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The role of metagenomics in understanding the human microbiome in health and disease

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The term microbiome refers to the genetic material of the catalog of microbial taxa associated with humans. As in all ecosystems, the microbiota reaches a dynamic equilibrium in the human body, which can be altered by environmental factors and external stimuli. Metagenomics is a relatively new field of study of microbial genomes within diverse environmental samples, which is of increasing importance in microbiology. The introduction of this ecological perception of microbiology is the key to achieving real knowledge about the influence of the microbiota in human health and disease. The aim of this review is to summarize the link between the human microbiota (focusing on the intestinal, vaginal, skin, and airway body sites) and health from this ecological point of view, highlighting the contribution of metagenomics in the advance of this field.

Introduction

The term microbiota refers to the microbial population present within the human body,[1] including bacteria, viruses, archea, protozoans, and fungi. Every individual human harbors 10–100 trillion symbiotic microbial cells, with gut bacteria being the most abundant.[2] In this context, the microbiota is implicitly assumed to be similar to a multicelled organ. However, due to the abundance of microhabitats in the human body and the large number of interactions between the different species with the host and the external environment, microbiota can also be conceptualized as a dynamic ecological community (**Fig. 1**).[3] Indeed, each microbial community within the human body has its own structure, depending on the exact environment where it is localized.[4] Many essential body processes require the presence of diverse microorganisms, as they provide the host with essential nutrients, metabolize indigestible compounds, and defend against colonization by opportunistic pathogens, as well as possessing immunomodulatory properties (for more details see also ref. 5).

As with all ecosystems, a balance exists between the human body and the microbiota. However, this dynamic equilibrium can be altered at any time by environmental factors and external interferences, such as the use of antibiotics.[6] These alterations frequently result in microbial imbalances on or inside the body, a phenomenon also called dysbiosis. Thus, in some ecosystems, such as the gut, a high biodiversity is associated with a healthy status, while low biodiversity is more linked to pathological conditions.[7] On the contrary, in other ecosystems such as the vagina, high diversity is directly associated with illness such as vaginosis.[8] Nevertheless, a disruption of normal microbiota profile or biodiversity is frequently related with a physiopathological condition for the host. From this perspective, there is an increasing interest in the use of microorganisms (i.e., probiotics) to resolve dysbioses.

To achieve the study of this biodiversity, traditional methods were based for many years on culture dependent techniques. However, although the use of these techniques provided a large and interesting set of data, they also resulted in an erroneous view of the human microbiota composition in certain cases. Indeed, many microorganisms need special growth conditions, such as the extremely oxygen sensitive (EOS) bacteria, that makes their culturing and even their detection difficult;[9] whereas others have never been grown in culture and may require special, as yet unknown, growth conditions preventing their identification by culture-dependent methods.[4] Recently, several culture-independent techniques have been developed allowing for a qualitative and quantitative means of identification and are mostly based on PCR and DNA hybridization techniques. These simple methods have completely changed the notion of the human microbiota, opening the door to new and more complete fields such as metagenomics, which is the study of microbial genomes within diverse environmental samples.[10] Metagenomics was first introduced in 1998[11] and is now a widely used technique that has revolutionized the study of the microbiota as a result of its ability to generate a comprehensive catalog of microbial sequences present in various different ecological niches within a large host organism such as humans.

In the past, only single organisms were considered important regarding pathogenic interactions with humans. For this reason, only a few studies of either microbial communities or non-pathogenic bacteria were performed, since these bacterial types were believed not to impact on the well-being of humans. Fortunately, this incorrect concept and underestimation of the importance of the human microbiota has changed in the last few years.

Genomic Approaches to Study the Human Microbiota: Defining the Human Microbiome

The term microbiome refers to the genetic material of the catalog of microbial taxa associated with humans.[12] Being

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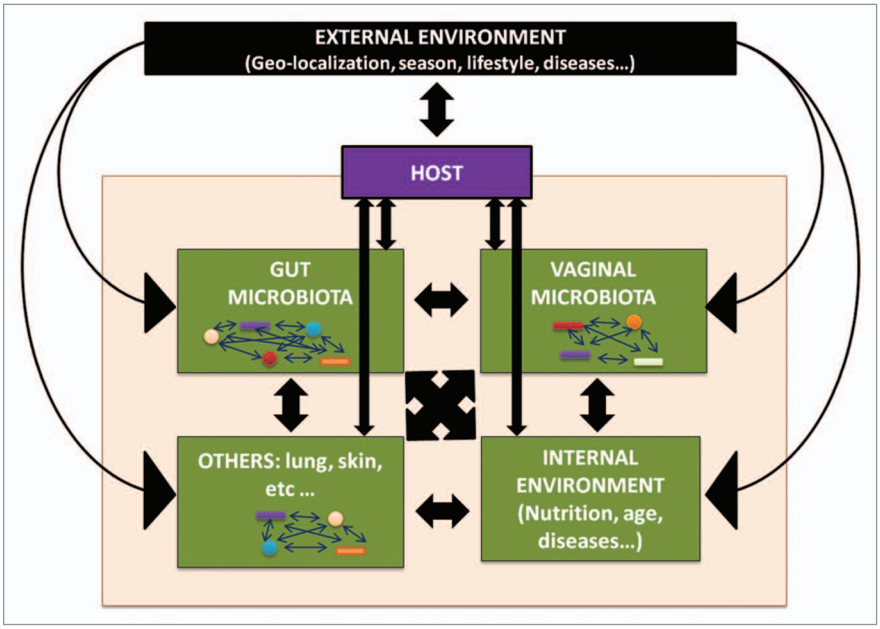

