MINIREVIEW

Implications of the human microbiome in inflammatory bowel diseases

Syeda M. Bakhtiar[1], Jean Guy LeBlanc[2,3], Emiliano Salvucci[4], Amjad Ali[1], Rebeca Martin[5,6], Philippe Langella[5,6], Jean-Marc Chatel[5,6], Anderson Miyoshi[1], Luis G. Bermúdez-Humarán[5,6] & Vasco Azevedo[1]

[1]Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (ICB/UFMG), Belo Horizonte, Brazil; [2]Centro de Referencia para Lactobacilos (CERELA-CONICET), San Miguel de Tucumán, Argentina; [3]Cátedra de Metodología de la Investigación Científica, Facultad de Medicina, Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina; [4]Facultad de Ciencias Exactas, Físicas y Naturales, Instituto de Ciencia y Tecnología de los Alimentos, Universidad Nacional de Cordoba, Cordoba, Argentina; [5]INRA, UMR1319 Micalis, Commensal and Probiotics-Host Interactions Laboratory, Jouy-en-Josas Cedex, France; and [6]AgroParisTech, UMR1319 Micalis, Jouy-en-Josas, France

Correspondence: Luis G. Bermúdez-Humarán, INRA, UMR1319 Micalis, Commensal and Probiotics-Host Interactions Laboratory, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France.
Tel.: +33 134 65 24 63; fax: +33 134 65 24 62; e-mail: luis.bermudez@jouy.inra.fr

Received 25 January 2013; revised 19 February 2013; accepted 19 February 2013. Final version published online 15 March 2013

DOI: 10.1111/1574-6968.12111

Editor: Andre Klier

Keywords
Microbiome; Human holobiont; Microbiota; Commensal.

Abstract

The study of the human microbiome or community of microorganisms and collection of genomes found in the human body is one of the fastest growing research areas because many diseases are reported to be associated with microbiome imbalance or dysbiosis. With the improvement in novel sequencing techniques, researchers are now generating millions of sequences of different sites from the human body and evaluating specific differences in microbial communities. The importance of microbiome constituency is so relevant that several consortia like the Human Microbiome project (HMP) and Metagenomics of the Human Intestinal Tract (MetaHIT) project are focusing mainly on the human microbiome. The aim of this review is to highlight points of research in this field, mainly focusing on particular factors that modulate the microbiome and important insights into its potential impact on our health and well-being.

Introduction

The term Microbiome, which refers to the total number of microorganisms and their genetic material, is frequently confused with the term microbiota, which is the microbial population present in many parts of the human body (Fig. 1). Joshua Lederberg first coined the term 'Microbiome' in 1977 to define the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space, arguing that microorganisms inhabiting the human body should be included as part of the human genome, as they directly influence the human physiology (Lederberg & Mccray, 1977). The human body has 100 trillion microorganisms in the gut, literally 10 times more than the cells in the human body (Fig. 1). These indigenous microbial communities explain critical features of human biology and also play an important role in human health and disease. Factors, which enable a bacterium to colonize the gut, are not yet fully explained, but there is a general acceptance about the mutual benefits for both the host and bacteria, which is the key for this successful partnership. New insights are emerging to explain this close association. The hologenome theory considers that the holobiont, an organism and all of its associated symbiotic microorganisms, including parasites, mutualists, synergists, and amensalists (microbiome) evolved as a result of symbiopoiesis, or codevelopment of the host and symbionts (Margulis & Fester, 1991; Zilber-Rosenberg & Rosenberg, 2008; Gilbert *et al.*, 2010).

Initially, the analysis of the human microbiome was carried out using culture-based techniques to study the diversity of commensal bacteria. The advent of culture-independent techniques was one of the most important steps for microbial diversity profiling in humans. Cul-

