The Microbiomes of Humans, Animals, Plants, and the Environment 1

Maria Gazouli
George Theodoropoulos *Editors*

Gut Microbiome-Related Diseases and Therapies

Springer

The Microbiomes of Humans, Animals, Plants, and the Environment

Volume 1

This series covers microbiome topics from all natural habitats. Microbiome research is a vibrant field of science that offers a new perspective on Microbiology with a more comprehensive view on different microorganisms (microbiota) living and working together as a community (microbiome). Even though microbial communities in the environment have long been examined, this scientific movement also follows the increasing interest in microbiomes from humans, animals and plants. First and foremost, microbiome research tries to unravel how individual species within the community influence and communicate with each other. Additionally, scientists explore the delicate relationship between a microbiome and its habitat, as small changes in either, can have a profound impact on the other. With individual research volumes, this series reflects the vast diversity of Microbiomes and highlights the impact of this field in Microbiology.

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Preface

The human digestive tract is colonized by a highly diverse ecosystem of microorganisms that comprise the gut microbiota. Microbiota has been acknowledged to play a crucial role in maintaining a healthy state, as well as in drastically modifying susceptibility and progression of common human diseases. Diverse mechanisms including, but not limited to, inflammation are implicated in this complex bidirectional crosstalk between the gut microbiota and the host. A substantial body of evidence has been progressively accumulated, has enlightened the mechanistic details involved in this crucial interaction, and has opened novel avenues on the ways we will envisage diagnosis and treatment of human pathologies. An in-depth understanding of this relationship will be vital not only to advance the human health but also to enhance our understanding of diseases and to highlight new therapeutic approaches.

The book primarily focuses on the host-gut microbiome interaction and on cause-effect mechanisms. The authors aspire to offer basic researchers and medical professionals a comprehensive insight on the concepts of microbiome-related diseases susceptibility and progression, on the significance of microbiota disturbances in gut dysbiosis, and on the array of interactions between the microbiome and the human genome and epigenome. This collective work, eventually, aims in aiding the reader to acquire profound knowledge on the interplay between the gut microbiota promoting and protective features and the pathogenesis of benign and malignant human diseases and their respective therapies.

Whether you are a clinician, biomedical researcher, student, or patient, or just interested in Gut Microbiome, we hope you enjoy reading this book as much as we have enjoyed researching, writing, and organizing it!

Athens, Greece — Maria Gazouli
Athens, Greece — George Theodoropoulos

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About the Editors

Maria Gazouli is Professor of Biology - Nanomedicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece. She was admitted as a PhD student in the Biology Department and Medical School of National and Kapodistrian University of Athens and was granted a honored Hellenic Pasteur Institute scholarship. She continued her postdoc training in Cell Biology Department, Georgetown University Medical Center, Washington DC, USA. Dr. M. Gazouli's work focuses on the molecular basis of diseases mainly autoimmune diseases and cancer, on the molecular detection of pathogens, and on the investigation of the pathogenesis of the diseases they cause to humans. These activities have produced more than 250 publications in peer-reviewed journals, 11515 citations (*h*-index: 55), more than 150 announcements in scientific congresses that were awarded in 17 cases, 1 granted International Patent, and 3 European Patent Applications. Recently Dr. Gazouli was involved in the incorporation of nanotechnology to targeted cancer detection, imaging, and drug delivery. She was honored with a Fulbright Scholarship for the Development of Nanotechnology-based Biosensor Arrays for the Detection of Circulating Colorectal Cancer Cells at the University of Maryland, College Park, MD, USA. The research has been recognized by distinguished awards and funded by national and international (EU) competitive research grants. Maria Gazouli has been actively involved in undergraduate and postgraduate training, as well as ERASMUS program, and her laboratory has trained a significant number of young scientists.

George Theodoropoulos was graduated from Athens Medical School in 1992. His PhD research was in Tumor Markers in Gastrointestinal Malignancies. He completed a 6-year residency program in General Surgery and a fellowship in Colon and Rectal Surgery in the USA. He is currently holding an academic post as an Professor of Surgery at Athens Medical School, Athens, Greece. He is a Diplomat and a Fellow (FACS) of the American Board of Surgery and of the American Board of Colon and Rectal Surgery (FASCRS). He completed a 6-month research fellowship in the Department of Colorectal Surgery, Cleveland Clinic Florida, Weston, FL, USA. He has set up and coordinated a clinic of Health-Related Quality of Life surveillance of colorectal cancer patients, has been supervising the Colorectal Unit of the Athens Medical School First Department of Propaedeutic Surgery, and has

established a multidisciplinary "Lower Digestive Tract Study Unit" in the hospital he is currently practicing.

He has performed about 3000 general surgery and colorectal surgery procedures. He applies a variety of minimally invasive techniques, and he is skilled at laparoscopic colorectal procedures for cancer and inflammatory bowel diseases, as well as management of common and complex anorectal pathologies. He has delivered presentations in more than 200 meetings and has been an invited speaker for 130 talks in congresses and workshops. He is the author/coauthor of 130 internationally cited peer-reviewed journal publications (5500 citations, *h*-index: 37).

Among other societies, he is a member of the European Association for Endoscopic Surgery (EAES) Research Committee and the International Committee of the American Society of Colon and Rectal Surgeons, while representing Greece as one of the committee members of a European COST (European Cooperation in Science and Technology) research platform on perioperative care of cancer patients.

The Human Microbiome

1

Nick-Panagiotis Andreou and Maria Gazouli

Abstract

Humans have coevolved with the trillions of microorganisms that inhabit their body, namely human microbiome. The human microbiome, especially gut microbiome, has gained an extensive interest over the last decades due to state-of-the--art technology and large-scale metagenomics studies that attempt to unravel the mystery of this complex, heterogenous ecosystem and its repercussions to host physiology. Bacteria have been the center of attention across research literature, but here an overview of the role of fungi, archaea, viruses, and protozoa is addressed as well. The aim of this chapter is to explore the diversity of taxonomic composition of human microbiota and their pivotal role in regulating host metabolism, immune system, and protection against invading pathogens. The chapter also focuses on the potential external factors (initial colonization, diet, lifestyle) prompting variable configurations of human microbiota that lead to imbalance of homeostasis (dysbiosis) and result in a broad spectrum of pathological diseases, such as obesity, inflammatory bowel disease, and *Clostridium difficile*-induced diarrhea.

Keywords

Microbiome · Microbiota · Dysbiosis · Diet · Antibiotics

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1.1 Introduction

The human body is inhabited by a vast number of microorganisms that live in concordance with their host and are commonly referred to as human microbiota of microflora. The human microbiota contains a collection of commensal, symbiotic, and opportunistic pathogenic bacteria, fungi, archaea, viruses, and protozoa (Sekirov et al. 2010). Bacteria are considered the most prominent group in the community, estimated to be approximately 10^{13} to 10^{14} microbial cells, with around 1:1 microbial cells to human cells ratio (Sender et al. 2016). Therefore, microbiome research has been mainly focused on bacteria, whereas fungi and viruses have recently started to gain more attention concerning their pivotal role in homeostatic regulation (Vemuri et al. 2020). The microbiota colonizes various sites of the human body including oral cavity, skin, genital organs, and respiratory and gastrointestinal (GI) tract (Lloyd-Price et al. 2016). The GI tract occupies a major surface, highly enriched in nutrients, creating a preferable environment for microbial growth and colonization. Additionally, the gut microbiota is not homogenous and microbial composition varies between sites or different layers of the same tissue, such as the intestinal epithelium, where the microbes present in the intestinal lumen are significantly distinct from the microbes attached to the epithelium or those entrapped within the mucus layer. The majority of intestinal microbiota is primarily comprised of strict anaerobes that dominate over anaerobes and facultative anaerobes and is classified to the four major phyla of *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, with minor proportions of species belonging to the phyla of *Fusobacteria*, *Tenericutes*, and *Verrucomicrobia* (Sekirov et al. 2010).