Figure 1. The human microbiome conceptualized as a dynamic ecological community. Interrelations between all the components of the ecosystem lead to an equilibrium state required to maintain the health status of the host.

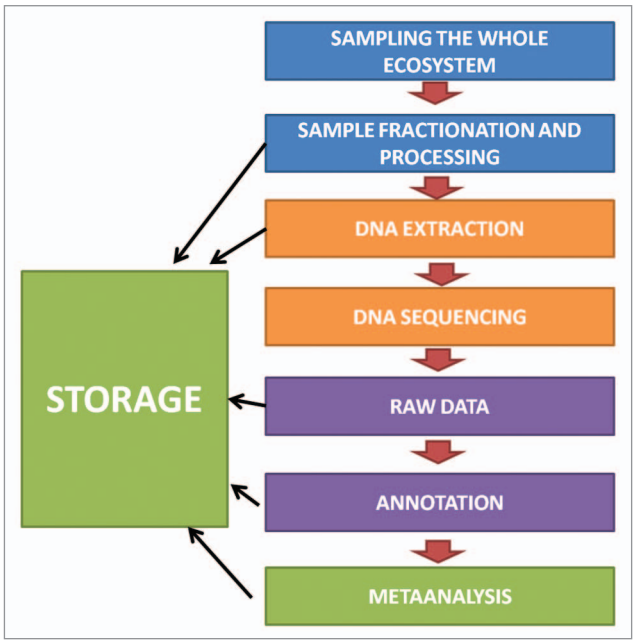

Figure 2. Metagenomic approach general flowchart. After sampling the whole ecosystem and processing the samples, DNA is extracted and sequenced generating raw data. The sequences generated are annotated and submitted to metaanalysis. There are several points to store samples or data.

frequently confused with the term microbiota, the microbiome was first defined by Joshua Lederberg in 2001[13] and is sometimes referred to as our second genome.[14] Although great effort has been applied to the study of the human microbiota (e.g., the Human Microbiome Project, which was launched in 2008 with a funding of $157 million to determine whether there is a shared core microbiome among individuals),[15] the differences among individuals is huge compared with genomic variations.[12] This fascinating reality has potential implications in personal medicine, whereby microbiota composition analysis could be used routinely in clinical practice to diagnose dysbiosis-related disorders.[16]

Although the term metagenomics was originally coined for the shotgun characterization of total DNA, it is also presently being applied for studies of marker genes such as the 16S rRNA.[12] Both shotgun characterization of total DNA and marker genes are used mainly to analyze community structure of the human microbiome. Sequencing the full-length 16S rRNA gene was performed classically by the Sanger dideoxy chain termination technique; today, next generation sequencing methodologies such as Ion Torrent PGM sequencing of 16S rRNA gene-based amplicons[17] reduces the cost and increases the depth. Although a bacterial species is hard to define, the current definition requires a minimum of 97% identity in the 16S rRNA.[4] However, although 16S rRNA sequence is the best measure of low-abundance organisms[18] and it has been widely used allowing for cross-study comparisons,[15] more comprehensive results are found without focusing on target regions such as the 16S rRNA. Shotgun characterization allows either the cataloging of genes of organisms present in a community[4] or the analysis of individual genomes in the ecosystem under study.[19] All these powerful tools provide information on community diversity and structure[20] even if caution is necessary in the sampling method. Huge variations can be introduced by methodological bias.[17] The flowchart diagram (**Fig. 2**) briefly describes the main steps for metagenomic analysis. Several microecological processes have been defined using metagenomics such as microbiota establishment,[21] effect of diet on gut microbiome,[22] and microbiota changes in inflammatory bowel disease (IBD)[23] and obesity.[23,24]

