The Human Microbiome Handbook

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Preface

THE term "microbiome" has been in use for over 50 years but only in the last 15 years has it gained popularity in the health community. The word describes the totality of microorganisms in and on a particular environment. In humans, this totality includes the gastrointestinal tract (including the mouth), the skin, the respiratory tract, the genitalia, and even the ocular surface. But while this singular concept has garnered significant attention, our understanding of the scope in terms of public health and medicine continues to be enigmatic.

For over a century we have known microbes play a role in our lives, although for the majority of this span, the focus has been on infection or, ecologically speaking, parasitism. We now know the number of pathogens amounts to only a tiny fraction of the entirety of the microbial species on earth and less than one-tenth of the microbes associated with the human body. The rest have been primarily studied outside of the realm of human health with discoveries limited to journals focusing on microbiology rather than medicine.

Over the last 40 years, we have seen a burgeoning increase in the number of scientific articles examining the interaction of microbes and humans in terms of "commensalism" as well as "mutualism"; ecological terms that now also apply in the field of medicine and public health because of a deeper appreciation of the microbial ecology of the body. We are not solely made up of 37 trillion human cells; we also have microbes totalling up to three times that number. Through observation at the lab bench, in animal models, and clinical trials, we are learning how these two very different organisms—mammal and microbe—in-

teract. More importantly, we have a growing understanding of how this interkingdom interface affects acute as well as chronic health outcomes.

The Human Microbiome Handbook was conceived as an examination of our knowledge about the microbial influence in public health. Though the amount of data continues to increase at a staggering rate, many trends of microbe-human interaction have become solidified. These are duly explored within the pages of this book. The range of topics encompasses many branches of medicine from gastroenterology to metabolism to immunology and mental health. In each chapter, the authors, all of whom are experts in their individual microbiome fields, provide the latest findings and, where applicable, mechanism-based explanations. All told, this compilation will provide any medical or health professional with the necessary knowledge and applicable references to ensure a well-rounded appreciation of the microbiome and its impact on our health.

Many health professionals have only a rudimentary understanding of the microbiome. This book has been designed to ensure all individuals can access the most pertinent information in the field. This has been accomplished by separating the book into three sections, beginning with a general overview of the microbiome and gradually moving to specific mechanisms, including discussions on disease and possible therapeutics. In this way, it is our hope that any reader, regardless of academic background, will be able to gain enough information for use in their future work and practice.

The first section provides an introductory perspective on the microbiome in which a more general observation of the knowledge is provided. Chapter 1, by one of the pioneers of microbiome research, Sydney Finegold, is historical in nature, taking us through his journey in the field over five decades. Chapter 2, by Dutch researchers Kaludyna Borewicz and Hauke Smidt, provides an overview of the microbiome as a part of the human body. This chapter also introduces the concept of ecology in which microbial populations, not solely singular species, are now the focus of research. The final section provides an overview of the concept of our microbiome as more than a static entity. Chapter 3, headed by Paul O'Toole from Ireland, provides a longitudinal examination of the nature of the gut microbiome from birth to death.

The second section of this book examines the trends of microbial influence on our bodily processes. Vicky De Preter and Kristin Verbeke from Belgium examine first the microbial side of the interaction.

Chapter 4 takes a look at the life cycle of bacteria and how certain by-products can act not as waste but as useful stimuli for several associated biological systems. The effect of microbes and mental health is next examined in Chapter 5 by Canadian scientists, Aadil Bharwani, John Bienenstock, and Paul Forsythe. These researchers are forging the path to our understanding of how microbes in the gastrointestinal tract can affect our mental state and influence pathologies such as depression. The key to this may lie in immune system interactions, and Chapter 6 by Leando Lobo, Rosana Ferreira, and Caetano Antunes, from Brazil, explores this concept. Although much has already been learned as a result of traditional, infection-based work, incorporation of the microbiome into this field of study may lead to the development of microbially-mediated immune therapies. Finally in Chapter 7, Tinting Ju, Jiaying Li, and Benjamin Willing, from Canada, provide an examination of how microbes can modulate our metabolism. In the context of human health, microbes have a significant influence and may be the key to several chronic illnesses such as obesity, diabetes, and cardiovascular disease.

The third section deals specifically with disease and therapies. The theme in this section is "balance". As in all ecological environments, equilibrium of species is needed in order to attain harmony, and when this balance is disrupted, problems may ensue. We now understand the same applies to the human body and several diseases once thought to be mysteries have been elucidated on the basis of this lack of ecological balance. In addition, when the ecology is restored, balance can be re-established and health can be returned.

In Chapter 8, Spanish researchers, Claudia Herrera, Virginia Robles-Alonso, and Francisco Guarner examine the effects of microbes on our gastrointestinal health and how a change in ecology may lead to chronic health problems including inflammatory bowel disease, liver diseases, and antibiotic-mediated illnesses. In Chapter 9, Holly Ganz and Dawn Kingsbury, from the United States, explore one of the most hotly debated topics in microbiome research: epigenetics. Though this field is still relatively new, we are beginning to appreciate how microbes are not only influencing our cellular world, but also our genes. This chapter will examine what is already known and as well will explore several hypotheses to explain potential mechanisms behind some of our most problematic diseases.

In contrast to disease, Rowena Almeida and Elaine Petrof, from Canada, provide an in-depth look at one of the most discussed medical procedures today. Known as fecal microbiota transplantation, or FMT, this

process of restoring a balanced ecology in the gastrointestinal tract has of late gained significant notoriety. Chapter 10 will unveil the mechanisms, reveal the benefits and drawbacks, and dispel the myths. Apart from FMT, the other major interest for health professionals is the realm of probiotics. Canadian scientist Gregor Reid, in Chapter 11, will provide an examination of the nature of probiotics—what they are, what they are not—and will explore the beneficial properties of these special microbes. He will also provide a critical perspective on questions associated with their use and where gaps in our understanding may be filled.

The end of this book offers a positive outlook for the future. We are still only beginning to understand the scope of microbial influence on our health and illness. As we continue to explore the once-hidden ecology within our bodies, we will unveil even more incredible mechanisms and possibly routes to novel and perhaps even revolutionary therapies. Although we have come far in the short period of time since Lederberg introduced the microbiome terminology to the world, we also know the journey will extend long into the future and change the face of health practice. *The Human Microbiome Handbook* will enable anyone to join the journey, if only as a witness, and to gain awareness and readiness for the marvels that undoubtedly will come. For those in pursuit of medical and health degrees or simply wishing to learn more about the involvement of microbes in their field, understanding the impact of the microbiome now will make for an even richer practice down the road.

We wish you a good read and a very balanced microbiome.

JASON TETRO
EMMA ALLEN-VERCOE

CHAPTER 1

Some Historical Notes on Bowel Microflora

SYDNEY M. FINEGOLD, M.D., MACP, D (ABMM)

SINCE so much of the bowel flora is anaerobic, it makes sense to start with what was known about anaerobic bacteria in the "olden days".

I graduated from UCLA in 1943 as a Bacteriology major. This school is one of good reputation. Still, I learned virtually nothing about anaerobes; just that clostridia were anaerobic bacteria and were responsible for some serious and often fatal infections, or intoxications, such as tetanus, botulism, and gas gangrene. There were laboratory sessions for most of the courses we took as bacteriology majors, but we didn't do anything with any clostridia and did not even see pictures of these organisms or of the serious clinical illnesses related to them in our textbooks. There might well have been concern about handling such bacteria in the laboratory since penicillin was only available for the military in 1943 and was in such short supply that urine was saved from patients receiving it so that penicillin could be recovered from it and used again, but there are many benign anaerobes that could have been used in college courses. (As a Navy Corpsman assigned to the clinical microbiology lab at Long Beach Naval Hospital from 1943 to 1945, I was assigned the task of collecting all urine from patients treated with penicillin.)