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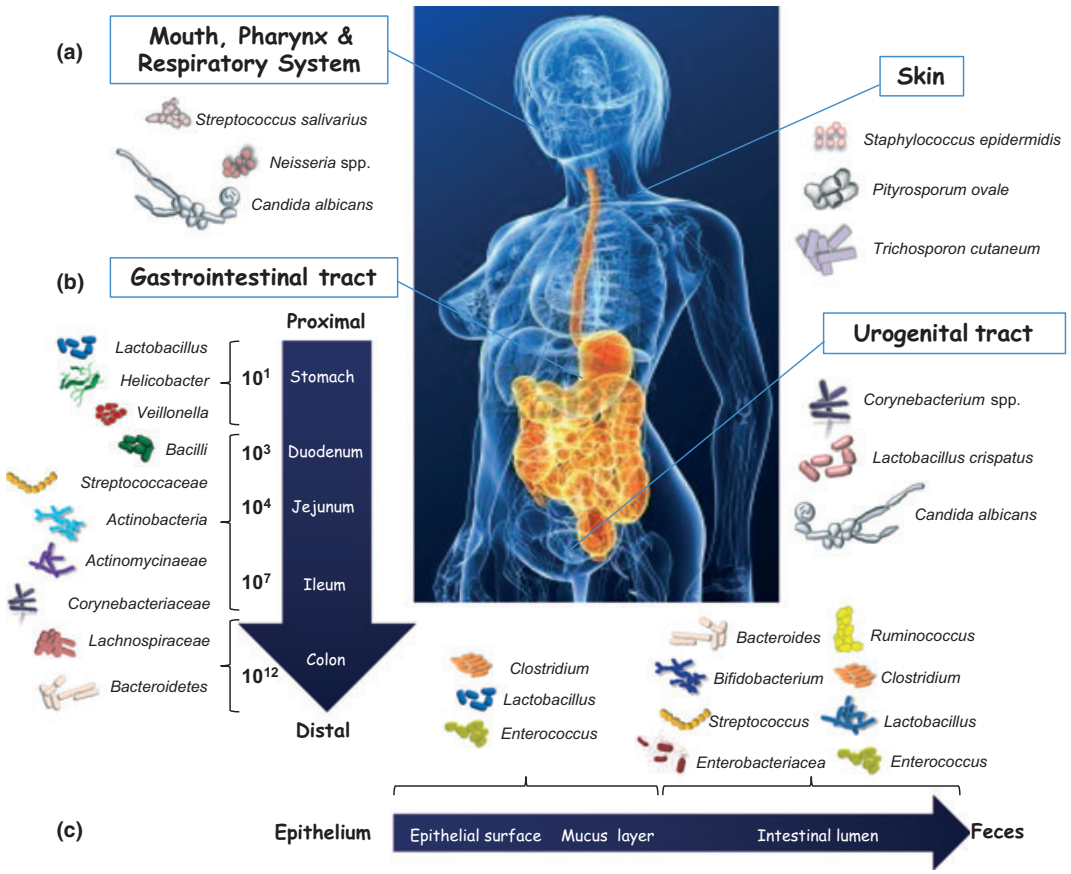

Fig. 1. Schema of microbial population present in the human body. (a) Spatial features of main microbial composition present in different parts of the human body. (b) Variations in bacteria numbers and composition across the length of the gastrointestinal tract. (c) Longitudinal variations in bacteria composition in the intestine (Microorganisms illustration not to scale).

ture-independent methods used for characterizing the microbiota along with a molecular phylogenetic approach provide the basis for researchers to compare microbial communities across environments within a unified phylogenetic context. Although new sequencing technologies and potent bioinformatics tools are essential for understanding the human microbiome, they will not be the focus of this current review.

The human holobiont

The diversity of the human microbiome was first studied by Antonie van Leeuwenhoek, who compared oral and fecal microbiota (Van Leewenhoek, 1684). Today, we know that more than 1000 different bacterial phylotypes comprise the human microbiome; composition of which is unique to each individual. Humans did not evolve as a single species instead they evolved with a complex-associated microbiome, building a kind of 'superorganism' or holobiont (Zilber-Rosenberg & Rosenberg, 2008). The human microbiome determines and defines the immune system, and it is an integral part of fundamental processes in the human body, such as vitamin production, digestion, energy homeostasis, angiogenesis, and maintenance of the intestinal barrier integrity (Dominguez-Bello & Blaser, 2008; Kau *et al.*, 2011; Rosenberg & Zilber-Rosenberg, 2011; Slonczewski & Foster, 2011). The microbiome of an individual is an highly variable and compartmentalized ecosystem (Costello *et al.*, 2009; Caporaso *et al.*, 2011; Salonen *et al.*, 2012). As such, the anatomical sites within the human body serve as important sources of variation. Although a 'core' set of specific bacterial taxa has been proposed (Tap *et al.*, 2009), this has yet to unequivocally demonstrated or proven (Ursell *et al.*, 2012). This fact implicates the importance of a particular pool of microbial genes, which can be found in a set of microbial species that yield similar functions (Ursell *et al.*, 2012). The diversity of the host's genome pales in comparison with that of the diversity of the microbiome (Qin *et al.*, 2010). For example, individuals can be classified by enterotypes based on their gut microbiome. In one study, 22 sequenced fecal metagenomes of individu-

als from four countries with previously published data sets were analyzed and three robust clusters were identified that are not nation or continent specific (Arumugam *et al.*, 2011). These clusters (enterotypes) are mostly driven by species composition; however, abundant molecular functions are not necessarily provided by abundant species, highlighting the importance of a functional analysis to understand microbial communities (Arumugam *et al.*, 2011).