The Role of Microbiome in Human Health

As we can deduce from **Figure 1**, where the human microbiota (microbiome by extension) has been represented as a collection of dynamic ecological communities, the perturbation of one of these communities has a direct impact on health and well-being of the host.[25,26] As a result of recent technological advances, the vagina and the gastrointestinal tract (GIT) have been well characterized (and are to date the most studied human micro-ecosystems) representing low and high complexity communities, respectively.[16] Although dysbiosis has been mainly linked to the GIT, it can take place on any exposed surface or mucus membrane such as the vagina, the skin, or the respiratory system. Indeed, this kind of variation in the microbial population can

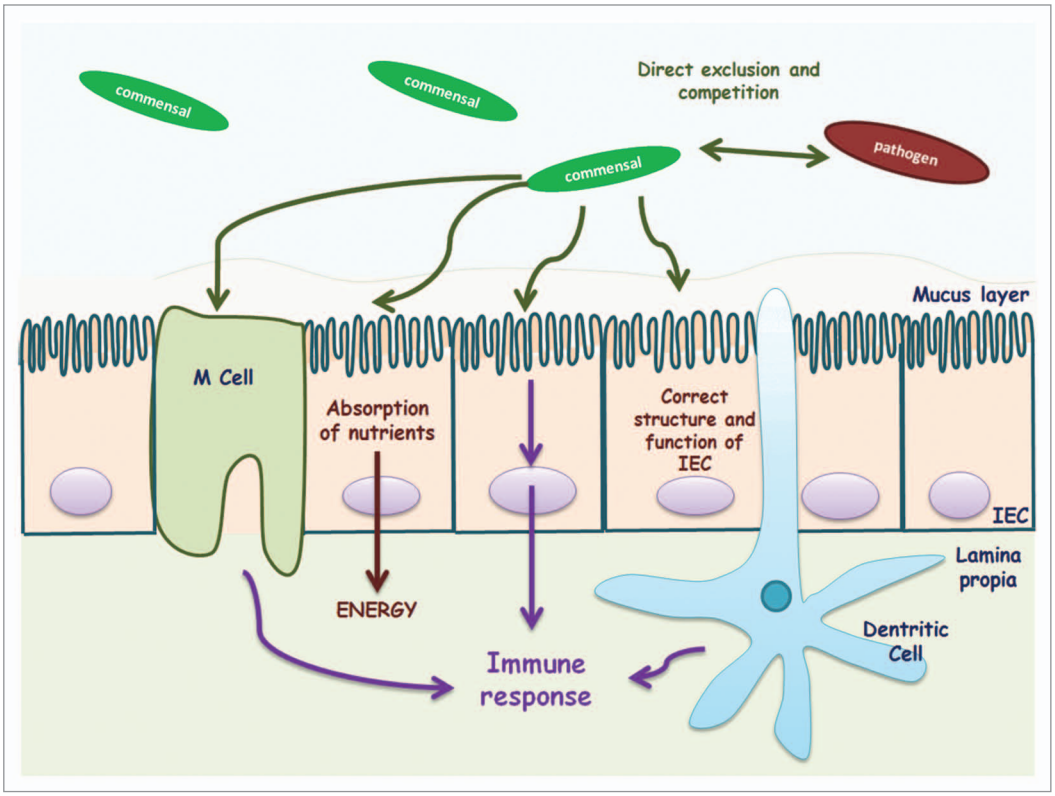

Figure 3. Commensal intestinal bacteria crosstalk with the host. Commensal bacteria supply the host with essential nutrients and defend the host against opportunistic pathogens. Commensals are involved in the development of the intestinal architecture as well as in immunomodulatory processes (Modified from Martín et al.).[5] IEC, intestinal epithelial cell.

strongly impact human health. Nowadays, the challenge of linking microbiome to human health and disease is being confronted by different research teams around the world. They are currently studying different disease states to identify potential correlations and ecological models of community structure and function in order to understand the dynamics of all ecosystems that comprise the human microbiome.

GIT ecosystem

The composition of the human intestinal microbiome is less diverse than other bacterial ecosystems, such as those found in soil or water, presumably reflecting the harsh physico-chemical conditions of this niche. Most of the microorganisms colonizing humans are bacteria present in the GIT at a density of approximately 10^{13}–10^{14} cells/g of fecal matter (particularly in the colon, 70% of total microbiota), and many of them are EOS.[27] The intestinal microbiome also contains a minority population of eukaryotic microorganisms (fungi, yeasts), viruses, and archae.[4] The human gut microbiome is considered to be beneficial for the host due to its key role in the stimulation and maturation of the immune system, promotion of mucosal structure and function, and providing colonization resistance against pathogen attack (**Fig. 3**).[5] Recently, microbiota functions have been reviewed thoroughly by Sommer et al.[28] and Serikov et al.[29]