In medical school (1945 to 1949), I worked part time in the surgical research laboratory of Dr. Edgar Poth who was well known for his studies on so-called "intestinal sulfonamides", used prophylactically in patients having bowel surgery. These compounds were tested initially in dogs and my job was to obtain fecal samples and study the fecal flora using a protocol that was set up previously. For anaerobic flora, we used Brewer plates (special Petri dishes whose lids came down to a

very short distance from the agar surface so that the air space was quite limited) with Brewer thioglycollate agar which supported the growth of many anaerobes. What was not known at that time (and I didn't know until sometime later) was that virtually all clinically significant aerobic and microaerophilic bacteria are facultative and grow well (often better) under anaerobic conditions. We did not know to test all organisms recovered on these Brewer agar plates for the ability to grow under nonanaerobic conditions. In fact, there was no identification of anything growing on those plates; we simply determined the "anaerobic counts" by counting colonies on these plates, not even counting different colony types. They had used these procedures for many years before I was involved.

In my postgraduate work in Minneapolis I worked with Dr. Wesley Spink and Dr. Wendell Hall. There was no specialty of Infectious Diseases yet, but I chose their program because they worked with brucellosis and other bacterial infections and I was still very interested in microbiology. During my clinical training, I had a patient with pleural empyema. I removed purulent pleural fluid by thoracentesis; it was putrid and I was surprised when the laboratory told me they didn't grow any bacteria from it. I looked at the Gram stain with the Chief of the Clinical Microbiology Laboratory and we couldn't decide that there were any bacteria present, just pink-staining pleomorphic "globs". I presented this patient at a conference attended by Faculty and students from several teaching hospitals in the city and no one had any suggestions as to what the cause of this infection was. Finally, one of my colleague Fellows, Gordon Riegel, from the University and VA hospitals in Minneapolis, timidly asked whether this might be an anaerobic empyema. Gordon had trained earlier at Johns Hopkins and remembered one professor talking about anaerobic infections and noting that the discharges were often foul smelling and it was difficult to grow these organisms. No one knew how to respond to the Fellow. I discussed the case further with the head of the Clinical Microbiology Lab and she had no other suggestions.

I had another period in military service from 1951 to 1953. Then I got my first real faculty position 62 years ago as a staff physician at the VA Hospital in Los Angeles and on the faculty of the UCLA School of Medicine in the Department of Medicine and the Department of Microbiology, Immunology, and Molecular Genetics. As luck would have it, we had another case of putrid empyema which did not grow any organisms. I recalled the patient from Minneapolis and I discussed the two

cases with Vera Sutter, Ph.D., head of the Clinical Microbiology Lab at the VA hospital where the patient was being treated. We looked at the Gram stains together and found the same questionable pleomorphic bacteria. This time I decided I needed to pursue these anaerobes. Vera said she remembered seeing an anaerobic jar in the basement somewhere and searched until she found it. We again cultured pus from this patient, both on plates in the anaerobic jar and on aerobic plates. We again grew no aerobes but recovered two different gram-negative anaerobic bacilli from the plates incubated in the anaerobic jar. I was very lucky that no bacteria grew from either of the two putrid empyema patients. Anaerobic infections very commonly are mixed with aerobes as well as anaerobes. For that reason, anaerobic infections are often overlooked because the aerobic bacteria grow and the infection is attributed to them. I was also unlucky because if there had been gram-positive anaerobes (cocci, for example) present, I would have seen them on the Gram stain and with negative cultures I would have realized there was some kind of fastidious organism present.

I was finally launched on a many-years-long study of anaerobic bacteria. This was no easy task as it required classification, optimum methods of growing and preserving cultures, unique features of the bacteria, and clinical presentations of anaerobic infections. I was amazed to find anaerobes in so many different settings. Early on I found a small green book by Louis D.S. Smith of Montana on nonspore-forming anaerobic bacteria and their activities. As I got into literature searches, I became aware of centuries-old studies by French and German microbiologists in particular; I was amazed at how much they knew in the 1800s. I published *Anaerobic Bacteria in Human Disease* in 1977 summarizing our studies and those of others. My laboratory, with some outside collaborators, published the *Wadsworth Anaerobic Bacteriology Manual* in 1972, now in its sixth edition and called the *Wadsworth-KTL Anaerobic Bacteriology Manual*.

Early in my academic career, and overlapping my new-found major interest in anaerobic bacteria, I also became interested in bowel flora. Neomycin was a newly introduced antibiotic and it was noted that there was little absorption by the oral route, so the levels achieved in the gut were relatively huge. This led to an interest in using this and similar drugs for preoperative preparation of patients for bowel surgery. With my background from Dr. Poth's laboratory, I was very much interested in studying this compound. I started by determining what the impact of oral neomycin was on the bowel microflora. This was so early in my

career that I still was not using the anaerobic jar routinely. I made serial 10-fold dilutions of feces and planted them onto various agar plates that would permit recovery of various known colonic bacteria and also planted them into a set of thioglycollate broths. The appearance of the cultures at 48 hours was really striking. There was no growth on any of the plates incubated aerobically, but the thioglycollate broths were turbid all the way out to 10^{12}/ml! Aerobic subcultures from these broths were sterile, but subcultures incubated in anaerobic jars yielded many anaerobic bacteria of various types.

We subsequently learned about other systems for growing anaerobes, including watch glasses placed on the surface of inoculated plates by Professor Haenel of Potsdam, East Germany. These watch glasses were close to the agar surface and early growth of aerobes soon converted the space to an anaerobic environment. It was tedious working with this setup but Haenel managed to do excellent studies of bowel flora with it. Initially we used line gas (methane) in our anaerobic jars; fortunately, it was not so toxic to anaerobes in Los Angeles and we could grow some of them (but didn't know what we might be missing). Later, commercial kits to provide an anaerobic atmosphere with carbon dioxide in jars became available, as did catalysts to remove traces of oxygen. We ultimately switched to anaerobic chambers when these became available, and to tanks of pure nitrogen, hydrogen, and carbon dioxide gases, individually and in appropriate mixtures. Learning to identify anaerobes, even by the crude techniques available at that time, was a problem. Initially, we called them "gray colonies" (the *Bacteroides fragilis* group, it turned out), "clear colonies" (some of these were *Fusobacterium* we later found out), and brown or black "pigmented" colonies on blood or hemoglobin-containing media.

In comparison to the rapid, wide spectrum of analyses performed on a day-to-day basis, this work may seem minimal. Yet, back then, everything was. Take the mere concept of sharing results and/or communicating with colleagues. Today, the communication possibilities are great and one can phone or e-mail anyone and expect to usually get responses that are very helpful and save much time. At present, one can usually easily arrange to visit other laboratories briefly or even arrange to spend several months or even years studying with someone who has perfected techniques and procedures to deal with problems you have not yet coped with yourself. And textbooks and current literature are presently readily available. One can travel to scientific meetings to listen to and even meet leaders in various fields that may be of inter-

est. When I was starting out, these communication benefits were not so readily available. I did write to and subsequently briefly visited several leaders in the United Kingdom, France, and Germany when I had the opportunity to do so. I was fortunate to meet such notable Professors as Garrod, Beerens, and the grand master of anaerobes, Professor André Prévot, who unfortunately was ill on the day I met him and couldn't meet with me for more than half an hour. But in that short period of time, one could gain a wealth of knowledge and even find a direction for future work. Also, it is so much more personable than any electronic media; you have to exist in order to communicate.

Of course, a half-hour talk does little in the context of the second generation systems currently used to study the microbiome. Using a machine such as the Illumina permits rapid detection and identification of complex microbial floras. These can then be catalogued in databases and analyzed using a number of different software methods. This has indeed helped us to better understand the microbes such as those seen in the human colon. But we were able to do many important studies older methods combined with a DNA sequencer and real-time PCR machine. I will comment on some of these studies in the remainder of Chapter 1.

We studied small bowel fluid from a patient with blind loop syndrome and found six different anaerobes and a total anaerobic count one log higher than the total aerobic count. We developed and evaluated several selective media; we improved gas liquid chromatographic procedures for quantitation of fatty acids and alcohols; and we compared the efficiency of anaerobic jars, the Anoxomat system, and anaerobic chambers. We found that antibiotic susceptibility patterns of various anaerobes were useful as guides to classification and characterization of certain anaerobes and studied these patterns with various anaerobes as a guide to therapy of infections with these organisms. We studied the effect of various antimicrobial drugs on the normal bowel flora of patients. We studied the toxins of *Clostridium difficile* and the epidemiology of disease due to this organism in the hospital setting. We studied an outbreak of enterocolitis in our hospital due to phage type 54 staphylococci resistant to kanamycin, neomycin, paromomycin, and chloramphenicol. We studied the normal flora of ileostomy and transverse colostomy effluents and the flora of the maternal cervix and the newborn's gastric fluid and conjunctivae.