The intestinal microflora is involved in host physiology, regulating digestion, vitamin production, xenobiotic drug metabolism, immunological responses as well as conferring protection against pathogen perturbation (Gouba et al. 2019). Changes in the balance of healthy microbial communities, namely dysbiosis, are often associated with numerous pathological conditions, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and obesity (Gouba et al. 2019). The gut microbiota community is dynamic (Li et al. 2016), meaning that not all microorganisms can colonize the gut permanently, hence homeostasis relies on maintaining the microbial biodiversity, which is characterized by its species evenness (the different kinds of species) and richness (the number of different species) (Vemuri et al. 2020). This is challenging for studies focusing on humans, since biopsy sampling is infeasible and the majority of data is obtained by fecal specimens, which may contain occasional species (Sam et al. 2017). Consequently, the use of "humanized" gnotobiotic animal models could provide insight into the mechanisms of microbiome regulation, evaluate potential therapeutic treatment in microbiome-related diseases and assess the pharmacological monitoring of the selected treatment (Kho and Lal 2018).

The composition and the properties of human microbiome were formerly poorly characterized due to technology limitations regarding lack of optimized techniques for noncultivable microbial species and curated reference databases (Gouba et al. 2019). Advances in sequencing technology (e.g. NGS) and bioinformatic tools

enabled large-scale sequenced-based microbiome projects such as Human Microbiome Project (HMP) and Metagenomics of Human Intestinal Tract (MetaHIT), funded by the United States National Institutes of Health (NIH) and the European Commission, respectively, that resulted in reference genome mapping, metagenomic assembly, gene cataloging, and metabolic reconstruction of human microbiome (Kho and Lal 2018). Analysis of HMP samples along with lifestyle information has revealed that life history features and microbiome composition are considerably intertwined (Cresci and Bawden 2015). Microbial establishment in the human gut begins promptly after birth, hence delivery and feeding method of the infant determine initial colonization, and it is assumed that this initial colonization sets the ground for the composition of intestinal microbiota throughout adulthood. Dietary habits and use of antibiotics can directly affect the gut microbiome composition, while host genetics is suggested to have an indirect impact, probably by altering host metabolism. Notably, composition of intestinal microflora remains fairly stable at the phylum level and the four dominant groups are highly conserved across individuals, despite their proportional variation. Functional redundancy within those groups allows for interindividual variation of microbial species while preserving the maintenance of proper function (Sekirov et al. 2010).

A remarkable progress has been made to elucidate the relationship between the commensal microbiome and its host, as well as their subsequent impact on dysbiosis--related disease and therapeutic approach. However, human microbiome research is still in its infancy and further investigation is required to unravel the mystery of this field. The aim of this review is to compile information from various studies in order to redefine the composition and the function of the human microflora, depending on colonization site, and exemplify the dysbiotic features that are associated with a particular set of diseases.

1.2 Microbiome Composition

The composition of the human commensal microbiome exhibits a large variety of microorganisms with distinctive characteristics. Researchers were formerly constricted to culture-based methods for classification, performing biochemical tests, using different growth media to select specific populations and staining for phenotypic identification under microscope (e.g. Gram stain for bacteria, lactophenol stain for fungi) (Gouba and Drancourt 2015). These methods have a limited ability in providing sufficient information since more than 80% of the gut microbiome and mycobiome are unculturable under standard laboratory conditions (Eckburg et al. 2005). However, combination of high-throughput cultivation followed by MALDI--TOF-MS and 16S rRNA identification allows for “culturomics” to be still widely used (Gouba et al. 2019; Lagier et al. 2012).

Since the advance of molecular, genomic, and bioinformatic tools, research has been focused on genome sequencing approaches, “fingerprinting” methods, DNA microarrays, FISH, and qPCR to avoid culture bias (Sekirov et al. 2010). These techniques require the use of relatively small genes as markers of genetic diversity,

providing that they maintain balance of conservation and variance (Peterson et al. 2008). Microbial classification is based on the 16S ribosomal RNA (rRNA) sequence, while fungal characterization targets the 18S rRNA or the internal transcribed spacer (ITS) sequence (Suhr and Hallen-Adams 2015). Targeted sequences are then clustered into Operational Taxonomic Units (OTUs), based on their sequence identity and compared with existing databases (Gouba et al. 2019). Each technique has its benefits and its drawbacks and the selection is determined by the application. "Fingerprinting" methods, such as denaturing gradient gel electrophoresis (DGGE), are primarily used for comparative studies, but they are limited by the resolution of fragments on gel. Microarrays, FISH, and qPCR have been proved useful as screening tools for clinical applications, yet are incapable of identifying novel species of microorganisms. Next generation sequencing (NGS) technology has significantly decreased the cost of full-length (Sanger) sequencing and expanded our knowledge in microbiome diversity, though it demands extensive data analysis (Sekirov et al. 2010).

Despite the continuously growing number of identified commensal microbes in the human body, there was inadequate reference regarding their roles in human physiology, and numerous species were still unculturable or uncharacterized. Consequently, the National Institutes of Health (NIH) and the European Commission initiate the Human Microbiome Project (HMP) and the MetaHIT (METAgenomics of Human Intestinal Tract), respectively, to address these issues. Metagenomic analysis provided information from the collective genomes of a community about the organisms' composition and their function in the community. Therefore, both projects established a microbial genes record depending on specific body sites, revealed the implications of microbiome on human diseases, and they developed new tools and reference databases for organization, storage, and comparative analysis (NIH HMP Working Group 2009; Qin et al. 2010; Weinstock 2012).

The human body is inhabited by trillions of microorganisms that symbiotically live and have coevolved with the host, rendering this ecosystem as one of the most important mediators of human health and disease (Lloyd-Price et al. 2016). These commensal microbes are referred as microbiota or microflora and are comprised of bacteria, viruses, archaea, and eukaryotes, mainly fungi and protozoa (Lederberg and McCray 2001). They reside in the gastrointestinal (GI) tract (25%), the oral cavity (25%), the skin (21%), the airways (14%), and urogenital tract (9%) (HMP). The most well-studied microbiota in humans are bacteria, with the majority of them belonging to the phyla of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Rajilić-Stojanović et al. 2007). Although bacteria were initially thought to predominate, it is now recognized that the healthy human gut is inhabited by 10^{15} bacteriophages, making viruses the most prevalent microorganisms (Lozupone et al. 2012). Less extensive references considering the archaea demonstrate that they are mostly methanogens (methane-producing organisms) and they play an important role in gut function (Gaci et al. 2014; Matijašić et al. 2020). The eukaryotic community is mainly represented by fungi (also referred as mycobiota) which belong to the phyla of Ascomycota, Basidiomycota, and Zygomycota (Sam

et al. 2017; Huseyin et al. 2017), followed by protozoan parasites with *Blastocystis hominis* being the most common (Matijašić et al. 2020).