Metagenomics approach to study the intestinal microbiome

The development of molecular ecology, with emphasis on 16S rDNA-based approaches, has dramatically changed our vision of the gut microbiome. Recent reviews have described the metagenomic exploration of the human intestinal microbiome through the availability of the reference gene catalog and mainly from the European project MetaHIT[23] and the American Human Microbiome Project.[30,31] The MetaHIT consortium has reported 3.3 million non-redundant genes in the human gut microbiome alone[23] and much effort has focused on defining a core human gut microbiome (i.e., a set of features shared across all or the vast majority of gut microbiomes).[32] The average human's intestinal microbiome is now better defined and comprises a huge diversity of bacterial species present in each individual (approximately 160).[23] Firmicutes and bacteroidetes are both dominant phyla representing 90% of the human microbiome. However, the microbiome composition differs along the GIT (from mouth to the rectum), as well as between individuals.[33] In spite of this relative heterogeneity, through fecal metagenomic analysis, it is possible to distinguish three main robust clusters named "enterotypes" in the gut microbiome, which are determined by species composition. Each of these three enterotypes is identifiable by the variation in the levels of one of three genera *Bacteroides*, *Prevotella*, and *Ruminococcus*.[34] Their abundance and proportions vary between individuals and is associated with long-term dietary habits.[35] However, the enterotype is quite a complicated concept because of the necessity to consider the intestinal microbiome according to different factors that could eventually impact its composition (e.g., aging, geographical origin, nutritional needs

and habits, physiological variations and the impact of westernization, etc.).[36] Thus, the enterotype concept is one possible way to simplify the microbiome complexity.

Intestinal microbiome: a component of health

Intestinal bacteria were first qualified as commensals. This adjective comes from the Latin: cum ("with") and mensa ("table"), which means sharing of the meal. Indeed, the host supplies the energy substrate for microorganisms via food intake. In exchange, the intestinal microbiome (through its large repertoire of enzymatic activities), constitutes a complementary metabolic directory of the human digestive system. The co-evolution of the GIT and the microbiome led to the selection of adapted bacteria developing a beneficial cohabitation with the human host.[27,37,38] The metagenomic approach allows a better knowledge of this symbiotic relationship. The intestinal microbiome (the collective genomes of the microorganisms that reside in our GIT) consists of 150-fold more genes than the human genome itself, suggesting the importance and the impact of our "second genome" on human physiology.[23] For instance, the understanding of the ability of the microbiome to metabolize fiber has made some progress, due to the exploration of functional libraries using *Escherichia coli* as host.[39] Besides, the composition of the intestinal microbiota is widely influenced by diet. For example, a study using high-throughput 16s rDNA and comparing the microbiota of children with a western diet to children with a fiber-rich diet (in a rural African village of Burkina Faso), demonstrated that bacterial composition was clearly different. Indeed, the bacterial microbiome of the inhabitants of Burkina Faso was more adapted to the degradation of cellulose.[22]

The gut microbiota represents a real functional barrier allowing the inhibition of pathogen growth and colonization. Metagenomic sequencing has enabled systematic and unbiased characterization of microbial populations including the viral spectrum and it will enable the development of therapeutic strategies and/or vaccines in the near future.[40] Indeed, the use of this approach also allows the identification of new potential pathogens. For example, a novel virus (bat papillomavirus) was discovered and characterized using 454 sequencing from rectal swabs randomly collected from asymptomatic wild, food, and pet animals.[41] A modification of the GIT′s microbial barrier could be an influencing factor in diseases such as IBD, one of the most studied diseases in this field. Recent advances in "omics" approaches (i.e., genomics, transcriptomics, proteomics, and metabolomics) have opened the door for further investigation of the structure and function of the gut microbiome without the need to cultivate, identifying some promising approaches for future therapeutic and diagnostic applications.[42] For instance, the beneficial effects in the physiopathology of Crohn disease (CD, a type of IBD) patients of the commensal bacterium *Faecalibacterium prausnitzii*[43] on one side and the undesired effects of the opportunistic pathogen, adherent and invasive *Escherichia coli* (AIEC)[44] on the other, are two well-illustrated examples of the potential of these therapeutic and diagnostic applications. Indeed, *F. prausnitzii* could be considered as a sensor of human intestinal health, with different studies based on metagenomics methods reporting a reduction of this anti-inflammatory bacterium in CD patients and in other patients with intestinal disorder.[9] In contrast, it was recently shown by DNA pyrosequencing that in TLR5-deficient mice, AIEC colonization might induce lasting changes in the microbiota.[45] Metagenomic approaches to analyze microbiome composition in a genetically susceptible host, through the detection of a pathobiont in a developing microbiome can predict the development of chronic inflammation.[45] In the same way, the reduction in diversity of fecal microbiome in CD patients was revealed by a metagenomic approach.[7] Some authors have already proposed the identification of novel specific diagnostic targets for CD patients through integrated metagenomics/metaproteomics approaches.[46] IBD is not the only example of the link between disease and microbiome; metabolic disorders, celiac disease, irritable bowel syndrome (IBS), and colorectal cancer can also be cited.[9] It would be interesting to determine the link between enterotypes and pathologic phenotypes. In fact, if an enterotype is shown to be related to disease, long-term dietary interventions may allow modulation of an individual's enterotype for improving health.[35]