We were the first to isolate *Acidaminococcus fermentans* and *Megasphaera elsdenii* from normal human feces. Our group studied the bacteriology of infections in patients undergoing head and neck cancer

surgery that provided guidance for the type of antimicrobial prophylaxis that would be most effective in prophylaxis for such patients. We studied the impact of a partially chemically defined diet on the bowel flora of humans. We also had the opportunity to study stool specimens from two patients presenting with d-lactic acidosis; one patient had previously had most of the small bowel removed because of mesenteric thrombosis and the other patient had previously undergone a jejunoileal bypass. The stool flora of both patients was quite abnormal on admission with predominantly gram-positive anaerobic bacilli, *Eubacterium*, *Lactobacillus*, and *Bifidobacterium*, which produced primarily d-lactic acid. The patients responded well to oral vancomycin therapy.

A very important study that we did in collaboration with Dr. Ernst Drenick, an internist and nutritionist, and Dr. Edward Passaro, Jr., a general surgeon, concerned patients undergoing jejunoileal bypass surgery for obesity. The really unique approach of this study was to obtain specimens from patients in the operating room who did not receive any preoperative antimicrobial prophylaxis. Specimens were obtained during surgery from the proximal jejunum and distal ileum. The plan was to obtain similar specimens from any patients who might require surgery for complications relating to the original surgical procedure. We could also compare the data from patients who had only specimens from after the bypass procedure since they were all processed in the same way.

Among eight patients from whom we had baseline studies, the proximal jejunum was sterile in five. The other three had a predominantly aerobic flora with low counts. Only one patient had anaerobes in the jejunum and counts were low. Ileal contents were sterile in two patients; the other six had variable counts. The ileal contents had higher counts than the jejunal contents; the flora resembled fecal flora qualitatively but with lower counts and a higher ratio of aerobes to anaerobes. Only one of the original patients required repeat surgery; he had a sterile jejunum at the first surgery but at re-operation the functioning small bowel was colonized with fecal-type organisms with a total count of $10^{7.5}$/ml.

Looking at the three patients with no baseline studies, one had a high total bacterial count of $>10^9$/ml., another had *Fusobacterium varium* outnumbering the *B. fragilis* group in both the functioning small bowel and in the blind loop. The third case yielded only *E. coli* from the excluded loop. This latter patient, despite a sparse flora, had severe complications suggesting that perhaps toxin production or metabolic behavior might account for some complications. The various complications that may be seen in these bypass patients include an inflamma-

tory bypass enteritis, pneumatosis cystoides intestinalis, impaired liver function, and even fatal hepatic coma, polyarthritis, skin lesions, eye complications, etc. Metronidazole typically was quite effective therapeutically.

We also did microbiology studies in 10 patients undergoing so-called biliopancreatic bypass (Scopinaro procedure). Collection of specimens, only from the bypassed segment (biliopancreatic bowel segment), was done in the operating room at the start of the procedure and with no antibiotic bowel preparation preoperatively. Counts of organisms recovered were relatively low (10^2 to 10^7/ml. Three subjects developed diarrhea that was moderate to severe which responded promptly to metronidazole given orally.

The final notable study we performed was a comparison of bowel flora in different populations with different incidences of colon cancer—Japanese with their traditional diet, Seventh Day Adventists with variable incidences of meat consumption, people on the standard American or Western diet, and people with colonic polyps. This study went on for years thorough bacteriologic studies on their stools as we could in the 1970s. This important study, however, really should be done again with second generation sequencing techniques.

In the past 15 years, we have been studying the fecal flora of children with regressive autism,of autistic children in comparison with that of normal control children, and with that of their siblings. Our first publication (with Sandler *et al.* 2000) was a small open-label study of oral vancomycin but it was important because of the dramatic improvement in virtually all the 10 children treated. All the subjects relapsed after the short treatment course was stopped, but this study established that the clostridia recovered from their stools played a key role in the disease.

A study published in *Clin. Infect. Dis.* in 2002 showed the importance of clostridia and included small bowel aspirates as well as stool. We documented the presence of clostridia by quantitative culture, real-time PCR, and analysis of 16-23 S space region. Bacteria found that were much more frequently found in autistic children than in the control patients were *Clostridium bolteae*, sp. nov., and perhaps some closely related species. A pyrosequencing study was performed and published in 2010. This study led to recognition of five *Desulfovibrio* spedies as role players in autism, the findng that *Bifidobacterium* counts were low in stools of autistic children as compared to controls. We have confirmed the work of others as to the importance of certain *Sutterella* species in autism and of a protective role for *Akkermansia*, as well as *Bifi-*

dobacterium, but have not published this as yet. We have recently found that an unusual clostridial toxin plays a role in autism.

As to where we stand with the colon and indeed the microbiome, even after all the years of work, I have realized we are only at the beginning. Our laboratory has detected a number of novel taxa and studied, named, and reported them, with various colleagues. Included were: *Bilophila wadsworthia, Sutterella wadsworthensis, Clostridium bolteae, Cetobacterium somerae, Anaerotruncus colihominis, Anaerofustus stercorihominis, Clostridium bartlettii, Porphyromonas uenonis, Bacteroides nordii, Bacteroides salyersae, Fastidiosipila sanguinis, Parabacteroides goldsteinii, Porphyromonas somerae, Alistipes onderdonkii, Alistipes, shahii, Peptoniphilus duerdenii, Peptoniphilus koeneneniae, Peptoniphilus gorbachii, Peptoniphilus olsenii, Anaerococcus murdochii, Blautia wexlerae, Porphyromonas bennonis, Murdochiella asaccharolytica, Gemella asaccharolytica*, and *Corynebacterium pyruviproducens*. Along with Paul Lawson and others, we have even recommended reclassification of a few organisms, notably the *Ruminococcus* group. This group is now being regarded as one of the three major enterotypes of the gut microbiome. That means all this work is only one-third of the information we have now. As we continue to learn more with even higher levels of analysis, this fraction may diminish even further. Although this may appear at first to be disheartening after over six decades of work, I am happy. While the microbiome continues to expand in its scope, much of which will be discussed in this book, it all started with a general look at the colon and the belief there was much more to the picture. As we continue to learn, that picture is larger than we might have ever imagined.

CHAPTER 2

Ecology of the Human Microbiome

KALUDYNA BOREWICZ and HAUKE SMIDT

2.1. OVERVIEW

RECENT technological and conceptual developments in culture independent approaches targeting bacterial 16S ribosomal RNA (rRNA) genes have offered a new way of looking at microbial ecosystems. This in turn has contributed to the current expansion in the number of research projects aiming at characterizing microbiota composition and function in health and disease. Healthy human microbiota is composed of many complex and diverse microbial ecosystems, with estimated 10^{14} microbial cells inhabiting the human body (Savage 1977). These microbial ecosystems are also unique between different body sites and between individuals, and this variation in microbial composition can be attributed to many factors including host genetics, environment, diet, and early life microbial exposure (Human Microbiome Project 2012). Despite taxonomic differences in microbial community structure, the core metabolic and functional pathways carried out by these ecosystems seem to be relatively stable, suggesting that the role of microbiota in health and disease may be largely due to disturbances in microbial function, rather than changes in microbiota composition alone (Human Microbiome Project 2012).

2.2. MICROBIOTA OF THE GASTROINTESTINAL TRACT

The human gastrointestinal (GI) tract is by far the most densely colonized and best studied microbial ecosystem found in the human body. It

is estimated that 1,000–1,500 species of bacteria can inhabit an average adult GI tract, but this number could be even higher (DiBaise 2008). Each person carries approximately 160 bacterial species and about 10 million microbial genes, which give each individual a unique microbial make up (Li 2014). Host genetics may contribute to these individual variations in microbiota, and it has been shown to be an important factor affecting bacterial community composition and function (Moreno-Indias 2014).