The fastidious environment of the human gut suggests that co-evolution of vertebrates and their microbial consortia, over hundreds of millions of years, has selected a specialized community of microorganisms (Ley *et al.*, 2008). Regardless of the increasing knowledge about bacterial communities that colonize the human gut, little is known about other groups, like methanogenic archaea, viruses, and mobile elements. An important characteristic of prokaryotes is their capability to exchange genes with one another through horizontal gene transfer. Bacteria comprising the microbiome have mobile elements that include plasmids, transposons, integrons, and bacteriophages that constitute the 'mobilome'. This genetic pool and the horizontal gene transfer within the microbiome are a key factor of the microbiome activity and constitute the dynamic response to the environment leading to the adaptation of the holobiont (Siefert, 2009; Jones, 2010). The human microbiome is rich in diversity and abundant in microbial species, which are in close contact with the human body, especially in the gut, providing the best situation to facilitate the exchange of genetic material between these microorganisms. In fact, conserved genes and transposon families, among human microorganisms, suggest that horizontal gene transfer occurs in the human microbiome (Lozupone *et al.*, 2008).

Analyses of the relationship between human microorganisms from different human body sites can be achieved by constructing a human microbe interaction network based on the horizontal gene transfer events among human microorganisms. Kanhere and Vingron have analyzed the function of horizontal gene transfer candidate prokaryotes and found that the majority of genes, which are transferred, have functions related to metabolism and translation (Kanhere & Vingron, 2009).

Changes in microbiome composition with age

The establishment of the human microbiota and microbiome starts at birth and reaches its maximum complexity at adolescence, finally remaining relatively stable in healthy adults. In the later stages of life, the microbiome becomes comparatively less diverse with reduced stability (Biagi *et al.*, 2010).

The microbiota acquired by an infant depends on the mode of delivery (Dominguez-Bello & Blaser, 2008; Scholtens *et al.*, 2012). Just after the birth, the microbiota of vaginally delivered babies is similar to that of the mother's vagina and in case of C-section delivery, the baby's microbiota resembles that of the mother's skin (Dominguez-Bello *et al.*, 2010). Another important factor for the establishment of the microbiome is the mode of feeding, as the microbiota of breast- and formula-fed babies is significantly different, both in composition and diversity. *Bifidobacteria*, known for their beneficial properties, are present in the microbiota of breast-fed babies, while with formula-fed babies *Escherichia coli, Clostridium difficile, Bacteroides fragilis,* and lactobacilli predominate (Penders *et al.*, 2006). Pregnant women of normal body weight and healthy microbiota profiles, (gut and breast milk microbiota) have greater opportunities to pass on compounds, antigens modified by the mother's gut, and other agents, that promote the development of a healthy immune system in the breast-fed infant (Isolauri, 2012). Genome analysis shows that *Bifidobacterium longum* ssp. *infantis* can use milk-derived molecules that are not of nutritional importance for the baby (Sela *et al.*, 2008), while some *Bacteroides* such as *Bacteroides thetaiotaomicron* can live on milk glycans as a sole carbon source (Marcobal *et al.*, 2010). In fact, metatranscriptomic studies show that carbohydrate metabolism is the most prominent function of the transcripts, and also revealed that these genes are expressed at higher levels in breast-fed babies than formula-fed babies (Klaassens *et al.*, 2009).

A gradual increase in phylogenetic diversity of the microbiome with time has been reported to exist. The ecosystem stabilizes, being more complex in adult life, and is dominated by the phyla *Bacteroidetes* and *Firmicutes* (Rajilić-Stojanović *et al.*, 2009). Aging results in reduced ecosystem stability making it more dynamic, with the dominating phyla at this stage shifting from *Firmicutes* to *Bacteroides* accompanied by an increase in the number of *Proteobacteria* and reduction in *Bifidobacteria* (Biagi *et al.*, 2010). The core microbiota of elderly individuals significantly differed from young adults, with a characteristic shift toward a *Clostridium*-dominated community (Claesson *et al.*, 2011).

Interaction between microbiome and environment

The interaction between the human microbiome and the environment is dynamic. Nevertheless, human microbial communities can strongly differentiate individuals and can act as their signatures (Fierer *et al.*, 2010). For example, the microbial community structure during the neonatal period is referred to as 'chaotic' in relation to composition

and structure, after exposures to specific antigen, diet, chemical, human, and animals, affecting the overall microbiome. For example, the presence of porphyranase and agarase, unusual active products against polysaccharides of marine red algae, in Japanese individuals supports this idea, as Japanese people prefer consuming nutritional seaweeds, which resulted in acquisition of these genes (Hehemann *et al.*, 2010).