Vaginal ecosystem

The microbiome associated with the vagina has an important influence on human development, physiology, and immunity. This community of mutualistic bacteria constitutes the first line of defense for the host by excluding non-indigenous microbes that may cause sickness.[47] A mature microbiota is already established in early adolescence after the hormonal changes typical of this period[48] and it includes some microorganisms also present in the GIT, even if the relative frequencies are different. The first microbiological study of the human vagina reported lactobacilli as the dominant microorganisms of this cavity,[49] being more than 70% of all microorganisms isolated from vaginal exudates of healthy and fertile women (and 100% in some cases).[47,50,51] Nevertheless, the species found may vary depending on the methodology used for the identification. In this context, following culture and phenotypic characterization, the most dominant species found are *Lactobacillus acidophilus* and/or *L. fermentum*,[52-54] but when genetic methods are applied, the predominant species reported are *L. crispatus*, *L. gasseri*, and *L. jensenii*.[55-58]

Metagenomics to study the vaginal microbiome

Understanding bacterial composition and the interrelationships of constituent species is necessary to understand the role of the vaginal ecosystem, as well as the effect of different habits and practices. Although the vaginal ecosystem is dynamic as a result of its physiological function (menstrual cycle) and personal habits (contraception and hygiene practices), it remains stable over the long-term as a result of physiological and microbiological factors. In this sense, the Vaginal Human Microbiome Project (at the Virginia Commonwealth University) aims to investigate the complex vaginal microbiome and its link to human health and disease as well as its variability with different physiological conditions.[59] This fundamental knowledge is needed to diagnose and properly assess the risk of disease.

Just as it has been described by culture-dependent methods, metagenomic approaches also describe the vagina as an ecosystem rich in lactobacilli. While 20 species of lactobacilli have been isolated from the vagina, normally only one or two species

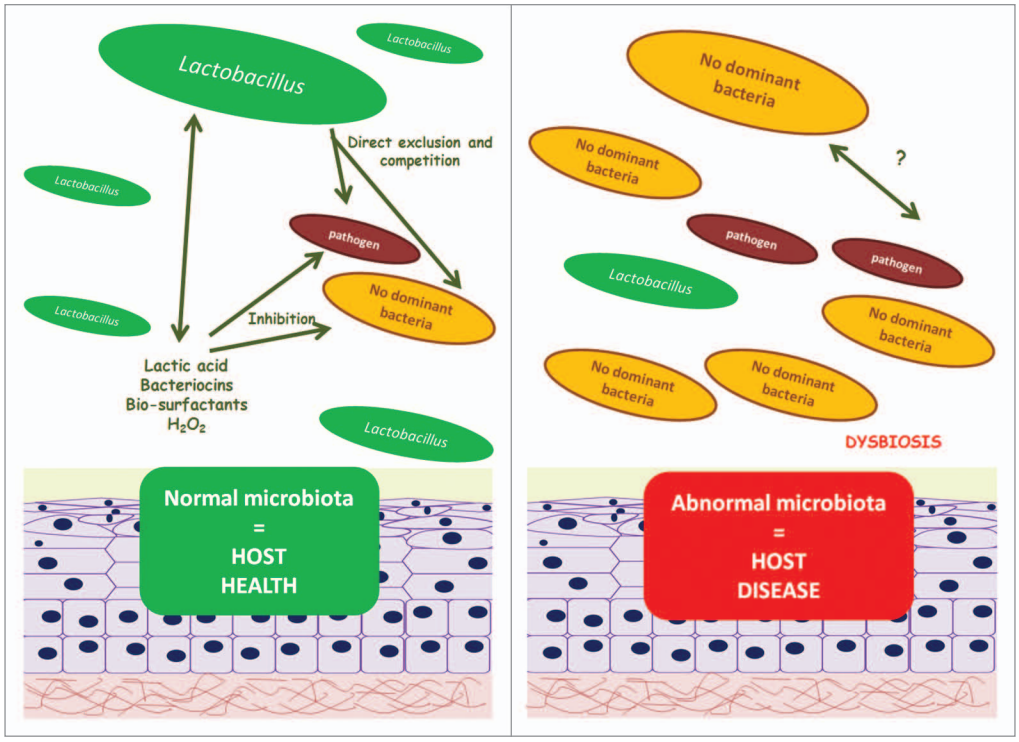

Figure 4. Beneficial effect of lactobacilli on the vaginal ecosystem. Lactobacilli protect the host epithelium as a result of two main mechanisms: (1) exclusion, driven by the competition to epithelial cell receptors and (2) inhibition of growth, due to generation of antimicrobial compounds. When the vaginal microbiota is dominated by lactobacilli a health status is found in this ecosystem; alternatively, when no dominant species predominates in the vaginal ecosystem this dysbiosis normally leads to a disease state.