Microbial colonization of the GI tract in healthy humans starts at birth and is influenced mainly by the mode of delivery (vaginal versus Caesarean section) and the method of feeding (breast milk versus formula) during infancy (Moreno-Indias 2014). An adult-like microbiota becomes established with introduction of solid foods and begins to resemble microbiota of adults during the first year of life, after which it remains relatively stable throughout adulthood. Diet, infections, antibiotic use, and other environmental conditions can temporarily disturb the normal gut microbial ecosystem, however, these disturbances tend to be temporary and in most cases, the microbiota is able to recover back to its former state. Microbial composition changes in elderly, as the diversity and stability of gut microbiota decrease with age (Moreno-Indias 2014).

Despite the individual variation in microbial composition, the majority of bacterial species found in the human gut belong to two phyla: *Bacteroidetes* and *Firmicutes* (Mariat *et al.* 2009). Most species in the phylum *Bacteroidetes* belong to the class *Bacteroidetes*, and more specifically to the genera *Bacteroides* and *Prevotella*. Most species in the phylum *Firmicutes* belong to *Clostridium* clusters IV and XIVa, which include genera *Clostridium*, *Eubacterium*, and *Ruminococcus*. Other detected phyla include *Proteobacteria, Actinobacteria, Fusobacteria, Spirochaetes, Verrucomicrobia*, and *Lentisphaerae* (Gerritsen *et al.* 2009). In addition to bacterial groups, *Archaea* (methanogens) and eukaryotic microorganisms (fungi) are also part of healthy human gut microbiota.

Metagenomic sequencing data suggests that even with individual differences in microbiota composition, the metabolic pathways remain stable in the GI tract of healthy subjects (Human Microbiome Project 2012). This collection of microbes forms a dynamic ecosystem which is known to exert important metabolic, physiological, and immunological functions on its host, as well as to provide protection from pathogens through so-called colonization resistance (Wade 2013). The host, on

the other hand, offers the microbes a stable environment and nutrients necessary for their survival. The general understanding of the microbial ecosystem function has increased tremendously in the recent years, however, the details are still largely unknown. It is becoming clear that the network of interactions, whether these are positive or negative, is very complex and we are now only at the beginning of understanding the roles of different bacterial groups, and how their functions influence the host.

In order to understand how microbial ecosystems contribute in health and disease, we should first know which microbes comprise the healthy human microbiota. More importantly, we need to ascertain the specific roles they perform and how their presence can impact the host. In the following sections we will first give an overview of the key microbial groups and their functions in different regions of a GI tract of healthy adults. Later, we will discuss how changes in microbiota correlate with selected types of diseases.

2.3. MICROBIAL COMPOSITION IN THE GI TRACT OF HEALTHY ADULTS

The human GI tract can be divided in anatomical regions, each characterized by a different set of physicochemical conditions which create a unique environment for microbial growth. The most important factors influencing intestinal microbiota include pH, redox potential, nutrient content, motility, and presence of host secretions such as digestive enzymes, bile, and mucus. The environment at each anatomical region can be further divided into the luminal content and the mucosal layer. The mucosal layer forms a lining along the GI tract and consists of a single sheet of epithelial cells and an irregular coating of mucus that protects the cells from direct action of host secretions, food, and pathogens found in the lumen. The mucosal layer also provides a site of attachment for commensal microbiota. In the following sections, we will describe microbial ecosystems with respect to different regions of the GI tract.

2.3.1. The Oral Cavity

The oral cavity comprises many different niches that provide unique conditions for microbial growth. Most microbes are associated with the mucosal surfaces on the cheeks or tongue, and hard surfaces of teeth, braces, or dentures, and there is no resident microbiota in the lumen,

because the passage time of food in the mouth is very short. The oral microbial ecosystem is very diverse, with about 10^{12} bacterial cells of about 1,000 different species belonging to phyla *Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes, Synergistetes*, and *Tenericutes*, candidate phylum TM7, and the uncultured divisions GN02 and SR1 (Wade 2013; Tlaskalova-Hogenova *et al.* 2011; Soro *et al.* 2014; He *et al.* 2015). The relative distribution of each microbial phylum differs between individuals and between location in the mouth (Zaura *et al.* 2009). The most predominant genera include *Actinomyces, Streptococcus, Neisseria, Veillonella, Porphyromonas*, and *Selenomonas*. In addition, viruses, protozoa, fungi, and a small number of methanogenic *Archaea* are also members of the normal microbiota. The microbial composition at the species level is highly variable between individuals and can be influenced by factors such as age, diet, oral health, and hygiene (Wade 2013).

2.3.2. The Upper Gastrointestinal Tract

The upper gastrointestinal tract includes the esophagus, stomach, and duodenum. In humans, microbial ecosystem composition and function in the upper GI tract are still largely unknown, due to poor accessibility of these areas and the need for invasive procedures to obtain samples. In the surveys on microbiota of the distal esophagus, members of six phyla, namely *Firmicutes, Bacteroides, Actinobacteria, Proteobacteria, Fusobacteria*, and *TM7*, were found in the mucosal layer, and most common genera included *Streptococcus, Prevotella*, and *Veillonella* (Pei *et al.* 2004; Fillon *et al.* 2012). Research shows that the distal esophagus is inhabited by a complex but conserved microbial community, with composition resembling the oral microbiota of the host (Pei *et al.* 2004). Similar to the oral cavity, food does not stay in the esophagus long enough to allow for establishment of resident microbiota. The stomach is the first part of the GI tract that holds food for longer periods of time. Thus, the microbial distribution in the stomach, and in the descending regions of the GI tract, is spatially specific, with different microbes associated with the gastric content and with the mucosal layer (Wang and Yang 2013). Because of its low pH which can only be tolerated by certain acid-resistant bacteria, the bacterial counts in the stomach content are generally low, with about 10^3–10^4 bacterial cells per mL (Tlaskalova-Hogenova *et al.* 2011). The microbiota of gastric content can vary depending on diet or influx of bacteria from the mouth,

esophagus, and duodenum, however, these factors affect to a lesser degree the mucosa-associated microbiota which is protected in the mucus and much more stable (Wang and Yang 2013). Culture independent studies on stomach microbiota showed that in the mucosal layer *Firmicutes, Proteobacteria, Bacteroidetes*, and *Fusobacteria* were the most abundant phyla, and *Streptococcus, Prevotella, Porphyromonas, Neisseria, Haemophilus*, and *Veillonella* were common genera, but the distribution of taxa at genus level was highly variable between individuals (Stearns *et al.* 2011; Bik *et al.* 2006; Li *et al.* 2009). One of the important, and certainly most well-studied species found in about 50% of the human population is *Helicobacter pylori*, which has been associated with gastric diseases such as gastritis and cancer (Wang and Yang 2013). The duodenum is the last part of the upper GI tract and the first part of the small intestine, and it is discussed in Section 2.3.3.

2.3.3. The Small Intestine

The small intestine is the site where most of the host enzymatic digestion and absorption of nutrients, in particular lipids and simple carbohydrates, takes place. Studies on microbial composition are again very limited with the majority of findings being based on biopsy specimens in association with various GI disorders. The duodenal lumen forms a unique environment characterized by a low pH, fast passage time, and the presence of antimicrobial bile and digestive enzymes, making it an unfavourable place for microbial growth. No culture independent studies up to date focused on resident microbiota in human duodenal content. On the other hand, biopsy samples provided insight in microbiota in the duodenal mucosa. In a recent study using 16S rRNA gene-targeted HITChip analysis of duodenal biopsies from children, 13 phylum-like level bacterial groups were detected, and *Proteobacteria, Bacilli*, and *Bacteroidetes* were the most abundant taxa, with each individual subject showing a different and unique microbial profile (Jing Cheng *et al.* 2013). The predominant genus-like groups included *Sutterella wadsworthensis et rel.*, *Streptococcus mitis et rel.*, *Aquabacterium, Streptococcus intermedius et rel.*, and *Prevotella melaninogenica et rel.* (Jing Cheng *et al.* 2013). In a study using sequencing of 16S rRNA gene clone libraries, the most abundant phyla detected in biopsies from children and adult subjects were *Firmicutes, Proteobacteria, Bacteroidetes*, and also *Actinobacteria, Fusobacteria*, and *Deinococcus-Thermus* (Nistal *et al.* 2012). Most sequences were classified as *Streptococcus* and *Prevotella*