The human GI tract is extremely colonized by microbes and the gut microbiome has received the greatest attention so far. The GI tract is comprised of esophagus, stomach, small intestine, and large intestine thus providing an enormous surface for microbial colonization. There are 10 to 10^2 CFU/ml of microbes starting from the stomach and duodenum (*Lactobacilli*, *Helicobacter*, *Streptococci*, *Veillonella*, Yeasts), 10^4 to 10^8 CFU/ml moving on to jejunum and ileum (*Bacteroides*, *Bifidobacteria*, Coliform bacteria, *Fusobacteria*, *Lactobacilli*, *Streptococci,* members of Actinomycetaceae and Corynebacteriaceae) and 10^{10} to 10^{12} CFU/ml reaching the colon (*Bacteroides*, *Bifidobacteria*, *Clostridia*, Coliform bacteria, *Eubacteria*, *Fusobacteria*, *Lactobacilli*, *Proteus*, *Pseudomonades*, *Staphylococci*, *Streptococci*, *Veillonella*, members of Enterobacteriaceae, Lachnospiraceae, Prevotellaceae, and Methanobacteriaceae, Yeasts, Protozoa) (Sekirov et al. 2010; Lloyd-Price et al. 2016; Cresci and Bawden 2015). Longitudinal variations can also be observed in the intestine with the epithelium and the intestinal lumen governed by particular species (*Clostridium*, *Enterococcus*, *Lactobacillus* and *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterobacteria*, *Enterococcus*, *Lactobacillus*, *Ruminococcus*) (Sekirov et al. 2010). The composition of the gut mycobiome has been relatively unstable with great interindividual variability, therefore predominant species differ among various studies (Hallen-Adams and Suhr 2017). However, there are some species often encountered in the GI tract, but it is not clear whether they are true inhabitants or they are "passing through" (Sam et al. 2017). These include *Candida* and *Phialemonium* in stomach gastric fluid, *Cladosporium* in ileum and fecal samples, *Galactomyces* and *Geotrichum* in stool samples, *Dothideomycete* sp., *Galactomyces geotrichum*, and *Ustilago* sp. in colon mucosa, as well as species of *Aspergillus*, *Debaryomyces*, *Penicillium*, *Saccharomyces*, and *Trichosporon* (Sam et al. 2017; Hallen-Adams and Suhr 2017; Witherden et al. 2017).

The oral cavity is the second most habituated body part following the gut and most individuals share a common core oral microbiome at the genus level. The microbial communities of the mouth consist of viruses (*Herpes simplex*, Human Papilloma Virus) (Scott et al. 1997), protozoa (*Entamoeba gingivalis*, *Trichomonas tenax*), archaea (*Methanobrevibacter oralis*, *Methanobacterium curvum/congolense*, *Methanosarcina mazeii*) (Matarazzo et al. 2011), fungi (*Aspergillus*, *Aureobasidium*, *Candida*, *Cladosporium*, *Cryptococcus*, *Fusarium*, members of Saccharomycetales) (Ghannoum et al. 2010) and bacteria (Wade 2013). The dominant bacterial phyla are Actinobacteria (*Actinomyces*, *Angustibacter*, *Corynebacterium*, *Kineococcus*, *Rothia*), Firmicutes (Gemella, Paenibacillus, *Selemonas*, *Streptococcus*, *Veillonella*), Proteobacteria (*Aggretibacter*, *Alysiella*, *Kingella*, *Neisseria*), Bacteroidetes (*Capnocytophaga*, *Tannerella*, *Porphyromonas*), Spirochaetes and Fusobacteria (Dewhirst et al. 2010). There are no significant geographical differences suggesting that diet and environment do not affect the oral microbiome composition (Wade 2013; Solbiati and Frias-Lopez 2018).

The skin represents the largest organ of the human body, with each body surface providing various microenvironments for microbe colonization depending on pH, moisture, sebum content, etc. (Segre 2006). It has been observed that the skin microbiota communities retain their stability regardless of environmental changes with the exception of eukaryotic DNA viruses that exhibit high intraindividual variance (Oh et al. 2016). Once again bacterial colonization is enriched in the skin with species of the lipophilic *Propionibacterium* dominating sebaceous sites and species of *Staphylococcus* and *Corynebacterium* thriving in moist areas (Segre 2006). Interestingly, bacteriophages associated with *Propionibacterium* and *Staphylococcus* are persistently present in every skin site studied, whereas no core DNA virome is found to be conserved (Oh et al. 2016; Byrd et al. 2018). The less abundant mycobiome exert great similarity across the body with *Malassezia* being the most prevalent in core body and arm sites. *Malessezia* spp. is prevalent in dandruff-affected scalps (Park et al. 2012) and is implicated in atopic dermatitis (Zhang et al. 2011). Conversely, foot sites are susceptible to transient fungal colonization of diverse species (*Malassezia*, *Trichophyton*, *Aspergillus*, *Cryptococcus*, *Epicoccum*, *Rhodotorula*) and this might also explain the remarked variability of eukaryotic DNA viruses at that site (Byrd et al. 2018). Bacterial communities on hands belong to the phyla of Firmicutes (classes Bacilli and Clostridia, families Staphylococcaceae and Streptococcaceae), Actinobacteria (families Corynebacteriaceae and Propionibacteriaceae), Proteobacteria (classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria), Bacteroidetes (classes Bacteroidiia, Flavobacteria, and Sphingobacteria) and Fusobacteria, while fungal communities included *Malassezia*, *Aspergillus*, *Candida*, and *Saccharomyces* (Edmonds-Wilson et al. 2015).

The vagina hosts a dynamic microbial ecosystem that alters its composition in consideration of numerous factors such as age, menstrual cycle, and types of birth control. The main phyla present in the vagina is Firmicutes, with the predominance of the *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus gasseri* and *Lactobacillus jensenii*. These four species are well adjusted to the vaginal environment, have different properties than nonvaginal species (e.g. lower %G + C content, inability to metabolize glycogen), differentiate across ethnicity groups (found in 91% of Caucasian vs. 68% of African women) and depend on estrogen and glycogen levels. Studies during pregnancy reveal that pregnant women have higher abundance of *L. crispatus* and *L. iners* and also confirm that there is a positive correlation between increase of estrogen levels and stability of vaginal communities (Nunn and Forney 2016). Other species that may flourish in the vaginal environment include *Gardenerella vaginalis*, *Atopobium*, *Bifidobacterium*, *Corynebacterium* (Actinobacteria), *Enterococcus*, *Megasphaera*, *Peptostreptococcus*, *Staphylococcus*, *Veillonella* (Firmicutes), *Prevotella* (Bacteroidetes), *Escherichia* (Proteobacteria), and *Candida* spp. Microbial invasion of amniotic cavity is a common cause of intra--amniotic infection and the usual suspects are *Mycoplasma hominis* and *Ureoplasma urealyticum* from the phylum of Tenericutes. Additional species may include *Fusobacterium*, *Leptotrichia*, *Sneathia* (Fusobacteria), *Gardenerella vaginallis*

(Actinobacteria), *Bacteroides* (Bacteroidetes), *Streptococcus* (Firmicutes), and *Candida* spp. (DiGiulio 2012; Zhou et al. 2004).