predominate at the same time in each ecosystem, the most frequently reported being *L. crispatus*, *L. inners*, *L. jensenii*, and *L. gasseri*.[60-66] Although the paradigm of the association between lactobacilli abundance and vaginal health seems to be true for the majority of women, it does not necessarily apply to all. In fact, it has been shown that a typical vaginal environment (i.e., pH > 4.5) is usually rich in lactobacilli. In this sense, vaginal ecosystems dominated by other lactic acid-producing bacteria, such as *Bifidobacterium* sp., *Atopobium vaginae*, *Megasphaera* sp., and *Leptotrichia* sp. have also been described.[57,62,67] A higher vaginal pH has been reported in some racial and ethnic groups[68-70] as well as the absence of lactobacilli and the presence of *Gardnerella vaginalis*, *Prevotella* sp., *Pseudomonas* sp., and/or *Streptococcus* sp. being predominant.[57] In fact, several studies have demonstrated the presence of different microbiome profiles named vagitypes, many of which are dominated by a single bacterial taxon.[59] However, caution has to be taken with this interpretation, since it is not possible to completely rule out a transition state between disease and health in these atypical microbiomes.[71,72]

Metagenomics has also identified differences in vaginal microbiome profiles depending on geographical origin,[67,69,70] as well as during the menstrual cycle and the period of a woman's life, mainly due to hormonal changes.[48] Thus, estrogen deficiency (typical of post-menopause states) leads to a reduction in lactobacilli population.[73-75] The vaginal microbiome also suffers changes due to other environmental factors and sexual practices.[76,77] Genetic polymorphisms related to normal signaling of the innate immune system have also been associated with vaginal microbiota changes, promoting the presence of less healthy microbiota.[78-80] During pregnancy, changes in the vaginal ecosystem (estrogen and progesterone levels, epithelium thickness, and extra glycogen production)[81,82] lead to a significant reduction in diversity and richness of the vaginal microbiota.[83]

Vaginal microbiome: a component of health

The vaginal microbiota, primarily lactobacilli, has been found to assert its beneficial effect against pathogens by two main mechanisms: (1) exclusion, driven by the competition for epithelial cell receptors and (2) inhibition of growth, due to generation of antimicrobial compounds[84] (**Fig. 4**). The first mechanism results from the ability of lactobacilli to compete for receptors against urogenital pathogens such as group B *Streptococcus* (GBS), *Staphylococcus aureus*, *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*, and *Actinomyces neuii*.[85-90] Impairment of adherence by treatment of lactobacilli or epithelial cells with proteases, lipases, or periodic acid suggests that the bacterial adhesins and cellular receptors are proteins, lipids, or polysaccharides respectively.[86,90-92] Furthermore, identification of the proteins anchored to the bacterial cell wall has provided a list of polypeptides putatively involved in mucous adherence.[93-95] In addition, some lactobacilli can co-aggregate with potential pathogens, such as *E. coli*, *C. albicans*, and *G. vaginalis*, which may help in their clearance.[86,88]

Regarding the second mechanism, lactobacilli are able to produce several antimicrobial compounds which are mainly organic