The most important factor influencing the composition of the microbiome is the use of antibiotics. When these compounds are used against pathogens they also affect the host innate microbiota and impact the stability of the microbiome. The responses to antibiotic treatment are individualized and are influenced by prior exposure of an individual to the same antibiotic. In fact, only after 1 week of treatment with ciprofloxacin, gut microbiota can regain its composition, returning to its pretreatment state (Dethlefsen & Relman, 2011). In contrast, clindamycin treatment (an antibiotic with strong antianaerobic activity) can reduce *Bacteroides* diversity, after a week of consecutive use, and its impact can last up to 2 years in healthy individuals. On the other hand, a different impact on microbiota has been observed for 4 years after usage with broad spectrum antibiotics, such as clarithromycin and metronidazole (Jernberg *et al.*, 2010).

The genetic diversity found within the human gut microbiota also affects the microbiome, as it allows the digestion of compounds using several metabolic pathways that are not explicitly coded for in the mammalian genome (Turnbaugh *et al.*, 2006). Each individual human microbiome provides new genes to digest new dietary products (Hehemann *et al.*, 2010). A number of studies support the observation that the microbiota of the human gut earns energy from the polysaccharides and peptides, which are indigestible by human enzymes (Guarner & Malagelada, 2003). For instance, the human genome lacks genes coding for enzymes required for degrading plant polysaccharides with high carbohydrate containing xylan, pectin, and arabinose, which usually humans consume. However, this capability is provided by the microbiome, which enables humans to utilize sucrose, glucose, galactose, fructose, and mannose as well as participate in the synthesis of essential amino acids and vitamins (Bäckhed *et al.*, 2005; Gill *et al.*, 2006; LeBlanc *et al.*, 2012). Indeed, the transformation of sugars into butyryl-CoA butyrate, a short-chain fatty acid that is the main energy source of colonocytes and establishes a barrier that maintains a healthy intestinal state, is carried out by the microbiome (Topping & Clifton, 2001). Metagenomic, metatranscriptomics, and metaproteomics analyses suggest that *Firmicutes* are helpful in carbohydrate metabolism, whereas Bacteroidetes are reported to be involved in amino acid transport and metabolism (Ottman *et al.*, 2012).

Food habits also play an important role in the establishment of the microbiome. *Firmicutes* and *Proteobacteria* dominate the microbiome of European individuals as European food is usually high in animal protein, sugar, starch, and fat, but low in fiber, contrasting with the vegetarian diet rich in carbohydrates, fibers, and nonanimal protein consumed in Africa, which results in a microbiome dominated by *Actinobacteria* and *Bacteroidetes* (De Filippo *et al.*, 2010). Despite the fact that certain bacterial phyla always dominate the human gut, variations in the relative percentages of *Firmicutes* and *Bacteroidetes*, within the human population, reflect the dietary effects and macronutrient consumption (Ley *et al.*, 2005). The microbiome of individuals also depends on the genetic background, as the microbiome of twins has been reported to be more similar, when compared to their parents or a third individual (Turnbaugh *et al.*, 2009).

Recent evidence from molecular ecology has also shown that prebiotics (nondigestible food ingredients that stimulate the growth and/or activity of specific microorganisms) can influence the species composition of the intestinal microbiota both in short-term dietary interventions and in response to habitual long-term dietary intakes (Flint, 2012; Saulnier *et al.*, 2013).

Another method to modify the microbiome is through fecal microbiota transplantation (FMT), which re-establishes a balanced intestinal flora with resultant cure of recurrent *Clostridium difficile* infection (CDI), and has also been used to treat other gastrointestinal diseases including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and chronic constipation and a variety of non-GI disorders (Aroniadis & Brandt, 2013). A recent study showed the statistical analyses of pooled deep-sequencing data from successful FMT recipients revealed the dynamic changes in the microbial community that occur pre- and post-FMT (Shahinas *et al.*, 2012). At the level of phyla, the healthy gut microbiota consists predominantly of *Bacteroidetes*, whereas CDI patients in this study demonstrate an overabundance of *Proteobacteria* and a marked decreased in richness and diversity. Healthy post-FMT recipients appear to 'adopt' certain elements of the donor microbiota caused by an eradication of key *Proteobacteria* species and a corresponding restoration of key *Firmicutes* and *Bacteroidetes* species, which may well be critical to 'outcompeting' or depriving *C. difficile* of fitness in some way.