Bacteria are the prominent members of the human microbiome and therefore extensively studied, yet there is a growing interest about the viruses and the archaea that cohabit the human gut. The human virome includes phages, prophages, eukaryotic viruses, and retroviruses (Vemuri et al. 2020), while it is also considered that a "core-phageome" exists and consists mainly of double-stranded DNA viruses of the order Caudovirales (families Myoviridae, Podoviridae, Siphoviridae) and single--stranded DNA viruses (family Microviridae) (Manrique et al. 2016). The eukaryotic virome contains species of the families Adenoviridae, Anelloviridae, Astroviridae, Parvoviridae (genus *Bocavirus*), Picornaviridae (genus *Enterovirus*), and Picobirnaviridae (Vemuri et al. 2020; Matijašić et al. 2020). Considering the archaea, there are four commensal species with *Methanobrevibacter smithii* as the dominant species of the gut, followed by *Methanosphaera stadtmanae*, *Methanomassiliococcus luminensis* (fecal samples) and *Methanobrevibacter oralis* (oral mucosa). There are also two nonmethanogenic species found namely *Haloferax massiliensis* and *Haloferax assiliense* and several members of the orders Methanosarcinales, Methanobacteriales, Methanococcales, Methanomicrobiales, Methanopyrales, Desulfurococcales, Sulfolobalales, Thermoproteales, Nitrosphaerales, and Halobacteriales (Matijašić et al. 2020).

1.3 Function of the Microbiome

A wide range of microbes reside in the human body, composing a complex and dynamic system that is associated with numerous functions such as vitamin production, metabolic processes, regulation of the immune response, and protection against pathogens perturbation (Li et al. 2016; Kho and Lal 2018). Most of these microorganisms have developed a symbiotic relationship with the host and they are not harmful, yet some of them are potential pathobionts, meaning that under certain conditions or relocation can be responsible for various diseases. At this point it should be noted that even though the terms microbiota and microbiome are interchangeable throughout international literature they are equally distinct. Microbiota refers to the community of microorganisms that live in an individual's body and is composed of bacteria, archaea, viruses, fungi, and other eukaryotes, whereas microbiome refers to the collection of genomes and genes present in the microbiota (Gordon 2012).

The gut microbiota is responsible for the fermentation of complex carbohydrates, indigestible polysaccharides, and insoluble dietary fibers resulting in the production of short chain fatty acids (SCFAs) (Donia and Fischbach 2015; Lee and Hase 2014). SCFAs (acetate, propionate, and butyrate) serve as energy metabolites for colonocytes, as their implication in water and electrolyte absorption contributes to a large extent in the mitochondrial ATP production (Dumas 2011), prevent impairment of intestinal barrier and provide protection against pathogens (e.g. butyrate inhibits yeast to hyphae transition of *C.albicans*) (Swidergall and Ernst 2014). Energy is

also provided from the glycosaminoglycan degradation and is supplied to liposaccharides (LPS) synthesis, which are vital components of the outer membrane of Gram-negative bacteria (Poole 2002).

The gut bacteria are also essential in the metabolism of bile acids, the production of antimicrobial proteins (AMPs), and the synthesis of essential amino acids and vitamins. Primary bile acids are synthesized in the liver, secreted into the intestine tract where they are mostly reabsorbed, while the unabsorbed part is bioconverted to secondary bile acids by bacterial enzymes (e.g. from *Clostridium perfringens*) and the secondary bile acids are then transported back to the liver (Ajouz et al. 2014; Gopal-Srivastava and Hylemon 1988). Epithelial cells of the gut, skin, and respiratory tract produce a group of proteins with antimicrobial properties (AMPs) that act as natural antibiotics. Defensins, cathelicidins, and C-type lectins are among the most common AMPs that aim to the disruption of the microbial cell wall (or membrane). Apart from their direct actions against pathogens, AMPs act as mediators of inflammatory responses through their chemotactic activity on leukocytes and interaction with TLR ligands (Gallo and Hooper 2012).

Vitamins are indispensable for metabolic processes and gut microbiota along with food-supplied lactic acid bacteria help producing them in the human body. Species from the genera of *Lactobacillus*, *Bifidobacterium*, *Bacillus* and *Escherichia* are involved in the synthesis of menaquinone (vitamin K_2), riboflavin (vitamin B_2), pantothenic acid (vitamin B_5), folate (vitamin B_9) and cobalamin (vitamin B_{12}) (LeBlanc et al. 2013). Vitamin K is essential in reducing vascular calcification, increasing HDL and decreasing cholesterol levels thus confining the risk for cardiovascular disorders (Geleijnse et al. 2004; Kawashima et al. 1997). Members of the vitamin B complex act as coenzymes for key metabolic pathways and it is worth mentioning that vitamins B_5 and B_{12} are exclusively synthesized by the gut microbiome (Andrès et al. 2004; Gominak 2016).

Aside from bacteria, archaea participate in the anaerobic fermentation producing SCFAs, CO_2, and H_2 (Samuel and Gordon 2006). Methanogens then use H_2 and CO_2 for methanogenesis, a process that results in improved bacterial fermentation, complete anaerobic degradation of organic substances, and inflammatory responses. It has been recently documented that *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* are implicated in monocyte-derived dendritic cell maturation and their subsequent pro-inflammatory cytokine release (Chaudhary et al. 2018), whereas *Methanomassiliococcus luminyensis* could degrade trimethylamine (TMA) (Borrel et al. 2017) and reduce TMA-N-oxide plasma levels impeding cardiovascular and chronic kidney diseases (Liu et al. 2015; Tang et al. 2015).

Interaction between intestinal microflora and host immune system is being extensively studied since disturbance of this homeostatic relationship could lead to pathogenesis. It has been reported that a key regulator of intestinal homeostasis is the balance between T regulatory cells (T_{reg}) and T helper 17 cells (T_{H17}). Firmicutes as well as *Bacteroides fragilis* and *Bifidobacterium infantis* promote maturation of T_{reg} cells, which suppress aberrant T_{H17}-induced inflammation. Hence T_{reg}/T_{H17} ratio, along with SCFAs, maintain the integrity of the intestinal barrier against immune inflammatory response (Atarashi et al. 2008; Chen et al. 2017; El Aidy et al. 2012;

Lawley and Walker 2013; Paust et al. 2004; Peng et al. 2007). Enteric nervous system (ENS) is comprised of enteric glial cells (EGCs) which are astrocyte-like cells that control exocrine/endocrine secretions, gut motility, blood flow, and inflammation (Ochoa-Cortes et al. 2016; Yu and Li 2014). Malfunction of ENS and EGCs could lead to disruption of intestinal barrier, motility disorders (e.g. constipation), various GI disorders (e.g. IBD, IBS), or infection-induced gut inflammation (Kho and Lal 2018).