spp. in both age groups, and 5% of sequences that were found only in healthy children could not be assigned to any known genus. Bacterial community richness was higher in the adult group as compared to the juvenile group, with members of *Veillonella, Neisseria, Haemophilus, Methylobacterium*, and *Mycobacterium* present in adult mucosa. It is interesting to note that overall duodenal microbiota composition seems to resemble the microbiota found in the oral cavity and esophagus, and less so the microbiota found in the lower GI tract (Wacklin *et al.* 2013). The number of bacterial cells and diversity increase along the intestine, and it is estimated that the jejunum harbors 10^5–10^6 bacteria per mL of content (Tlaskalova-Hogenova *et al.* 2011). An earlier study examining mucosa biopsies of human jejunum showed that *Streptococcus* and *Proteobacteria* were the most abundant taxa and contributed respectively to 68% and 13% of all microbiota detected (Wang *et al.* 2005). A more recent study showed that ileostomy effluent samples can provide a good representation of microbial composition in the human jejunum/proximal-ileum without the need for invasive sampling (Zoetendal 2012). The most predominant (common core) taxa in ileostoma-effluent and in jejunum included *Bacilli* (*Streptococcus* spp.), *Clostridium* cluster IX (*Veillonella* spp.), *Clostridium* cluster XIVa, and *Gammaproteobacteria* (Zoetendal 2012). Similar findings came from an earlier study on ileostoma-effluent where the most abundant species were members of the *Lactobacillales* and *Clostridiales*, mainly *Streptococcus bovis*-related species and the *Veillonella* group, as well as species belonging to *Clostridium* cluster I and *Enterococcus* (Booijink *et al.* 2010). However, the ileum-associated *Bacteroidetes* and *Clostridium* clusters III, IV, and XIVa were reduced in ileostoma-effluent samples. Bacterial numbers increase to about 10^8–10^9 cells per mL of ileal digesta. Biopsies and catheter-collected lumen samples revealed that the bacterial community in the human ileum is dominated by species belonging to *Bacteroidetes* and *Clostridium* clusters IV and XIVa and resembles the microbiota found in the colon (Tlaskalova-Hogenova *et al.* 2011; Wang *et al.* 2005). Similar to the ileostomy-effluent samples, ileum microbiota is also characterized by short and long term fluctuations in microbial profiles within individuals and large interindividual variability between patients (Booijink *et al.* 2010).

2.3.4. The Large Intestine

The large intestine is separated from the small intestine by the il-

eocecal valve, and it can be divided into the cecum; the ascending, transversing, and descending colon; the rectum, and the anal canal. The cecum is the first region of the large intestine that receives food from the small intestine. It is also connected with the appendix—a small and rudimentary projection, which in humans has no function in food digestion, but it may play an important role as a reservoir of microbiota and in stabilizing and restoring the colon microbial ecosystem, especially after disturbance, for example due to antibiotic use (Laurin *et al.* 2011; Bollinger *et al.* 2007). Unlike the small intestine, microbial composition and function of the human large intestine has been studied to great extent, mostly because of the ease of collecting fecal samples, and because of the high density of microbial cells, estimated to be around 10^{11}–10^{12} per mL (Tlaskalova-Hogenova *et al.* 2011). The most predominant microbial groups found in the human large intestine include *Bacteroides*, members of the various *Clostridium* clusters, *Bifidobacterium, Enterobacteriaceae*, and *Eubacterium*. Even though the large intestine can be divided into five anatomical regions, the microbial composition is very uniform, and fecal material seems to represent well the microbiota in the entire region (Gerritsen *et al.* 2011). However, just like in other parts of the GI tract, in the large intestine there is a large difference between microbial ecosystems found in the lumen and mucosal layer. Fecal samples represent the luminal fraction only, and the mucosal layer is much less explored due to the need for more invasive methods in collecting biopsy samples. Large intestinal microbiota is very diverse, highly unique to each individual, and relatively stable over time (Lahti *et al.* 2014). Factors such as age, disease, or the use of antibiotics may permanently alter the microbial composition (Lahti *et al.* 2014). Recent studies utilizing large cohorts of subjects suggested that the fecal microbiota composition in healthy adults can be categorized into three major enterotypes dominated by different bacterial populations, in particular *Bacteroides, Prevotella*, and *Ruminococcus* (Arumugam *et al.* 2011; Benson *et al.* 2010). These enterotypes are independent of age, ethnicity, gender, and body mass. However, this division is still controversial, and some studies failed to detect presence of enterotypes in both the elderly (Claesson 2012) and in adult research populations (Huse *et al.* 2012).

Another large study suggested an alternative to the enterotype theory (Lahti *et al.* 2014). The authors noted that in fecal samples of Western adults, certain bacterial groups, namely *Dialister* spp., *Bacteroides fragilis, Prevotella melaninogenica, P. oralis*, and two groups of uncultured

Clostridiales cluster I and II, were bimodally distributed in the healthy human population, representing so called "tipping elements" (Lahti *et al.* 2014). These bistable bacterial groups were either very abundant or almost absent, and unstable at their intermediate abundance levels (Lahti *et al.* 2014). In addition, the condition of the bistable groups, especially the *Bacteroides* and *Prevotella*, seemed to correlate with the shifts in other bacteria, and as a result they were believed to be driving the overall composition of the colonic ecosystem towards specific enterotypes (Lahti *et al.* 2014).

2.4. MICROBIAL ECOSYSTEM FUNCTION IN THE GI TRACT OF HEALTHY ADULTS

Metagenomic studies provide insight on the functional potential of microbiota by analyzing microbial genes, collectively known as the microbiome. A recent study reported that each person carries about 10 million bacterial genes in their GI tract, the majority of which are involved in bacterial metabolism (Li *et al.* 2014; Turnbaugh *et al.* 2009). Additional information about microbial activity can be obtained from metatranscriptomics, metabolomics, and metaproteomics analyses. These approaches provide insight about microbial gene regulation and expression, as well as the production of metabolites, proteins, vitamins, and regulatory elements. Similar to compositional diversity, there is a large functional variation in different microbial ecosystems, but the core metabolic and functional pathways carried out by the same types of ecosystems seem to be relatively conserved and stable (Human Microbiome Project 2012). It is also common for the same metabolic functions to be carried out by different bacterial groups, meaning that correlating the compositional and functional changes in the ecosystem maybe less straightforward because changes in the composition and the function of a given microbial ecosystem can be independent from each other (Zoetendal 2008).

2.4.1. The Oral Cavity

The oral cavity is the first point of contact between microbiota, diet, and host. Despite regular influx of food ingested by the host, the majority of nutrients for the oral commensal microbes are derived from glycoproteins present in saliva and gingival crevicular fluid (Homer *et al.* 1999). Complete breakdown of these glycoproteins requires coopera-

tion between different species of bacteria. For example, oral streptococci (e.g., *S. oralis, S. sangiunis*) remove oligosaccharide side chains and break down the protein core by their proteolytic, endopeptidase, and glycosidic activity, while other Gram-negative anaerobes (e.g., *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens*, and *Peptostreptoccus micros*) further break down proteins into peptides and amino acids (Homer *et al.* 1999; Wickstrom *et al.* 2009; Bao *et al.* 2008). Amino acids can then be fermented to short chain fatty acids (SCFA), including branched chain fatty acids, which are further degraded by other bacteria and by methanogenic Archaea (Wade 2013). Certain food components, such as gluten or nitrate can also be degraded/transformed by microbial enzymes, and the processes and products are crucial for the health and well-being of the host, while breakdown of these functions can be linked with host diseases (Hezel and Weitzberg 2013; Helmerhorst 2010; Zamakhchari 2011). As already mentioned, the mouth is an open environment and commensal bacteria create a barrier against colonisation with transient microbes and any opportunistic pathogens that can enter with food or water. An *in vitro* study on oral microbiota from mice provided a good illustration of how the cooperation of different commensal species can leverage a community response to pathogen invasion. The study proposed that cooperation of three different species of oral streptococci were involved, with *S. saprophyticus* sensing the presence of an invader, and initiating the defence pathway, *S. infantis* acting as a mediator, and *S. sanguinis* producing hydrogen peroxide and acting as a killer (He *et al.* 2014). Besides colonization resistance, oral microbiota plays an important role in maintaining host-microbe homeostasis, by interacting with host mucosal cells and training the host's immune system to recognize and destroy pathogens, while downregulating the proinflammatory immune response towards the commensal bacteria normally present in the mouth (Srinivasan 2010).