Disease and dysbiosis

It is well know that our microbiome (*e.g.* intestinal microbiota) plays a major role in the functions of the intestinal epithelium and provides resistance to colonization by

pathogenic microorganisms. Intestinal epithelial cells (IECs) constitute the first line of contact of the gut microbiota and act as a barrier to prevent the translocation of substances systemically (Turner, 2009). IECs are also the interface between the external environment and the most extensive host lymphoid compartment, the gut-associated lymphoid tissue (GALT), a tissue rich in cells of the innate and adaptive immune system. To maintain intestinal homeostasis, GALT should process large quantities of information at the interface between the luminal side and the host, distinguishing between commensal bacteria and pathogenic microorganisms (Quigley, 2010). GALT interacts with intestinal bacteria that are sampled by dendritic cells (DCs) and IECs through pattern recognition receptors such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD; Coombes & Powrie, 2008; (Kelly & Mulder, 2012). These innate immune receptors recognize conserved microbial structures found on microorganisms referred as microbial-associated molecular patterns (MAMPs) both on pathogens and commensal bacteria. An essential symbiotic relationship exists thus between our intestinal epithelium and commensal bacteria, and although some intestinal bacteria are potential pathogens, the relationship between the intestinal microbiota and the human host is mostly symbiotic in healthy individuals (Hwang *et al.*, 2012). Disruption of this interaction and/or alterations of the microbiome could potentially affect human health and promote disease states or dysbiosis (Hojo *et al.*, 2009; Rogler, 2010). The gastrointestinal epithelium is covered by a protective mucus containing predominantly mucin glycoproteins that are synthesized and secreted by goblet cells. Intestinal microorganisms may directly affect goblet cell functions through the local release of bioactive factors, such as those that are released during the fermentation with beneficial microorganisms (de Moreno de LeBlanc *et al.*, 2008).

There are many diseases connected to the gut microbiota and their imbalance such as obesity, IBD, chronic periodontitis, IBS, tropical enteropathy, antibiotic-associated diarrhea, vaginosis, etc. However, the most evident impact of microbiota in human health is provided by studies on IBD, a group of diseases characterized by a chronic and relapsing inflammation of the GIT, which is prevalent in Western countries. The two most common forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC; del Carmen *et al.*, 2012).

An increase or decrease in the number of different bacterial groups has been reported to be associated with IBD. For example, decrease in *Firmicutes,* such as *Faecalibacterium prausnitzii*, is well documented in the case of CD patients (Sokol *et al.*, 2008 a,b). On the other hand, 16S rRNA gene pyrosequencing studies performed by Willing *et al.* (2010) revealed a clear division of microbial composition in healthy adults and UC patients. Rajilić-Stojanović *et al.* (2009) reported an analysis of fecal microbiota of UC patients and confirmed the reduction in bacterial diversity in these individuals. Similarly, obesity-associated metabolic disorders like metabolic syndrome, type 2 diabetes, etc. may be associated with microbiome compositions. Ley *et al.* (2006) reported an increase in microbial count of *Firmicutes* and decrease in *Bacteroidetes*, which was corroborated by many groups later on (Nadal *et al.*, 2009; Zhang *et al.*, 2009; Schwiertz *et al.*, 2010).

The dominating four bacterial phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* are the best studied groups in the microbiome, however, archaea, viruses, fungi, and eukarya, including helminths, also constituents of this community; their study is necessary for better understanding of the relationship between the microbiome and host, as a whole in addition to their complex intertwined metabolic networks. The emergence of several diseases such as IBD can be understood under the holobiont paradigm, with consideration of the Bioma Depletion Theory. Both theoretic frames consider the host and their microbiome evolved as a 'superorganism' (Kinross *et al.*, 2008; Rook, 2009). In this revolutionary approach that considers any organism a result of integration, the immune system can be considered as an interface, with their symbiotic organisms that have co-evolved more than a defense against invading organisms. Improvements in medical care and technology have increased the occurrence of allergic disorders, autoimmune diseases and left us an over-reactive immune response caused by a loss and separation of our partners, our microbiomes that normally interact with our immune system (Garn & Renz, 2007; Kau *et al.*, 2011).

The genetic background necessary to develop any of these illnesses is directly and closely related to and influenced by the metabolism of the microbiome (Proal *et al.*, 2009; Tilg & Kaser, 2011). The benefits of different foods, that have been known for centuries (such as the yogurt), led to the discovery of different bacterial strains, mainly lactic acid bacteria (LAB), that have been proposed, after many studies, to act as probiotics: live microorganisms which, when administered in adequate amounts, confer a beneficial health effect on the host (FAO/WHO, 2001). Autoimmune diseases like type 1 diabetes (T1D), arthritis, lupus and inflammatory related diseases (such as IBD and IBS), and asthma may be treated with a biome restoring process that could be done by probiotic administration.

In conclusion, human microbiome (which is a direct consequence of the mutualism between the host and its microbiota), is fundamental for the maintenance of the homeostasis of an healthy individual.

MPH and MetaHIT projects

After the completion of the Human Genome project, the next step was to sequence microorganisms present in and on the human body. In this context, the Human Microbiome Project (Peterson *et al.*, 2009) and the European MetaHIT (Metagenomics of the Human Intestinal Tract) project (Qin *et al.*, 2010) were launched and focused on all aspects of the human metagenomics, from protocol design to data analysis and visualization, and finally to disease correlation.