Commensal fungi are also involved in the immune system both directly by interacting with the immune cells and indirectly by regulating essential metabolites (Lee and Mazmanian 2010). The role of Candida species is ambiguous as *Candida kefyr* reduces IL-6 production thus attenuating gut inflammation (Takata et al. 2015), whereas *Candida albicans* produces ligands (e.g. β-1,3 glycan) for pattern recognition receptors (PRRs) that stimulate host cells to secrete prostaglandins and inflammatory cytokines (Lee and Mazmanian 2010). *C. albicans*-produced prostaglandin E2 is transferred through the bloodstream to the lungs where it acts on macrophages inducing allergic airway inflammation (Kim et al. 2014). Conversely, *Saccharomyces boulardii* stimulates intestinal anti-toxin IgA (Qamar et al. 2001), IL-10, and EGF production (Thomas et al. 2011) and decreases the secretion of proinflammatory cytokines (e.g. TNFα, IL-6) exerting a protective role against gut inflammation (Thomas et al. 2011).

Intestinal microbiota accounts for the defense of the host against perturbation of pathogenic invaders or overgrowth of pathobionts. This could be achieved through competition of human microbiome and pathogens for common habitats and nutrients ("competitive exclusion") or by activating the host immune system (Kho and Lal 2018; Belzer and de Vos 2012). Competition is often observed between *Lactobacillus* and fungal overgrowth in the gut or vagina (Rizzo et al. 2013). In terms of immune system modulation, *Saccharomyces boulardii* secretes enzymes to inactivate toxins produced by *Clostridium difficile* and *E. coli* (Buts et al. 2006; Castagliuolo et al. 1999) and inhibits proliferation of *C. albicans*, *Salmonella typhimurium*, and *Yersinia enterocolitica* (Enaud et al. 2018). Therefore, trans--kingdom interactions are responsible for maintaining the balance of the healthy human microbiome (Lloyd-Price et al. 2016).

Skin microbiota has been assigned to survive in an acidic environment, with ultraviolet light exposure and minimum nutrients (basic proteins and lipids). Sweat, sebum, and stratum corneum are their main resources and microbes have been adapted to utilize them for their benefit. Keratinocytes are in the first line of defense and occupy PRRs that can sense pathogenic microbial molecules and promote the excretion of AMPs to attack potential invaders. Moreover, recruitment of T cells in response to microorganisms' presence could occur in the absence of classical inflammation ("homeostatic immunity") (Byrd et al. 2018).

Oral cavity is heavily colonized by commensal microbiome and an inquisitive potential of oral bacteria is the reduction of nitrate to nitrite contributing to cardiovascular health. Oral bacteria facilitate the fermentation of dietary carbohydrates, which leads to reduction of pH. Microbial species of oral cavity as units are unable to process complex substrates, so instead they cooperate and combine their

enzymatic activities for food digestion. Streptococci can remove oligosaccharides and glycoproteins, Gram-negative anaerobic species (e.g. Prevotella, Porphyromonas) cleave proteins to peptides, whereas Fusobacterium and Peptostreptococcus ferment amino acids producing SCFAs. Disturbance of the oral cavity microenvironment could cause a shift in the composition of oral microbiome resulting in dental caries or other periodontal diseases. Opportunistic infections by *Candida* and *Staphylococcus* can still be caused, especially following antimicrobial treatment (Wade 2013).

Vagina confers an excellent residence for microorganisms as vaginal secretions are loaded with amino acids, carbohydrates, mucins, proteins, and glycoproteins. However, this content is highly influenced by the host physiology thus directly affecting the composition of vaginal microbiome. Estrogen levels control the accumulation of glycogen and the proliferation rate of *Lactobacillus*. Glycogen is depolymerized by α-amylase into simple sugars which in turn are fermented by vaginal *Lactobacilli* to produce lactic acid. Lactic acid creates an acidic environment which is not favorable for nonindigenous microorganisms. The origin (human or microbial) of α-amylase and whether glycogen is indirectly supplied to *Lactobacilli*, after it is metabolized by other microbes, or is accumulated due to the inability of *Lactobacilli* to directly use it remains uncertain and future studies would elucidate these issues (Nunn and Forney 2016).

State-of-the-art technology has conferred great advantages toward data acquisition, and considering the aforementioned, it is obvious that microbiota is an indispensable part of the human physiology and that several pathologies occur as a consequence of the disturbance in the dynamic equilibrium between host and microbes.

1.4 Microbiome and Dysbiosis

Research in the field of commensal gut microbiome ecology attempted to identify a group of microbial taxa universally present in healthy individuals but this pursuit proved infeasible. Conversely, the alternative hypothesis of a healthy "functional core" was proposed, describing a complement of metabolic and other molecular functions that are performed by the microbiome within a particular habit but are not necessarily provided by the same organisms in different people (Shafquat et al. 2014). In accordance to this statement, a healthy-associated microbiome requires a degree of resistance against external (e.g. dietary, pharmaceutical) or internal (e.g. age) changes and the ability of resilience afterwards. Therefore, microbial health comprises not a single static state but rather a dynamic equilibrium (Lloyd-Price et al. 2016).

Perturbation of this equilibrium exerts imbalance in the composition and regulation of microbial communities, a term which is widely known as dysbiosis. Dysbiosis is more likely to occur in response to insufficient presence of commensal microbes, loss of regular microbial diversity or competition between commensal microbiome and pathogenic species for the same colonization sites and/or nutrients supply

(Tamboli et al. 2004). Other external factors that contribute to the progression of dysbiotic features include malnutrition or lack of dietary fibers and vitamins, certain food additives (e.g. preservatives, emulsifiers), chronic alcohol consumption, use of drugs or pharmaceuticals (antibiotics, anti-inflammatories, contraceptives, chemotherapy), exposure to toxic environmental substances (chemical toxins, heavy metal, radiation), and stress levels (anxiety, depression). Dysbiosis is implicated in diverse pathologies, a number of which are briefly reported in the following sections.

1.4.1 Diet

Consumption of food is related to providing the body with a range of nutrients in order to perform fundamental metabolic processes. Anthelme Brillat-Savarin, in 1826, wrote in his book The Physiology of Taste, "Tell me what you eat and I will tell you what you are," implying that eating what is regarded as being healthy your organism will be healthy as well. Bearing in mind that intestinal microbiota is involved throughout the route of food processing, presuming that gut colonization by beneficial microbial communities is favored by the consumption of healthy nutrients (e.g. plant fibers, complex carbohydrates) supports further this argument. Diet is a complex concept that depends on geographical restrictions, ethnic and cultural customs, or even moral constraints, but irrespective of what lifestyle individuals choose to follow as adults, their gut microbiome is established from the very moment they were born.

Microbes are present in the placenta (DiGiulio 2012), amniotic fluid (Satokari et al. 2009) and umbilical cord blood (Jiménez et al. 2005) and their colonization starts in utero, although the adult-like configuration occurs after the first three years of life (Yatsunenko et al. 2012), therefore delivery mode and feeding methods of infants seems to have higher impact. Vaginally delivered infants acquire their mother's vaginal microbiome, whereas caesarean delivered infants are encountered with the skin microbiota of the mother. Infants born vaginally have higher prevalence of Bacteroidetes over Firmicutes compared to infants delivered thought caesarean section (Dominguez-Bello et al. 2010), while the latter show higher microbial diversity, delayed colonization of Bacteroidetes (Jakobsson et al. 2014) and an enrichment of pathobionts such as *Enterobacter cancerogenus*, *Haemophilus* spp, *Staphylococcus* spp, and *Veillonella dispar* (Dominguez-Bello et al. 2010; Bäckhed et al. 2015).

