulator (Then et al. 2020). Cihua Zheng et al. discovered that the abundance of *B. acidifaciens*, *Akkermansia muciniphila*, and *Pseudomonas xiamenensis* decreased at the species level in the IBS group compared to the control group, whereas *Staphylococcus lentus* significantly increased. The findings indicated that it could lead to heightened sensitivity of internal organs, inflammatory responses, damage to the intestinal

mucosal barrier, and disorders of the intestinal microbiota (Zheng et al. 2023).

Immunoglobulin A (IgA) is crucial in protecting the intestinal mucosa against harmful pathogens. Previous studies indicate that *Bacteroidetes*, the primary phylum of gut microbiota alongside Firmicutes, have an impact on IgA production (Macpherson et al. 2018). Akihito Nakajima and colleagues have profiled the species distribution among *Bacteroidetes* and identified some specific species linked to intestinal IgA induction in their research study (Nakajima et al. 2020). In mice fed a soluble high-fiber diet (sHFD), the gene encoding activation-induced cytidine deaminase (AID) showed increased expression in the lamina propria of the large intestine. AID plays a crucial role in IgA levels and IgM-to-IgA class switch recombination. The most abundant *Bacteroidetes* species in both sHFD and normal diet-fed mice was *Bacteroides acidifaciens*. A high-fiber diet was found to be associated with increased intestinal IgA levels and greater abundance of species within the *Bacteroides fragilis* group, including *Bacteroides faecis*, *Bacteroides caccae*, and *Bacteroides acidifaciens*. Conversely, mice who consumed a low-fiber diet had a decreased proportion of *B. acidifaciens* compared to other *Bacteroidetes* species. To assess the effect of *B. acidifaciens* on IgA production, we created mono-associated mice and observed an increase in intestinal IgA production and AID expression. Additionally, soluble dietary fiber was found to increase *B. acidifaciens* group and IgA production, potentially improving intestinal immune function and offering protection against intestinal pathogens while reducing the incidence of inflammatory bowel diseases. *Patient 9* reported symptoms of constipation and bloating and had previously consulted a dietitian for advice on maintaining a healthy diet. A healthy diet has the potential to improve IBS function, protect against pathogens, and reduce the incidence of inflammatory diseases. Our patient's low abundance of *B. acidifaciens* triggered her IBS symptoms. This finding also sheds light on our patient's regular nutrition. The patient's abundance of *B. acidifaciens* was low, indicating inadequate fiber consumption. While a healthy diet has a positive correlation with the abundance of other bacteria, *B. acidifaciens* specifically provides information on fiber consumption.

5.10 *Bacteroides plebeius*

Bacteroides plebeius was present only in *patient 3*.

Bacteroides plebeius is a microbe commonly found in the human gut, particularly in Japanese individuals. Seaweeds are recognized as an excellent source of dietary fiber. Recently, a

polysaccharide utilization locus (PUL) was discovered in *Bacteroides plebeius*, which targets the porphyran and agarose of red seaweeds. *B. plebeius* has the endo-type β -agarase BpGH16A, which belongs to the glycoside hydrolase family and cleaves the β -1,4-glycoside bonds of agarose, producing neoagarooligosaccharides.

Studies on *B. plebeius* bacteria are not available for IBS patients. However, it survives symbiotically in the intestine. It may be beneficial bacteria for IBS or it may have a pathogenic effect. We need more detailed studies on this bacterium.

5.11 *Bifidobacterium longum*

Bifidobacterium longum was present only in **patient 10**.