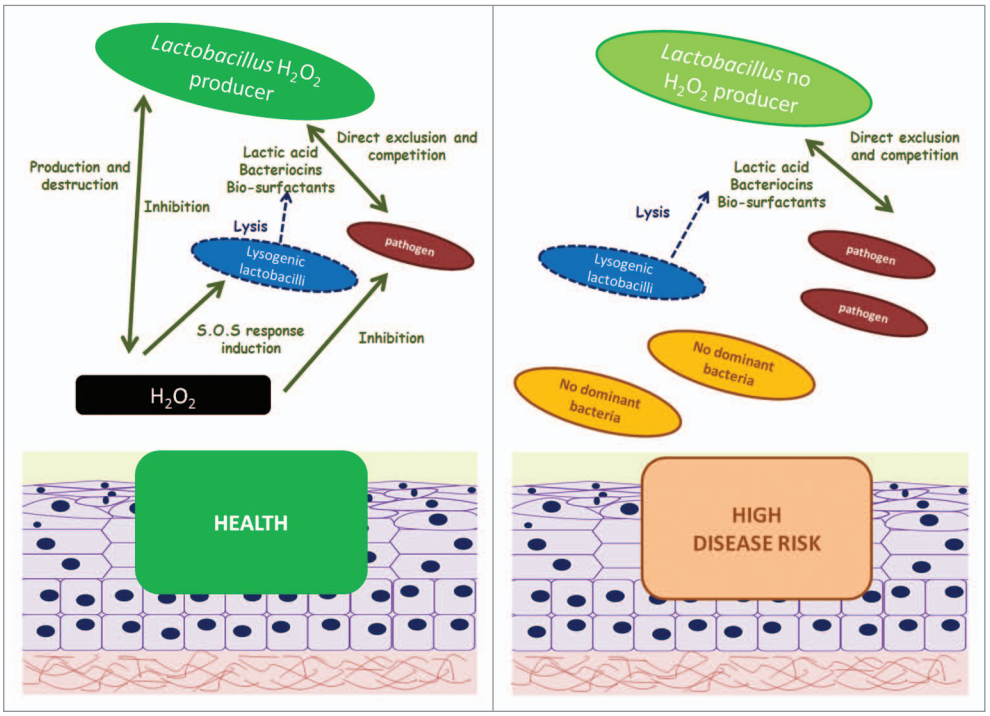

Figure 5. Role of hydrogen peroxide in the vaginal ecosystem. Hydrogen peroxide is an antimicrobial substance also known to counterbalance lactobacilli population due to a SOS response-mediated prophage induction in lysogenic lactobacilli.[105,106] Some lactobacilli are able to destroy this substance.[99] An overall reduced number of H_2O_2 producing lactobacilli enhances the risk of disease.

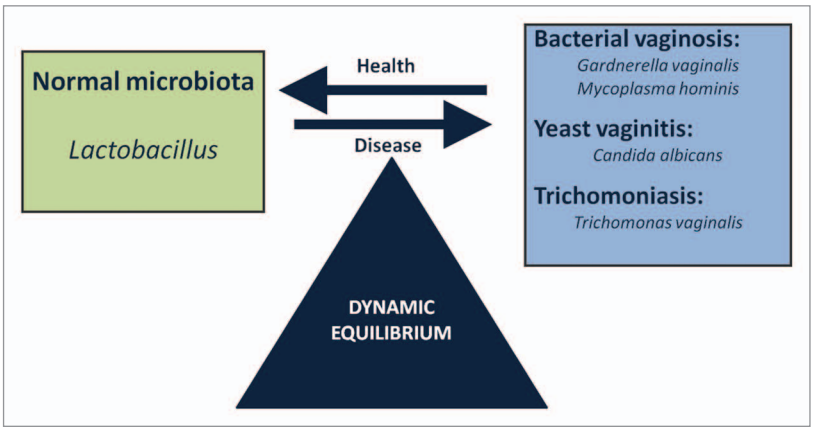

Figure 6. Vaginal equilibrium. A reduction in lactobacilli or overgrowth of some pathogens or no-dominant commensal bacteria can lead to a dysbiosis-related illness.

acids produced from the fermentation of sugars, that lead to the typically low pH of the vagina which inhibits the growth of most pathogens.[96] Furthermore, vaginal lactobacilli are also able to produce bacteriocins, bio-surfactants,[97,98] and hydrogen peroxide (H_2O_2).[99] At least three bacteriocins have been identified in vaginal *Lactobacillus* strains: Lactocine 160, Salivaricine CRL 1328, and L23.[99,100-103] Indeed, the prevalence of H_2O_2 producing strains has been correlated with reduced incidence of bacterial vaginosis (BV) and vaginal infections.[50,77] However, the exclusion capacities of this compound are controversial, as the antimicrobial activity of H_2O_2 can be neutralized by semen and cervix–vaginal fluids.[104] Hydrogen peroxide has also been postulated to be simultaneously regulated by and regulate the lactobacilli population, due to the ability of some lactobacilli to destroy this molecule[99] and the existence of a H_2O_2-mediated prophage induction mechanism that leads to lysis of the host lactobacillus.[105,106] The role of H_2O_2 in this ecosystem is an example of how a single modification in one element of the ecosystem (including an environmental one) can alter the health status of the host in this dynamic ecological community (**Fig. 5**).

Abnormal vaginal microbiota can occur because of sexually transmitted pathogens or overgrowth of resident organisms.[107] The most common pathologies are BV, the proliferation of *Candida* sp. (mainly *C. albicans*) (candidiasis) and *Trichomonas vaginalis* (trichomoniasis)[47] (**Fig. 6**). BV is the most frequent vaginal imbalance and was shown by molecular methods to be associated with a high microbiota diversity.[60] and the presence of unfamiliar bacteria such as *Mobiluncus* sp., *Atopobium* sp., *Megasphaera* sp., and *Ureaplasma urealyticum*.[8,57,108-110] Metagenomics has also been used recently to identify some uncultivable organisms associated with BV, the presence of which has been proposed as a diagnostic alternative to traditional culture-dependent methodologies.[8,60,107,111] Epidemiologically, vaginal dysbiosis such as BV has been associated with preterm birth, development of pelvic inflammatory disease, and acquisition of sexually transmitted infections.[112]