Breastfeeding favors the growth of Bifidobacterium, Bacteroides, and microbes that are transmitted after contacting the maternal skin (Dominguez-Bello et al. 2010; Zivkovic et al. 2011). Human breast milk is a complex of undigestible oligosaccharides that serve as a resource of prebiotics especially for *Bifidobacterium* species (*B. breve*, *B. adolescentis*, *B. longum*, *B. bifidum*, *B. dentium*) (Martín et al. 2009). Formula-fed infants are often colonized by *E. coli* and *Clostridium difficile* (Penders et al. 2006) and their fecal samples contain more anaerobic or facultative anaerobic microbes compared to that of breast fed infants (Stark and Lee 1982). Early establishment of infant gut with SCFA-producing species, such as *Bacteroides*, *Bifidobacterium*, *Lactobacillus* and *Faecalibacterium*, is indicative of a healthy

microbiome (Byrne et al. 2015). Dietary changes, illness or antibiotic treatment could induce a shift in the microbial composition during infancy which is associated with higher risk of asthma, atopic eczema (Abrahamsson et al. 2012) and allergic rhinitis (Bisgaard et al. 2011).

Bacterial community composition gradually shifts from Bifidobacterium--dominated in infancy to Bacteroidetes and Firmicutes dominance in adulthood and remains relatively stable (Ottman et al. 2012). However, recession of gastrointestinal function over senescence affects gut microbiome, with limited presence of *Bacteroides*, *Bifidobacteria*, and *Clostridium* cluster IV in elderly, yet higher prevalence of Bacteroidetes compared to the abundance of Firmicutes in younger adults (Zwielehner et al. 2009). As opposed to age, nutritional value has a greater influence on microbiome configuration. High protein intake is associated to increased *Bacteroides*, *E. coli*, and *Enterobacteria*, while growth of *Candida* species is positively correlated with carbohydrate consumption and negatively correlated with saturated fatty acids (Hoffmann et al. 2013). Vegetarian or vegan diet is enriched in carbohydrates and insoluble fibers that are fermented into SCFAs, leading to lower luminal pH, which is inhibitive for *E. coli* or *Enterobacteria* (Cresci and Bawden 2015) but favorable for the plant pathogenic *Fusarium*, and the fungal species of *Malassezia*, *Aspergillus*, and *Penicillium* (Hoffmann et al. 2013). Dietary habits are also affected by the availability of food resources. A study comparing European and African children concluded that there are differences in their gut microbiomes, with higher levels of Firmicutes and Proteobacteria in European compared to predominance of Actinobacteria and Bacteroidetes in African (De Filippo et al. 2010). Although SCFA-producing species were found in both groups, African children were exclusively colonized by *Xylanibacter*, *Prevotella*, *Butyrivibrio*, and *Treponema*, which utilize xylene, xylose, and carbomethylcellulose to produce SCFAs, resulting in fourfold increase in levels of butyrate and propionate (Flint et al. 2008).

Obesity is a medical condition where energy intake (food) exceeds the energy expenditure (thermogenesis) resulting in excess body fat accumulation (Maruvada et al. 2017) and is associated with abnormalities in the composition of human microbial communities. Significantly increased abundance in the butyrate-producing Firmicutes and reduction in Bacteroidetes has been observed in distal colonic microbiome of obese patients. Elevated levels of Firmicutes are attributed to higher levels of class Mollicutes (phylum Tenericutes) species (Turnbaugh et al. 2006). Biodiversity of fungal species is also altered, notably decreased in the Zygomycota phylum, with prevalence of *Nakareomyces*, *Candica*, *Penicillium*, and *Pichia* in obese patients compared to *Mucor*, *Candida*, and *Penicillium* in non-obese (Mar Rodríguez et al. 2015).

Type 2 diabetes (T2D) is a metabolic disorder of insulin resistance that is linked to obesity and changes in the gut microbiome are implicated in T2D development (Karlsson et al. 2013; Larsen et al. 2010; Qin et al. 2012). Increased Bateroidetes/Firmicutes ratio, abundancy of Betaproteobacteria species and significantly lower proportion of *Clostridia* have been documented in T2D patients versus nondiabetic controls (Larsen et al. 2010). Higher percentage of butyrate-producing species such

as *Feacalibacterium prausnitzii*, *Roseburia intestinalis*, and *R.inulinivorans* has been also observed in healthy individuals compared to greater colonization of pathobionts including *Eggerthela lenta*, *Clostridium symbiosum*, and *E. coli* in T2D patients (Qin et al. 2012). Significant reduction of Verrucomicrobia has been noticed in prediabetes subjects suggesting that assessment of Verrucomicrobiaceae concentration could be potentially used as a diagnostic biomarker for progression of T2D (Zhang et al. 2013).

1.4.2 Antibiotics

Antibiotics are antimicrobial compounds that either target the bacterial cell wall/membrane or interfere with bacterial essential enzymes thus inhibiting their growth (bacteriostatic agents) or block bacterial protein synthesis and immediately kill them (bactericidal agents). Narrow-spectrum antibiotics affect specific types of bacteria (e.g. Gram positive), whereas broad-spectrum target a wider range of bacteria (Kohanski et al. 2010). Use of broad-spectrum antibiotics that affect anaerobic bacteria is correlated with growth of yeast flora in the gut compared to antibiotics with poor anaerobic activity (Samonis et al. 1993). Treatment with antibiotics could be detrimental not only for the targeted pathogen but also for the hosts' bacterial community resulting in both short- and long-term effects on human microbiome (Jernberg et al. 2010). One approach indicates the introduction of a new species, whereas the other suggests alteration in the bacterial resistance genes (Antonopoulos et al. 2009; Jakobsson et al. 2010; Robinson and Young 2010).

Resistance is categorized as active (e.g. adapting to a counterattack against an antibiotic) or passive (antibiotic-independent adaptations). Active antibiotic resistance is achieved through efflux of the drug from the cell via membrane-associated pumping proteins, modification of the drug target (e.g. mutation of rRNA) or synthesis of modifying enzymes that impede with the drug activity (Wright 2005). Gram-negative bacteria are shielded with a bacterial outer membrane, constituted of porins and liposaccharide (LPS), and that often confers intrinsic resistance to species like *E. coli*, *Pseudomonas aeruginosa*, *Burkolheria* sp., *Stenotrophomonas maltophilia*, and *Acinetobacter* sp. Antibiotic resistance genes are typically found in Firmicutes (52%), Proteobacteria (32%), and Bacteroidetes (15%). Recently, studies have identified 1093 genes that confer resistance to 50 of the total 68 antibiotic groups and most of these genes code for proteins that modify or protect the target of the antibiotic (Quinn 1998).