The species *Bifidobacterium longum* comprises three subspecies: *longum*, *infantis*, and *suis*, initially classified as independent species. However, further analysis revealed a high degree of sequence homology between them and led to their integration into a single species, *B. Longum* (Lee and O'Sullivan 2010). *Bifidobacterium longum* spp. *infantis* and *longum* have been isolated from the intestines of infants and adults, respectively. Numerous strains from both subspecies have undergone laboratory and clinical probiotic testing. *B. longum* strains are primarily prevalent in the intestines of breastfed infants (Underwood et al. 2015). Both quantitative and qualitative disturbances of the gut microbiota may occur in individuals with IBS. Studies have shown that the use of probiotics can help to regulate the intestinal microbiota (Hill et al. 2014). The genus *Bifidobacterium* is representative of the intestinal microbiota, which has important effects on general gut physiology and positive effects on the immune system (Milani et al. 2015). The metabolic activity of the subject results in the production of short-chain fatty acids (SCFAs) through the degradation of oligo-fructose and short-chain fats (Falony et al. 2006). Abdominal pain and discomfort are prominent features in patients with irritable bowel syndrome (IBS). In the study by Leonilde Bonfrate and colleagues, patients taking *Bifidobacterium longum* probiotics experienced significantly greater relief of symptoms compared with placebo. It has been speculated that in IBS subgroups at risk of dysregulation of the gut microbiota, probiotics may improve symptoms. This change should include harmful bacteria involved in the production of toxic metabolites, as well as beneficial lactobacilli and bifidobacteria (Bonfrate et al. 2020). In another study, the consumption of *Lactobacillus* & *Bifidobacterium* probiotics for 30 days resulted in a positive change in gut microbial composition, leading to an increase in beneficial lactobacilli and bifidobacteria (Ringel and

Carroll 2009). Jean-Marc Sabaté and colleagues noted a clinically significant increase in quality of life in patients with severe IBS who were given *Bifidobacterium longum*. This indicates that *B. longum* could have therapeutic potential for such patients (Sabaté and Iglicki 2022). O'Mahony et al. (2005) conducted a study employing the same probiotic strain and reported substantial improvement in quality of life compared to placebo. While most probiotics studies do not target a particular IBS subtype, this research analyzed the effect of *B. longum* treatment over 30 days, based on IBS subtypes. The treatment proved efficacious on all IBS subtypes regarding disease severity and quality of life. During the treatment period, a reduction in the occurrence of extreme stool types and an elevation of normal stool types were recorded based on the Bristol stool scale (O'Mahony et al. 2005).

Research shows that the abundance of *Bifidobacterium longum* species in the intestine is beneficial. The *Bifidobacterium* genus is representative of the intestinal microbiota, which has significant effects on general intestinal physiology and positive effects on the immune system. Patient 10 has a reduced abundance of the genus *Bifidobacterium longum*, which causes an increase in IBS symptoms. Furthermore, the probiotic properties of *Bifidobacterium longum* offer advantages in the intestine. It is plausible that the symptoms were aggravated due to the low count of beneficial bacteria in our patient. Additionally, the drugs used during chemotherapy may have a detrimental effect on these bacteria. Additionally, the drugs used during chemotherapy may have a detrimental effect on these bacteria. It is well-known that cancer treatment can negatively impact beneficial bacteria, leading to a reduction in their numbers.

5.12 *Ruminococcus bromii*

Ruminococcus bromii was present only in **patient 1**.

Ruminococcus bromii is an anaerobic, gram-positive coccus that solely relies on fermentable disaccharide, polysaccharide, or fructose for optimal growth (Rogosa 1971). This species is a prevalent member of the human gut microbiota. The metabolites and cultural characteristics of this species indicate that it belongs to the genus *Ruminococcus*, as amended. This is due to its capacity to produce acetic and formic acids from fermentable carbohydrates, as well as its complex nutritional requirements (Moore, Cato, and Holdeman 1972). A subset of patients with irritable bowel syndrome (IBS), develop symptoms following an enteric infection. The pathophysiology of this condition has been attributed to bacterial dysbiosis and mucosal