2.4.2. Upper Gastrointestinal Tract

Upper gastrointestinal tract microbiota function is still not well understood, and most studies to date focused on specific pathogens and their role in the aetiology of different diseases and to a lesser extent on the microbial interactions in a healthy ecosystem. Little is known about the ecology of microbiota inhabiting the esophagus and stomach, but its role in colonization resistance and protection from pathogens is likely to be an important one. Normal microbiota generates a microenviron-

ment that can inhibit growth of pathogens by competing for substrates and binding sites, stimulating host immune responses against invaders and production of antimicrobial substances. For example, *in vitro* and *in vivo* studies using animal models showed that stomach colonization with *H. pylori* is inhibited by the normal commensal microbiota and by probiotic strains of *Lactobacillus, Bifidobacterium*, and *Saccharomyces*, suggesting the importance of microbial interaction in pathogen resistance (Wang and Yang 2013). Other studies using human biopsy samples also reported changes in intestinal microbiota associated with gastric cancer, however, the exact function and causality of this association is still being investigated (Tlaskalove-Hogenova 2011). It is likely that microbial metabolites, bacterial lipopolysaccharides (LPS), lipoproteins, lipoteichoeic acids (LTA), flaggellins, and bacterial nucleic acids can interfere with the normal function of gastric mucosa, causing chronic inflammation, changes in mucin production, metaplasia, and eventually can lead to diseases (Tlaskalove-Hogenova 2011; Jing Cheng *et al.* 2013). The functions of the microbiota in the duodenum are still not well understood, but changes in microbial composition between Celiac disease patients and healthy controls suggest that the microbiota plays a role in immune response, inflammation, and maintaining gut homeostasis (Jing Cheng *et al.* 2013; Wacklin *et al.* 2013). The homeostasis of gut epithelia relies to a large extent on adequate activation of toll-like receptors (TLRs), which recognize microbe-associated motifs, regulate the immune response to pathogens, and affect the epithelial barrier by regulating the expression of tight junction proteins, mucin, and antimicrobial peptides by the host's intestinal cells (Jing Cheng *et al.* 2013).

2.4.3. The Small Intestine

The small intestine is the site where most of the host enzymatic digestion and absorption of energy from the diet takes place. Thus, diet is an important factor modulating microbial function, by selecting bacterial groups that are better equipped to break down different dietary substrates (Moreno-Indias 2014). For example, certain *Lactobacillus* spp. found in duodenum and jejunum have been associated with weight gain and leanness, and differ in their metabolic capacities to break down dietary carbohydrates and fats supplied by the host (Moreno-Indias 2014). The transit time in the small intestine is very short, and *Streptococcus* and *Veillonella* spp., which dominate the microbial ecosystem

in the jejunum and ileum, are well adapted to quickly metabolize a variety of available carbohydrates, first to lactate (*Streptococcus*) and then to acetate and propionate (*Veillonella*) (Booijink *et al.* 2010). Recent metatranscriptome analysis of the ileostoma effluent confirmed a high abundance of genes involved in the transport and metabolism of diet-derived simple carbohydrates and linked the task mainly to *Streptococcus* groups (Aidy *et al.* 2015). In addition to its function in carbohydrate metabolism, it was concluded that small intestine microbiota could also play a key role in immune system development and homeostasis. For example, the ileum is connected with a large mass of gut associated lymphoid tissue (GALT) and Peyer's patches, and commensal bacteria, such as different strains of streptococci, were shown to induce specific immune responses in the host (Aidy *et al.* 2015). The close contact between the microbiota and the host cells in the small intestine underlines the current hypothesis that microbially derived metabolites or toxins also modulate gene expression via the gut-brain neural circuit and may influence endocrine function (e.g., secretion of glucagon and incretins) and even show an effect on mood or behavior of the host (Moreno-Indias 2014; Aidy *et al.* 2015).

2.4.4. The Large Intestine

Large intestine microbial ecosystem function has been well studied, mainly due to the ease of collecting fecal samples, but also because it has been known for a long time that colonic microbial processes play an important role in human health. The most direct role is in the digestion and metabolism, as the large intestinal microbiota breaks down indigestible food components and provides the host with an otherwise inaccessible source of energy. It also produces SCFA which are the main source of energy for colonocytes (Leser and Molbak 2009). In addition, the colonic microbiota is a main source of vitamins K and B12, it prevents colonization by pathogens, and it plays an important role in regulating the host's immune responses (Moreno-Indias *et al.* 2014; Leser and Molbak 2009). A study on the fecal microbiome of healthy Japanese subjects was among the first to explore microbial ecosystem function in the human colon using culture-independent methods. The study revealed that a high proportion of genes present were related to carbohydrate metabolism and transport. The authors also noted an enrichment of peptidases and enzymes for anaerobic pyruvate metabolism and reduction in genes involved in fatty-acid metabolism. There were

also high levels of enzymes involved in energy storage, antimicrobial peptide transport, and multidrug efflux pump peptides (Kurokawa *et al.* 2007). The authors concluded that these enzymes may help certain commensal microbes to compete with each other and thus may be essential for maintenance of ecosystem balance. Enzymes for DNA repair were also enriched. On the other hand, there was a low abundance of genes involved in biosynthesis of flagella and chemotaxis and in oxygen take-up (Kurokawa *et al.* 2007). Interestingly, these patterns in gene distribution were not observed in unweaned infants, suggesting that infant microbiota is less complex and thus microbial ecosystem function is less stable, more dynamic, and highly adaptable. In adult microbiota a higher diversity of bacterial species exists with large interindividual variability in microbial composition, yet there is a shared functional core, which is believed to be stable and much more uniform between individuals (Turnbaugh *et al.* 2009; (Kurokawa *et al.* 2007). Recently, more in depth analyses showed that there could be functional differences correlating with different enterotypes found in the colon (Arumugam *et al.* 2010). For example, the *Bacteroides*-rich type has more bacterial species that are capable of producing vitamins C, B2, B5, and H. This group is dominated by species that utilize carbohydrate fermentation as the main energy source. On the other hand, the *Prevotella* type showed higher numbers of species producing vitamin B1 and folic acid, and included species that use mucin glycoproteins as a source of energy, similarly to the *Ruminococcus* type (Arumugam *et al.* 2010).

One of the important functions of colonic microbiota that received a lot of attention in recent years is the production of SCFA, and in particular butyrate, by bacteria from *Clostridium* clusters IV and XIVa. The main butyrate-producing species are believed to be *Eubacterium rectale* and *Faecalibacterium prausnitzii*, in addition to others in the genera *Coprococcus* and *Roseburia* (Louis and Flint 2009). The process provides a great example of synergic interaction between diet, microbes, and host, and the presence of butyrate producers in the colon has been shown to be negatively correlated with functional dysbiosis, reduction of the risk of infections with opportunistic pathogens, and the decrease in oxidative stress (Moreno-Indias *et al.* 2014). Butyrate producers can respond to different environmental conditions, such as diet or pH, and engage different fermentation pathways in which the final products are lactate, formate, hydrogen, and carbon dioxide. It has been shown that cross-feeding between bifidobacteria and butyrate producers is also possible: bifidobacteria break down polysaccharides

CHAPTER 12

Considering the Microbiome as Part of Future Medicine and Nutrition Strategies

EMMA ALLEN-VERCOE

12.1. INTRODUCTION

THE purpose of *The Human Microbiome Handbook* is to provide an overview of current knowledge as it pertains to the human microbiome. It demonstrates that a few areas of health research have received such a surge in interest over the last decade. Moreover, while this handbook provides a current review of our understanding, the field is advancing at an astonishingly rapid rate. These are undoubtedly exciting times, since until recently modern medicine has considered human beings to be strictly human; our microbial passengers have been ignored—or worse—persecuted. It is my hope that this book has highlighted the very many aspects of our human biology and physiology that are influenced—or even controlled—by our microbial symbionts.