Clostridium difficile infection (CDI) is a gastrointestinal disease, strongly correlated to antibiotic treatment, caused by the *Clostridium difficile*, with symptoms of diarrhea and pseudo-membranous colitis and is the most common cause of hospital-acquired diarrhea (Kho and Lal 2018; Di Bella et al. 2015). *Clostridium difficile* is a Gram-positive, anaerobic, sporogenic, and toxin-producing bacterium that belongs to the Firmicutes. Under steady state, overgrowth of *C. difficile* is prevented by colonization resistance of commensal gut microbiome, presumably by metabolizing primary bile acids to secondary bile acids. It is proposed that primary

bile acids (cholate derivatives) serve as germinant for *C. difficile* spores, while secondary bile acids (deoxycholate) inhibit its growth (Song et al. 2008). Antibiotic treatment results in lower diversity of secondary bile acids-synthesizing microbes (e.g. C. Scindens) and a subsequent reduction of microbial bioconversion of primary bile acids to secondary bile acids, allowing *C. difficile* overgrowth (Antonopoulos et al. 2009; Theriot et al. 2014). Secretion of toxins A and B (TcdA and TcdB) produced by *C. difficile* causes damage to the cytoskeleton and colonial epithelial barrier integrity (Genth et al. 2006; Pruitt et al. 2012), followed by severe inflammatory response that induce impairment in intestinal ion absorption leading to diarrhea (Kho and Lal 2018).

1.4.3 Lifestyle

Stress is a situation that triggers a biological response to a specific demand or threat. Physiological and psychological stressors activate the hypothalamic-pituitary--adrenal (HPA) axis (Lucassen et al. 2014: 100). The gut microbiota is sensitive to stress mediators responding to the release of stress-related neurotransmitters or acting as carriers of neuroactive compounds (Lyte et al. 2011). Exercise is a physiological stressor that is beneficial for the healthy microbiome, yet high intensity training is extremely stressful for the body and that may prompt alterations in microbial communities or intestinal barrier aggravation (de Oliveira et al. 2014). Professional athletes follow a strict dietary plan of high protein and caloric intake which positively correlates with enhanced gut microbial diversity and interestingly that was reflected by the presence of 22 bacterial phyla compared to 11 and 9 phyla in the low and high Body Mass Index (BMI) controls, respectively. However, prolonged excessive training may lead to intestinal hypoperfusion, increased intestinal permeability, and endotoxin translocation (Gleeson and Williams 2013).

The human GI tract function is governed by millions of neurons that comprise the enteric nervous system (ENS), which is the second largest pool of neurons, outside the brain (Spencer et al. 2018). The ENS propagates and receives signals from the central nervous system (CNS) through the parasympathetic (via the vagus nerve) and sympathetic (via the prevertebral ganglia) nervous systems, but has also the ability to operate independently, therefore it has been characterized as a "second brain" (Li and Owyang 2003). The interplay of biochemical signaling between ENS and CNS along with the association of gut microbiome is commonly described by the term "gut–brain axis" (Mayer et al. 2014). This axis includes neuronal, endocrine, immune and metabolic pathways that are intertwined and collectively regulate the functioning of each other, maintaining homeostasis. Alterations in microbial communities or other physical and psychological stressors that interfere with the proper function of the axis are held responsible for dysbiotic features (Sommer and Bäckhed 2013).

There are numerous mechanisms by which intestinal microflora affects the gut–brain axis contributing to the pathogenesis of functional gastrointestinal disorders (e.g. IBS) (Martinucci et al. 2015) or even CNS diseases (e.g. anxiety, depression)

(Pirbaglou et al. 2016). It is noted that gut microbiota is capable of producing neurotransmitters that can either act locally or cross the mucosal intestinal layer and exert their actions in other systems (Wall et al. 2014). *Lactobacillus* and *Bifidobacterium* synthesize and release GABA; Bacillus, *S. cerevisiae*, and *Penicillium chrysogenum* produce norepinephrine; while serotonin can be synthesized by *Candida*, *Streptococcus*, and *Enterococcus* spp. (Tetel et al. 2018) A study proposed that serotonergic enterochrommafin cells in the gut epithelium act as chemosensors and transduce chemosensory information to the nervous system (Bellono et al. 2017). *C. albicans* is also able to produce histamine, a neurotransmitter involved in appetite regulation, circadian rhythm, and cognitive activity (Voropaeva 2002).

Activity of HPA axis can also be impacted by commensal gut microbiome, probably through microbial secretion of cytokines (e.g. IL-1, IL-6) and subsequent acute release of cortisol by HPA axis stimulation (Dantzer 2006). Persistent activity of HPA axis and increased levels of cortisol are highly correlated with anxiety and depression. Decreased microbial richness and diversity is observed in patients diagnosed with depression along with changes in colonization by specific taxa. Depressed patients are characterized by higher levels of Bacteroidetes, Proteobacteria and Actinobacteria and lower levels of Firmicutes compared to controls. The same study revealed increased levels of Enterobacteriaceae (Proteobacteria) and Alistipes (Bacteroidetes) and reduced proportion of Faecalibacterium (Firmicutes) (Jiang et al. 2015). However, there is a limited number of human studies concerning the effect of gut microbiome in behavioral disorders and further research is required.

The oral microbiota is extensively affected by smoking (Monteiro-da-Silva et al. 2013) and eating disorders (ED) (Back-Brito et al. 2012). Smoking is a causal factor for periodontitis and many species are associated with this disease, such as *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Anaeroglobus germinatus*, *Eubacterium saphenum*, *Filifactor alocis*, *Porphyromonas endodontalis*, and *Prevotella denticola* (Kumar et al. 2003: 80). *Candida* is present in fecal samples of smokers (58%) more frequently than in nonsmokers (29%) (Jobst and Kraft 2006). Opportunistic oral candidiasis is common to ED patients and is attributed to nutritional deficiencies in Zn, Fe, vitamin K, and water-soluble vitamins (Ghannoum et al. 2010; Lo Russo et al. 2008). Although there is a link between alcohol and fungal colonization in gut, there was no association in oral cavity (Hoffmann et al. 2013).

1.4.4 Human Genetics

1.4.4.1 GI Tract

Inflammatory bowel disease (IBD) is a group of gastrointestinal inflammatory conditions, featuring Crohn's disease (CD), in which inflammation can occur anywhere in the GI tract and ulcerative colitis (UC), which affects mainly the colon (Baumgart and Carding 2007). IBD probably emerges as repercussion of the abnormalities in host defense against commensal microbiome of genetically predisposed subjects

(Kho and Lal 2018). Normally GI mucus layer and AMPs, such as human defensins, cooperate to hinder direct interaction between luminal gut microbiota and epithelial cells preventing inflammatory responses. Dysbiotic impairment of the intestinal mucus barrier induces the growth of mucolytic bacterial species (e.g. *Ruminococcus* sp.) (Png et al. 2010) promoting gut inflammation (Johansson et al. 2008: 70; Salzman et al. 2010).

A tendency for higher portions of Actinobacteria and Proteobacteria (family Enterobacteriaceae) with a subsequent decrease in Firmicutes (family Lachnospiraceae) and Bacteroidetes is observed in IBD patients (Frank et al. 2007; Willing et al. 2010). Firmicutes is comprised of important butyrate-producing and anti-inflammatory bacteria that reduce the secretion of pro-inflammatory cytokines (IL-12, IFN-γ) and induce the production of anti-inflammatory IL-10 (Machiels et al. 2014; Sokol et al. 2008). IBD patients have lower proportions of *Feacalibacterium prausnitzii*, *Roseburia* sp., *Dialister invisus* (Firmicutes) and *Bifidobacterium adolescentis* (Actinobacteria) (Willing et al. 2010; Machiels et al. 2014; Joossens et al. 2011). Conversely, colonization is favored for *Ruminococcus gnavus* (Firmicutes), which produces a glucorhamnan recognized by innate immune cells (Henke et al. 2019), *Bacteroides fragilis* (Bacteroidetes) and members of the Enterobacteriaceae family, which have both highly endotoxic LPS on their outer membrane (Darfeuille-Michaud et al. 1998).