Another goal of the HMP was to discover whether humans have an identifiable microbiome 'core' of shared components (Salonen *et al.*, 2012). Although microbial organisms showed variations between individuals, metabolic pathways necessary for human-associated microbial life were consistently present, forming a functional 'core' to the microbiome at all body sites (Turnbaugh *et al.*, 2009). The healthy microbiome achieves a consistent balance of function and metabolism that is maintained in health, but with fine-tuned details personalized by genetics, life events, and environmental factors such as diet and exposures (Rho *et al.*, 2012). Data from individuals without overt signs of disease serve as an excellent reference for disease-associated microbiome studies, while also providing a comprehensive baseline for comparison of western populations with disparate geographic, ethnic, and genetic cohort (Yatsunenko *et al.*, 2012). Bacteria are of course not the only mediator of dysbiotic disease, and metagenomic approaches can also be used to identify potential viral etiologies (Wylie *et al.*, 2012).

Conclusion

Although invisible to the naked eye, the microbiome should not be underestimated as a key determinant of health and disease. Host and microbiome have been created alongside one another through evolution as a superorganism and changes in the microbiome affect the host as a whole. Although the tools currently available have yet to be perfected, the prospects for developing a mechanistic understanding of the factors that underlie the plasticity of the microbiome and then manipulate the microbiome to improve health seem increasingly bright. For example, limited read length in high throughput sequencing technologies currently limits our ability to detect bacterial species and strains, and analysis of viruses and eukaryotes is still very much an emerging frontier. Microbiomics and metagenomics must collaborate to fully elucidate the nature of the microbiome under healthy and disease states, which will, subsequently, pave the way for more effective therapeutic and diagnostic techniques. Restoration of our depleted microbiome appears as the current challenge in the treatment of complex disorders emerging due to imbalances in our immune system.

Acknowledgements

Authors would like to thank the CONICET, ANPCyT, MinCyt, and CIUNT (Argentina); the CNPq, CAPES, CABBIO, and FAPEMIG (Brasil), and ECOS-Sud (France) for their financial support. This study was a part of FPARIS collaborative project selected and supported by the Vitagora Competitive Cluster and funded by the French FUI (Fond Unique Interministériel), the FEDER (Fonds Européen de Développement Régional), the Burgundy Region, the Conseil Général 21 and the Grand Dijon. This work was also supported by Merck Médication Familiale (Dijon, France) and Biovitis (Saint Etienne de Chomeil, France). The authors declares no conflicts of interest.

References

Aroniadis OC & Brandt LJ (2013) Fecal microbiota transplantation: past, present and future. *Curr Opin Gastroenterol* **29**: 79–84.

Arumugam M, Raes J, Pelletier E *et al.* (2011) Enterotypes of the human gut microbiome. *Nature* **473**: 174–180.

Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA & Gordon JI (2005) Host-bacterial mutualism in the human intestine. *Science* **307**: 1915–1920.

Biagi E, Nylund L, Candela M *et al.* (2010) Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS ONE* **5**: e10667.

Caporaso JG, Lauber CL, Costello EK *et al.* (2011) Moving pictures of the human microbiome. *Genome Biol* **12**: R50.

del Carmen S, LeBlanc JG & de Moreno de LeBlanc A (2012) Use of probiotics in the treatment of Crohn's disease. *Crohn's Disease: Etiology, Diagnosis and Treatment Options* (LeBlanc JG & de MdLA, eds), (In press). Nova Science Publishers, Inc., Hauppauge, NY, USA.

Claesson MJ, Cusack S, O'Sullivan O *et al.* (2011) Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *P Natl Acad Sci USA* **108**: 4586–4591.

Coombes JL & Powrie F (2008) Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* **8**: 435–446.

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI & Knight R (2009) Bacterial community variation in human body habitats across space and time. *Science (New York, NY)* **326**: 1694–1697.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G & Lionetti P (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *P Natl Acad Sci USA* **107**: 14691–14696.

Dethlefsen L & Relman DA (2011) Incomplete recovery and individualized responses of the human distal gut microbiota

to repeated antibiotic perturbation. *P Natl Acad Sci USA* **108**: 4554–4561.

Dominguez-Bello MG & Blaser MJ (2008) Do you have a probiotic in your future? *Microbes Infect* **10**: 1072–1076.

Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N & Knight R (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *P Natl Acad Sci USA* **107**: 11971–11975.

FAO/WHO (2001) Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.

Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK & Knight R (2010) Forensic identification using skin bacterial communities. *P Natl Acad Sci USA* **107**: 6477–6481.