This chapter considers the current outlook for microbiome research, particularly as it pertains to the gut microbial ecosystem, and predicts areas where this research will be leveraged to benefit health in the near future.

12.2. MINING THE HUMAN MICROBIOTA FOR NEW DRUGS

What defines a healthy gut and why do some people seem to be more susceptible to GI infection than others? It is well known that people who have recently suffered microbial ecosystem depletion through, for example, antibiotic use or acute enteroviral infection are more suscep-

tible to further gut infection during their convalescence (Croswell *et al.* 2009; Stecher *et al.* 2010). There are several reasons for this susceptibility, but the reduced ability for competitive exclusion of pathogens by a depleted microbiota has always been considered as a primary cause (Malago 2014). However, more recently there has been a growing appreciation for the role of the gut microbiota in maintaining homeostasis in the GI tract, through protective effects that include the secretion of chemical signals that modify pathogen behavior.

Microbes within an ecosystem interact dynamically and ecosystem cohesion may rely on microbial chemical "conversations" that inform ecosystem members of, for example, food substrate availability or type, and cross-feeding availability (El Aidy *et al.* 2013). Such chemical signals may also act as a signal for pathogens—both autochthonous opportunistic species as well as allochthonous species—to refrain from expression of virulence determinants, since this energetically expensive exercise is less likely to be fruitful for these pathogens in the face of an intact, protective microbial ecosystem. Antunes *et al.* (2014), demonstrated this principle recently by screening members of the normal gut microbiota for antvirulence activity against the well-studied food-borne pathogen, *Salmonella enterica*. By measuring expression of the *S. enterica* virulence global regulator, *hilA*, it was found that the spent culture supernatants of particular members of the *Lachnospiraceae* family in particular had repressive activity that was afforded by the secretion of an as-yet uncharacterized small molecule metabolite by these common gut microbial species.

This finding likely only scratches the surface of the potentially prophylactic chemical repertoire secreted by the healthy human microbiota, a pharmacopeia that is as-yet relatively untapped. The major barrier to this area of drug discovery lies in a general inability to culture many of our microbial symbionts; however, there are now several efforts underway to both bring recalcitrant species into *in vitro* study (reviewed in Allen-Vercoe 2013). In the future, we should expect to see an expansion in the development of drugs mined from gut microbial ecosystems.

12.3. PROTECTING THE GUT MICROBIOTA FROM COLLATERAL DAMAGE DURING ANTIBIOTIC EXPOSURE

The overuse of antimicrobial drugs has received a lot of recent attention, from the point of view that the targeted pathogens have evolved

widespread resistance to these drugs, minimizing their effectiveness and creating fears of a return to the preantibiotic era when a simple puncture wound could lead to a life-threatening infection (Barriere 2015). Unfortunately, antibiotic resistance is not the only consequence of antimicrobial overuse, and there is now a growing realization that the collateral damage inflicted on the microbiota during antibiotic therapy is taking a toll on our health. Several studies have now conclusively shown that the gut microbial ecosystem changes profoundly during antibiotic administration, and that there may not be a recovery to the preantibiotic state, particularly if broad-spectrum antibiotics, or combinations of such, are used (Antunes *et al.* 2011; Arboleya *et al.* 2015; Cotter *et al.* 2012; Iapichino *et al.* 2008; Jernberg *et al.* 2007; Mangin *et al.* 2010; O'Sullivan *et al.* 2013). The missing microbiota hypothesis, as set out by Blaser and Falkow, also posits that because some aspects of the microbiota are inherited (through, for example, the processes of birth and breastfeeding), the ecosystem damage wreaked by antimicrobial use may compound over generations (2009).

The solution to both antibiotic resistance and collateral damage issues is to simply stop the use of antimicrobials; however, antibiotics are life-saving drugs when used appropriately, and an important weapon in the fight against infectious disease. Another strategy, therefore, is to find ways to protect the healthy microbiota during treatment. Many broad-spectrum antibiotics are given as oral preparations, and this fact as well as their pharmacology means that the gut microbiota, of all the host-associated microbes, is usually under the greatest threat during treatment. This is well illustrated by the common onset of diarrhea during a course of oral, broad-spectrum antimicrobials, which reflects a sudden change to the microbial ecology of the gut microbiota and a concomitant upset of the normal physiological homeostasis (Varughese *et al.* 2013). Part of the issue is that, if pharmacology allows, it is convenient to supply most antimicrobials by mouth for systemic absorption; however, most targeted infections are not found in the gut itself. Another problem is that for some infections where pathogenic biofilms are a component of the disease, such as otitis media, antibiotic doses have to be higher than the minimum inhibitory concentrations to be effective (Belfield *et al.* 2015), with potentially even greater collateral damage.

In the future, antibiotic administration will be much more carefully targeted. For example, treatment of ear or tooth infections may be carried out using topical applications of drugs that are less likely to accumulate to damaging concentrations in the GI tract (Dohar *et al.*

2006; Purucker *et al.* 2001). The necessity for prophylactic treatment as a routine part of surgical procedures will be more carefully evaluated (Young and Khadaroo 2014). Broad spectrum antimicrobials may be used only in emergency situations, with greater attention paid to rapid diagnostics allowing more targeted, narrow-spectrum antibiotics to be used (Spellbuerg *et al.* 2015). Alternatively, broad-spectrum antibiotics may be delivered orally in conjunction with compounds designed to maintain the antimicrobial in an inactive form until absorbed, to reduce damage to the gut microbiota from direct contact.

12.4. MICROBIAL ECOSYSTEM THERAPEUTICS

A greater understanding of the role of a damaged gut microbiota in disease has led to a surge in interest in the use of probiotics, defined as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host," (Hill *et al.* 2014). There are many probiotics now on the market, although only a minority has had proposed beneficial effects clinically proven, and even then the effects are moderate at best (McFarland 2014). Eventually, probiotics may prove to be very useful, for example in extending remission in some types of inflammatory bowel disease, or for reducing the severity of traveler's diarrhea (Ghouri *et al.* 2014; Sarowska *et al.* 2013). Yet there are limitations to their effectiveness because, from an ecology point of view, the addition of a single or small group of similar species to the enormous diversity of the human gut is unlikely to have a dramatic effect on the ecosystem as a whole. Furthermore, because the gut microbiota is a cohesive ecosystem that can be thought of as a microbial "organ", the addition of incidental microbes in the form of probiotics does not add to the ecosystem; probiotics are unable to colonize the gastrointestinal tract and have an effect on the host only while they transit through the gut (Gonzalez-Rodriguez *et al.* 2013; Mills *et al.* 2011).

The principle of probiotic use is sound, and because the practice is generally regarded as safe, there is little reason for patients not to try it. But to view probiotics solely as a therapeutic regimen for one particular indication may exclude a greater potential. With the combined knowledge shared in *The Human Microbiome Handbook*, we have become aware of the ecological nature of the human microbiome. One particular direction involves using the combination of experimental and clinical evidence to identify the steps in development of an ecosystem rich in beneficial microbes. Alternatively, in the future, we could leverage

the accumulating knowledge of the human gut microbiota to discover novel probiotic species or to create whole probiotic ecosystems. We are only beginning to understand how this is possible. Perhaps we should turn our attention to the microbiotas of individuals from varied geographical and cultural backgrounds, which traditionally are considered to be very healthy, often with higher than globally average numbers of centenarians. An expansion of the concept of probiotic use will require both time and further experimentation, yet more importantly, may result in a shift of the microbial-based medical mindset from one of treat and cure to adapt and restore.