inflammation. J. Sundin et al. found 54 genus-like phylogenetic groups that differed significantly in the fecal microbiota of IBS patients compared to healthy controls (Sundin et al. 2015). Among these groups, *Faecalibacterium prausnitzii* and relatives were found to produce butyrate, which was associated with the maintenance of intestinal health. There was a decreasing trend in the levels of butyrate, which may be relevant to the increased visceral perception that is a characteristic of IBS (Hamer et al. 2008). It was discovered that the quantity of *Ruminococcus bromii* bacteria found in stool was greater in IBS patients than in healthy controls. *Ruminococcus bromii* contains acetate, ethanol, and hydrogen producers and is assumed to facilitate gas production, hence, potentially contributing to the development of IBS symptoms (Walker et al. 2011). Cremon et al. conducted a study on the impact of probiotics on IBS. Their results indicate that the probiotic significantly lowered the *Ruminococcus* genus, leading to a significant increase in fecal SCFA butyrate levels, and a reduction in the pro-inflammatory cytokine IL15 (Cremon et al. 2018). The presence of the *Ruminococcus* genus in the intestinal microbiota is heightened in IBS patients. Hence, the potential advantageous effects of probiotic consumption in IBS are exemplified by the decrease in the relative abundance of this taxon. Correlation analyses corroborate the predominant participation of gut microbiota from the genus *Ruminococcus* in IBS (Corrêa-Oliveira et al. 2016).

Studies have shown that an increase in *Ruminococcus bromii* can worsen symptoms in patients with IBS. *Ruminococcus bromii* produces acetate, ethanol, and hydrogen, which can lead to increased gas production and trigger IBS symptoms.

5.13 Lactobacillus

Among the samples, *Lactobacillus* was present in **2 and 7 patients**.

Lactobacillus is a gram-positive bacillus widely used as a probiotic ingredient. *Lactobacillus* organisms in the intestine produce acetic acid, lactic acid, and hydrogen peroxide and promote the secretion of antimicrobial substances (Vanderhoof et al. 1998). *Lactobacillus* may also compete with pathogens for mucosal surface receptors and reduce the incidence of antibiotic-associated diarrhoea and *Clostridium difficile* colitis (Vanderhoof et al. 1999). Both quantitative and qualitative disturbances of the gut microbiota can occur in individuals with IBS. Therefore, studies support the use of probiotics to modulate the intestinal microbiota. The *Lactobacillus* genus is a significant member of the intestinal microbiota and has positive effects on general intestinal physiology, including the immune system. Its metabolic activity is related to oligo-

fructose. (Khailova et al. 2014). The combination of certain strains of *Lactobacillus* bacteria with *Bifidobacterium* species can help to restore balance to the gut microbiota (Milani et al. 2015). Our second and seventh patients showed 100% *Lactobacillus*. It is possible that the device provided inaccurate results due to incorrect sequencing. In the second and seventh patients, 100% *Lactobacillus* was detected. *Patient 7* sought medical help for abdominal pain. They do not smoke and are on regular heart medication. *Patient 2* sought medical attention for complaints of abdominal and stomach pain. They do not smoke and are not taking any medication regularly. They have previously been treated for breast cancer and the treatment has been completed. The patient characteristics are as stated. Although they exhibited symptoms of IBS, no distinct bacterial groups were detected. While we expected to observe similar bacterial groups in other patients with IBS symptoms, the result was a single type of bacteria. We were unable to repeat the experiment due to a lack of samples. As there are no articles that focus solely on one type of *Lactobacillus* group, it is not possible to evaluate these patients.

6. CONCLUSION

Intestinal samples were collected from 10 IBS patients via colonoscopy biopsy and bacterial diversity was revealed. In our study, the bacterial groups found in each patient were compared and analyzed in detail, considering the relationship between the patients' symptoms. *Faecalibacterium prausnitzii*, *Prevotella copri* and *Gemmiger formicilis* bacteria were found to be common in all analyzed samples. Our study revealed that there is a direct relationship between these 3 bacterial species and IBS symptoms in all patients. A high rate of *Gemmiger formicilis* was detected in our samples. However, there are almost no studies on the relationship of this bacterial species with IBS. This relationship should be examined in future studies. This thesis reveals the extremely important relationship in this respect.

In addition, the study compared the effects of smoking, heart diseases and nutrition on the intestinal flora in detail with IBS symptoms. The presence of other bacteria in patients with IBS was also examined in this study. The findings suggest that these bacteria may serve as a biomarker for IBS and provide insights into treating its symptoms in the future.

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