Fungal dysbiosis has also been noticed on IBD patients, with higher Basidiomycota/Ascomycota ratio, abundance of *C. albicans*, *Malassezia symbodialis* and reduction in *Saccharomyces cerevisae*. It has been observed that fungal and bacterial interactions are higher in UC patients and lower in CD patients (Sokol et al. 2017). Studies documented that there was greater fungal richness and diversity in inflamed mucosa versus noninflamed mucosa of CD patients and compared to healthy controls (Li et al. 2014; Ott et al. 2008). CD patients had a positive correlation with *C.glaberata* (Liguori et al. 2016) and also anti-*Saccharomyces cerevisiae* antibodies (ASCA) have been detected in their serum (Main et al. 1988). In pediatric IBD patients there is a dominance of Basidiomycota (Mukhopadhya et al. 2015) compared to the prevalence in Candida parapsilopsis and Cladosporium cladosporoides in healthy children (Chehoud et al. 2015).

Archaeal overgrowth results in reduction of butyrate and increased removal of SCFA from biofilms, prompting bacteria to become endoparasitic and invade intestinal epithelial tissue, triggering gut inflammation (Gonçalves et al. 2018; White 2017). *Methanobrevivacter smithii* levels are lower in IBD patients compared to healthy individuals (Ghavami et al. 2018). Virome is also implicated in IBD pathology with higher proportions of phages affecting Bacterial Alteromonadales, Clostridiales (*C. acetobulicum*), and Herpesviridae (increase of HBx protein) (Pérez-Brocal et al. 2015; Ungaro et al. 2019). Decreased Vigaviridae and Polydnaviridae, Tymoviridae are detected in CD and UC patients respectively, whereas in the latter there is increased abundance of Pneumoviridae and Anelloviridae (Pérez-Brocal et al. 2015; Ungaro et al. 2019; Zuo et al. 2019). UC patients are also less colonized by *Blastocystis hominis* and *Dientamoeba fragilis* (Petersen et al. 2013).

Irritable bowel syndrome is a functional gastrointestinal disorder with three subtypes: constipation-subtypes (IBS-C), diarrhea-subtypes (IBD-D), and mixed-type (IBD-M) (Longstreth et al. 2006). IBS and IBD are two distinct conditions, despite sharing similar symptoms, yet they are both associated with gut microbiota dysbiosis. Enrichment of Firmicutes and reduction of Bacteroidetes is observed in IBS patients (Jeffery et al. 2012), with Lachnospiraceae (Krogius-Kurikka et al. 2009) and *Veillonella* (Malinen et al. 2005) expressing higher abundance in IBS-D and IBS-C patients respectively. IBS patients have also higher proportion of *Dorea*, *Ruminococcus*, *Clostridium*, and lower proportion of *Bifidobacterium*, *Faecalibacterium*, and methanogens compared to healthy controls (Rajilić--Stojanović et al. 2011). The pathogens Pseudomonas aeruginosa and Staphylococcus aureus are two possible candidates for IBS pathology (Kerckhoffs et al. 2011; Rinttilä et al. 2011). Moreover, IBS-C patients have greater abundance of methane producer archaea, especially *M. smithii* and *M. stadtmanae*, compared to IBS-D patients.

Individuals with IBD are at increased risk of developing colorectal cancer (CRC), consequently changes in composition of microbial communities are also implicated in this disease (Hu et al. 2015). Non-colitogenic *Fusobacterium nucleatum* and enterotoxigenic strains of *Bacteroides fragilis* are markedly enriched in CRC patients (Toprak et al. 2006; Wang et al. 2012; Wu et al. 2013). Conversely, butyrate-producing *Feacalibacterium* and *Roseburia* are less expressed, which is associated with partial impairment of immunosurveillance and enhancement of tumorigenesis (Wang et al. 2012; Wu et al. 2013). Considering fungal mycobiome, there is an increase of Basidiomycota/Ascomycota ratio, depletion of *S. cerevisae* and enrichment of *Rhodotorula*, *Malassezia*, *Acremonium*, and *Aspergillus flavus* in CRC patients. Mycobiota differentiation has also been noted according to adenoma size and stage. Advanced adenoma biopsy samples have less diversity and increased abundance of Saccaromycetales, while nonadvanced adenoma tissues have lower proportion of Fusarium and Trichoderma, compared to adjacent rectal tissue (Luan et al. 2015).

Celiac disease is a serious autoimmune disease that occurs in genetically predisposed people, where the ingestion of gluten leads to damage in the small intestine. Significant reduction in total Gram+/Gram− bacteria ratio is observed in all phases of celiac disease, with less *Bifidobacteria* and more *Bacteroides*/*Prevotella* groups (De Palma et al. 2010; Marasco et al. 2016; Nadal et al. 2007). Studies in human colon Caco-2 cells demonstrate that gliadin, a component of gluten, induces increased gut permeability and *Bifidobacterium lactis* protects the epithelial junctions from the adverse gliadin-induced effects (Lindfors et al. 2008), whereas *Bifidobacterium longum* and *Lactobacillus casei* can regulate the production of pro--inflammatory cytokines and reduce the risk for gliadin-induced enteropathy in animal models (Laparra et al. 2012).

1.4.4.2 Neurodevelopmental

Autism spectrum disorder (ASD) is a range of neurodevelopmental disorders including autism and Asperger syndrome. ASD is significantly associated with

intestinal dysfunction and microbiome dysbiosis (Wang et al. 2011) and impaired tyrosine kinase MET signaling is potentially implicated (Ieraci et al. 2002; Okunishi et al. 2005). Higher levels of *Clostridium histolyticum*, *Bacteroides*, *Lactobacillus*, and *Desulfovibrio* (a sulfate-reducing bacterial genus) (Finegold et al. 2012) and lower levels of *Bifidobacteria*, carbohydrate-degrading *Prevotella*, *Cryptococcus*, and unclassified Veillonaceae have been reported in ASD children compared to control (Adams et al. 2011; Kang et al. 2013; Parracho et al. 2005; Song et al. 2004). Increased levels of *Sutterella* (Proteobacteria) were solely reported in children experiencing both autism and GI dysfunction but not in children with mere GI dysfunction (Williams et al. 2012).

Intestinal microbiome dysbiosis appears evident in neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's (PD). Changes in SCFA concentration (Unger et al. 2016) and altered levels of species belonging to the families of Bifidobacteriaceae, Lachnospiraceae, Lactobacillaceae, Pasteurellaceae, Christensenellaceae, and Verrucomicrobiaceae are detected in PD patients (Hill-Burns et al. 2017). Likewise, AD patients with brain amyloidosis show low proportion of the anti-inflammatory Eubacterium rectale and higher proportions of the pro-inflammatory *Escherichia/Shigella* (Cattaneo et al. 2017).

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