To a certain extent, steps have already been made toward this goal. In the treatment of recurrent *Clostridium difficile* infection, where fecal transplant is rapidly emerging as an effective intervention (as discussed Chapter 3), concerns about the safety of using stool as medicine have driven us to try to determine the microbial components that are missing from the colons of patients, and then to effect a treatment by replacing these components in a defined way (Lawley *et al.* 2012; Perez-Cobas *et al.* 2014; Petrof *et al.* 2013; Shahinas *et al.* 2012; Shankar *et al.* 2014). Our prototype therapeutic, "RePOOPulate", or Microbial Ecosystem Therapeutic (MET)-1 is an example of this approach, where a 33-strain ecosystem, rich in Firmicutes, was applied to *C. difficile* patients (Petrof *et al.* 2013). *C. difficile* infection is known to correlate with a reduction in Firmicutes and a concomitant increase in Proteobacteria (Fuentes *et al.* 2014), and thus our defined ecosystem was introduced to try to redress this balance. Although only a pilot study, MET-1 rapidly cured two patients with severe, recurrent *C. difficile* infection; furthermore, 16S rRNA gene profiling of patient stool during the 6-month period after treatment revealed signatures that identified with MET-1 components, indicating that, unlike traditional probiotics, the delivered ecosystem was able to colonize for at least this long in the patients (Petrof *et al.* 2013). MET-1 was designed with microbial ecology in mind; the 33-strain mixture was derived from a single healthy donor (Petrof *et al.* 2013). We believe this to be important because these selected strains had formed part of a cohesive ecosystem in the donor. In other words, the gut environment of the donor had selected a groups of strains that could work together efficiently. Further work is underway to create more complex ecosystems from a series of different healthy donors with differing lifestyles (for example, various dietary practices), recognizing that different ecosystems may be optimal for diverse recipients.

Studies of the gut microbiotas of individuals from cultural back-

grounds not typically exposed to widespread antibiotic exposure may help us to determine diversity loss in the Western world (Grzeskowiak *et al.* 2012; Schnorr *et al.* 2014) and could be instrumental in developing METs to restore the "missing microbiota". Understanding the host-microbiota cross talk that allows a given microbial ecosystem to work optimally within its host is a current research goal, and already bioinformatics approaches are being used to try to understand microbiota function in the context of disease (Collison *et al.* 2012). In the future, this stream of research will allow for the rational design of METs for use in the gut as well as other body sites. With accumulated knowledge, we may discover treatment or prevention regimens for a wide range of diseases.

12.5. PREDICTING THE INFLUENCE OF XENOBIOTICS ON THE HUMAN MICROBIOTA

Diet, so far, is the greatest known modulator of the gut microbiota (Dore and Blottiere 2015); microbes come into contact with and are influenced by the food we eat during the process of digestion, and the colon is essentially a specialized chamber where food substrates that are indigestible through the actions of human enzymes and processes can be broken down by the microbiota through anaerobic fermentation, a highly complex activity (Louis *et al.* 2007). As such, the food that we eat is more than food for our human selves, and we should consider our gut microbiota as an organ that takes part in the digestive process.

Recently, however, research on the effects of certain food additives on the colonic microbiota has brought to light some disturbing oversights. While xenobiotics such as food additives are rigorously tested for safety, in the past these toxicity assays have rarely, if ever, taken into account the effects of these additives on the gut microbiota. Some artificial food additives, such as sweeteners and emulsifying agents, have now been shown to affect the balance of microbes within the gut (Chassaing *et al.* 2015; Palmnas *et al.* 2014; Suez *et al.* 2014), and in the case of some sweeteners, may actually contribute to a microbiota reminiscent to that seen in metabolic disease (Palmnas *et al.* 2014; Suez *et al.* 2014).

In the same way that food additives have been overlooked as gut microbiota modulators, many of the drugs we consume have likewise rarely been tested for their effects on the gut microbiota (Li and Jia 2013). Pharmaceutical companies invest billions of dollars in drug

discovery, and the added burden of testing for microbiome-associated effects (where every individual may be different) seems like an impossible achievement. However, drugs such as metformin, used to treat people with type-2 diabetes, serve as a good example of the role of the gut microbiota in modulation of pharmacological effects—this drug has been shown to directly affect the metabolic pathways of the microbiota, influencing the growth of some microbes over others, perhaps explaining why some individuals cannot tolerate the medication because of diarrheal side-effects (Lee and Ko 2014).

In the future, food additives and drugs will require more vigorous safety profiling, with predictions of effects on microbiota types from a wide range of individuals in addition to standard toxicology assessments. This will allow much more accurate assessments of detriment versus benefit and may alter the way that new and existing food additives and drugs are used or introduced.

12.6. LEVERAGING MICROBIOME KNOWLEDGE TO OPTIMIZE NUTRITION STRATEGIES

Simplistically, gaining nutrition from foods takes place via two pathways: (1) directly, through the actions of human enzymes and binding factors on the food and subsequent absorption of the breakdown/bound products through the gut; and (2) indirectly, through the actions of the microbiota on foods to yield host-absorbable substrates and metabolites. Until fairly recently, the second pathway has been generally ignored, however, there are important consequences of this pathway to nutrition.

At its most extreme, the gut microbiota is associated with malnutrition in both infancy and old age, with changes in the microbiota correlating with poor absorption of nutrients (Claesson *et al.* 2012; Ghosh *et al.* 2014; Kane *et al.* 2015; Lakshminarayanan *et al.* 2014; Subramanian *et al.* 2014). In childhood malnutrition, poor development of the gut microbiota, perhaps because of lack of exposure to a diverse diet, has been implicated in the disease (Subramanian *et al.* 2014). The gut microbial ecosystem becomes resistant to compositional change as successions in various taxa naturally decrease with age (Valles *et al.* 2014), and therefore a poorly developed microbial ecosystem may persist through childhood and contribute to malnutrition even in the face of dietary intervention.

At the other end of the scale, obesity and metabolic syndrome are

now understood to be associated with the microbial content of the gut, and studies of identical twins discordant to obesity implicate certain microbial taxa in the disease (Goodrich *et al.* 2014). Two recent studies highlight the importance of the gut microbial ecosystem in obesity. The first of these was a trial of the effectiveness of fecal transplant, as donated from a healthy, lean individual, on metabolic disease in obese men (Vrieze *et al.* 2012). In this study, a reduction in insulin dependence was noted in the obese recipients who received the lean donor's stool, compared to those who received their own stool back as a control. The second study is a case report of a woman of average BMI who received a stool transplant from her obese daughter to treat a *C. difficile* infection, and though this patient was cured of her infection, she went on to gain significant weight in the months following the procedure, potentially as a consequence of receiving an obese-type microbiota (Alang and Kelly 2015).

In the future, the use of microbiome-modulating therapies to treat these conditions may become a reality, with a greater understanding of the development of the microbiota, as well as the influence of diet on these microbes. Such therapies may range from directed prebiotic therapy, using food starches targeted to specific microbial groups to stimulate their growth and effect more efficient digestion (Scott *et al.* 2015), to full MET strategies as above, to replace or modify ecosystems that are contributing to metabolic disease or malnutrition.

Future nutritional therapies need not be confined to disease management. Along with a dawning recognition that everyone has a unique gut microbial ecosystem, there is an opportunity for food manufacturers to capitalize on personalized nutrition. For example, it may become possible to determine optimal prebiotic foods from an assessment of gut microbiota profiles on an individual basis; armed with this knowledge, a person may be able to select food at the supermarket that is compatible with his or her gut microbiota, and to understand which food substrates might be the most optimal for their microbial symbionts.

12.7. SUMMARY

As was predicted thousands of years ago with the advent of Chinese traditional medicine, wellbeing originates in the gut (Li *et al.* 2009). This was echoed over 100 years ago by Élie Metchnikoff who postulated that microbes may be key to a longer and healthier life. Although much time has passed, we are now playing a form of catch-up to best

understand and appreciate the involvement of our trillions of microbial passengers. Thankfully, this revolution is not limited to microbiology but is now widespread in medicine and incorporating numerous studies once considered unimaginable. As this book was published, researchers began to demonstrate the use of microbes to alleviate allergies to peanuts as well as in the remediation of psychiatric conditions. While the data is still scant and more work needs to be performed, these two studies alone demonstrate how microbes have transcended their initial denouncements as solely pathogens, and have become an integral part of our health and medicine. In the future, greater attention will be paid to our microbial symbionts and leverage their beneficial activities. In doing so, it is anticipated that our view of health will be expanded such that we no longer focus on our human selves, but rather on ourselves as human/microbial superorganisms that can maintain our wellbeing through support of all our biological systems, physiological, metabolic, immunological, neurological, endocrinological, and finally, microbial.

12.8. REFERENCES

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