Flint HJ (2012) The impact of nutrition on the human microbiome. *Nutr Rev* **70**(Suppl 1): S10–S13.

Garn H & Renz H (2007) Epidemiological and immunological evidence for the hygiene hypothesis. *Immunobiology* **212**: 441–452.

Gilbert SF, McDonald E, Boyle N, Buttino N, Gyi L, Mai M, Prakash N & Robinson J (2010) Symbiosis as a source of selectable epigenetic variation: taking the heat for the big guy. *Philos Trans R Soc Lond B Biol Sci* **365**: 671–678.

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM & Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. *Science* **312**: 1355–1359.

Guarner F & Malagelada JR (2003) Gut flora in health and disease. *Lancet* **361**: 512–519.

Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M & Michel G (2010) Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* **464**: 908–912.

Hojo K, Nagaoka S, Ohshima T & Maeda N (2009) Bacterial interactions in dental biofilm development. *J Dent Res* **88**: 982–990.

Hwang JS, Im CR & Im SH (2012) Immune disorders and its correlation with gut microbiome. *Immune Netw* **12**: 129–138.

Isolauri E (2012) Development of healthy gut microbiota early in life. *J Paediatr Child Health* **48**(Suppl 3): 1–6.

Jernberg C, Löfmark S, Edlund C & Jansson JK (2010) Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* **156**: 3216–3223.

Jones BV (2010) The human gut mobile metagenome: a metazoan perspective. *Gut Microbes* **1**: 415–431.

Kanhere A & Vingron M (2009) Horizontal Gene Transfers in prokaryotes show differential preferences for metabolic and translational genes. *BMC Evol Biol* **9**: 9.

Kau AL, Ahern PP, Griffin NW, Goodman AL & Gordon JI (2011) Human nutrition, the gut microbiome and the immune system. *Nature* **474**: 327–336.

Kelly D & Mulder IE (2012) Microbiome and immunological interactions. *Nutr Rev* **70**: S18–S30.

Kinross JM, von Roon AC, Holmes E, Darzi A & Nicholson JK (2008) The human gut microbiome: implications for future health care. *Curr Gastroenterol Rep* **10**: 396–403.

Klaassens ES, Boesten RJ, Haarman M, Knol J, Schuren FH, Vaughan EE & de Vos WM (2009) Mixed-species genomic microarray analysis of fecal samples reveals differential transcriptional responses of bifidobacteria in breast- and formula-fed infants. *Appl Environ Microbiol* **75**: 2668–2676.

LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D & Ventura M (2012) Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* **24**: [Epub ahead of print].

Lederberg BJ & Mccray AT (1977) 'Ome Sweet 'Omics– A Genealogical Treasury of Words.

Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD & Gordon JI (2005) Obesity alters gut microbial ecology. *P Natl Acad Sci USA* **102**: 11070–11075.

Ley RE, Turnbaugh PJ, Klein S & Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. *Nature* **444**: 1022–1023.

Ley RE, Lozupone CA, Hamady M, Knight R & Gordon JI (2008) Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat Rev Microbiol* **6**: 776–788.

Lozupone CA, Hamady M, Cantarel BL, Coutinho PM, Henrissat B, Gordon JI & Knight R (2008) The convergence of carbohydrate active gene repertoires in human gut microbes. *P Natl Acad Sci USA* **105**: 15076–15081.

Marcobal A, Barboza M, Froehlich JW, Block DE, German JB, Lebrilla CB & Mills DA (2010) Consumption of human milk oligosaccharides by gut-related microbes. *J Agric Food Chem* **58**: 5334–5340.

Margulis L & Fester R (1991) Bellagio conference and book. Symbiosis as Source of Evolutionary Innovation: Speciation and Morphogenesis. Conference–June 25–30, 1989, Bellagio Conference Center, Italy. Symbiosis (Philadelphia, Pa.) 11: 93–101.

de Moreno de LeBlanc A, Dogi CA, Galdeano CM, Carmuega E, Weill R & Perdigon G (2008) Effect of the administration of a fermented milk containing Lactobacillus casei DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunol* **9**: 27.

Nadal I, Santacruz A, Marcos A *et al.* (2009) Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. *Int J Obes* **33**: 758–767.

Ottman N, Smidt H, de Vos WM & Belzer C (2012) The function of our microbiota: who is out there and what do they do? *Front Cell Infect Microbiol* **2**: 104.

Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA & Stobberingh EE (2006) Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* **118**: 511–521.

Peterson J, Garges S, Giovanni M *et al.* (2009) The NIH human microbiome project. *Genome Res* **19**: 2317–2323.

Proal AD, Albert PJ & Marshall T (2009) Autoimmune disease in the era of the metagenome. *Autoimmun Rev* **8**: 677–681.

Qin J, Li R, Raes J *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**: 59–65.

Quigley EMM (2010) Probiotics in gastrointestinal disorders. *Hosp Pract (1995)* **38**: 122–129.

Rajilić-Stojanović M, Heilig HGHJ, Molenaar D, Kajander K, Surakka A, Smidt H & de Vos WM (2009) Development and application of the human intestinal tract chip, a phylogenetic microarray: analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. *Environ Microbiol* **11**: 1736–1751.

Rho M, Wu YW, Tang H, Doak TG & Ye Y (2012) Diverse CRISPRs evolving in human microbiomes. *PLoS Genet* **8**: e1002441.

Rogler G (2010) The importance of gut microbiota in mediating the effect of NOD2 defects in inflammatory bowel disease. *Gut* **59**: 153–154.

Rook GAW (2009) Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. *Immunology* **126**: 3–11.

Rosenberg E & Zilber-Rosenberg I (2011) Symbiosis and development: the hologenome concept. *Birth Defects Res C Embryo Today* **93**: 56–66.

Salonen A, Salojarvi J, Lahti L & de Vos WM (2012) The adult intestinal core microbiota is determined by analysis depth and health status. *Clin Microbiol Infect* **18**(Suppl 4): 16–20.

Saulnier DM, Ringel Y, Heyman MB *et al.* (2013) The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. *Gut Microbes* **4**: 17–27.

Scholtens PA, Oozeer R, Martin R, Amor KB & Knol J (2012) The early settlers: intestinal microbiology in early life. *Annu Rev Food Sci Technol* **3**: 425–447.

Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C & Hardt PD (2010) Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* **18**: 190–195.

Sela DA, Chapman J, Adeuya A *et al.* (2008) The genome sequence of Bifidobacterium longum subsp. infantis reveals adaptations for milk utilization within the infant microbiome. *P Natl Acad Sci USA* **105**: 18964–18969.

Shahinas D, Silverman M, Sittler T, Chiu C, Kim P, Allen-Verco E, Weese S, Wong A, Low DE & Pillaij DR (2012) Toward an Understanding of Changes in Diversity Associated with Fecal Microbiome Transplantation Based on 16S rRNA Gene Deep Sequencing. *Therap Adv Gastroenterol* **5**: 403–420.

Siefert J (2009) Defining microbiome. *Horizontal Gene Transfer Genomes in Flux*, **Vol. 532** (Gogarten MB, Gogarten JP & Olendzenski L eds), pp. 13–27. Humana Press, New York, NY.

Slonczewski J & Foster J (2011) *Microbiology: An Evolving Science*. W. W. Norton and Co., New York.

Sokol H, Lay C, Seksik P & Tannock GW (2008a) Analysis of bacterial bowel communities of IBD patients: what has it revealed? *Inflamm Bowel Dis*, **14**: 858–867.

Sokol H, Pigneur B, Watterlot L *et al.* (2008b) Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn's disease patients. *P Natl Acad Sci USA*, **105**: 16731–16736.

Tap J, Mondot S, Levenez F *et al.* (2009) Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol* **11**: 2574–2584.

Tilg H & Kaser A (2011) Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest* **121**: 2126–2132.

Topping DL & Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and non starch polysaccharides. *Physiol Rev* **81**: 1031–1064.

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER & Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**: 1027–1031.

Turnbaugh PJ, Hamady M, Yatsunenko T *et al.* (2009) A core gut microbiome in obese and lean twins. *Nature* **457**: 480–484.

Turner JR (2009) Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* **9**: 799–809.

Ursell LK, Metcalf JL, Parfrey LW & Knight R (2012) Defining the human microbiome. *Nutr Rev* **70**: S38–S44.

Van Leewenhoek A (1684) An abstract of a letter from Antonie van Leewenhoek, dated sept 12, 1683, about animals in the scurf of the teeth. *Philos Trans R Soc* **14**: 568–574.

Willing BP, Dicksved J, Halfvarson J, Andersson AF, Lucio M, Zheng Z, Järnerot G, Tysk C, Jansson JK & Engstrand L (2010) A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology* **139**: 1844–1854.e1.

Wylie KM, Truty RM, Sharpton TJ, Mihindukulasuriya KA, Zhou Y, Gao H, Sodergren E, Weinstock GM & Pollard KS (2012) Novel bacterial taxa in the human microbiome. *PLoS ONE* **7**: e35294.

Yatsunenko T, Rey FE, Manary MJ *et al.* (2012) Human gut microbiome viewed across age and geography. *Nature* **486**: 222–227.

Zhang H, DiBaise JK, Zuccolo A *et al.* (2009) Human gut microbiota in obesity and after gastric bypass. *P Natl Acad Sci USA* **106**: 2365–2370.

Zilber-Rosenberg I & Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol Rev* **32**: 723–735.