Skin microbiome

Skin represents a physical barrier to infection as a result of epidermis cohesion and more particularly to its cornified layer. Skin also harbors several physiological populations of microorganisms including commensal or symbiotic bacteria, fungi, parasites, and viruses known as the skin microbiota.[113] The presence of a complex ecosystem plays a well-documented role in preventing adherence and invasion by virulent pathogens through biological competition.[114] A better understanding of the skin microbiota's roles requires investigation beyond the taxonomic catalog of bacteria for the characterization of specific activities associated with functional gene products.[115] Advances in molecular technologies allowed the identification of a much greater diversity of cutaneous microbiota than what was revealed previously using culture-based methods.[113,116] Recently, the construction of shotgun metagenomic

libraries provided access to the functions performed by dominant skin colonizing taxa, including *Corynebacterium*, *Staphylococcus*, and *Propionibacterium*.[115] This approach revealed the specific capabilities of skin microbiota to interact with and exploit compounds from the human skin.

Moreover, metagenomics techniques led to the identification of a new virus, the human polyomavirus, although there was no correlation between its presence on the skin and pathology. In fact, this virus is present on healthy skin and in the majority of merkel cell carcinomas.[117,118] The characterization of the skin viral microbiota, using high throughput metagenomic sequencing (HTS) (a highly comprehensive method based on random sequencing of the entire DNA) would allow identification of microbiome patterns associated with particular skin conditions.[119]

Airway microbiome

The human respiratory system, from the nose to the lung, is the ecological niche for many commensal microorganisms and for potential respiratory or invasive pathogens. In contrast to the GIT, the respiratory tract of healthy individuals harbors a homogenous microbiota that decreases in biomass from upper to lower tract.[120] Although the colonization by potential pathogens of the upper respiratory tract microbiome, especially the nasopharyngeal microbiome, induces identifiable disease in only a small percentage of people, colonization represents a major source of secretions containing bacteria that spreads between individuals.[121] For instance, in the case of children the impact of age, season, type of child day care, number of siblings, acute respiratory illness, diet, and sleeping position have been described; whereas in adults, other factors have been also implicated such as contact with children, chronic obstructive pulmonary disease, obesity, immunosuppression, allergic conditions, acute sinusitis, etc.[121] For instance, a metagenomic study on the detailed composition and variability in nasopharyngeal microbiota in samples from young children revealed that it differs between seasons.[122] During fall/winter which tends to be associated with increased incidence of respiratory and invasive infections, a predominance of Proteobacteria and Fusobacteria was observed. However, in spring, Bacteroidetes and Firmicutes were more abundant and, among them, *(Brevi)bacillus* and *Lactobacillus* species that can protect against respiratory or invasive infections. Another component of the nasopharyngeal microbiome are viruses and approximately 30% of all presumed viral cases fail diagnostic tests for etiologic agents.[123] Thus, metagenomics could allow the detection of known viruses in this specific environment, as well as the detection of new ones.[124] In the same way, some authors propose to define "the human virome project", as a systematic exploration of the viruses that infect humans for an investigation of a novel pathogen, and provide a blueprint for comprehensive diagnosis of unexplained acute illnesses or outbreaks in clinical and public health settings.[125,126] For example, in the case of the 2009 H1N1 influenza virus, this kind of strategy was shown to have the potential to replace conventional diagnostic tests.[126]

Little is known concerning the recently described lower respiratory tract microbiome, even if it is likely to provide important pathogenic insights (cystic fibrosis, respiratory disease of the newborn, chronic obstructive pulmonary disease, and asthma).[127] Moreover, infectious agents are known to be or are suspected of having key roles in a number of chronic lung conditions. The bulk of published evidence demonstrates that phylogenetically diverse microbial communities in the lungs of healthy humans can be detected using high-throughput sequencing.[128-130] Better characterization of the lung microbiome could help in understanding its role in preserving health or causing disease particularly in specific groups of patients, for example in smokers.[131]

Concluding Remarks

Nowadays, we recognize the need to study the human microbiome as a whole ecosystem to better understand the relation between microbiota and host health or disease. For this reason, powerful methodologies are required to globally analyze these ecosystems, with metagenomic approaches being key for further analysis of the human microbiome. However, external influences, as well as methodological and sampling bias and inter-individual differences have to be taken into account in the data interpretation. For this reason, to define the average human microbiome, standard operating procedures are critically needed as well as metaanalysis studies, since it is reasonable to anticipate that communities would differ on the basis of the existence of inter-individual differences.

Due to the close relationship between the microbiome and health and the existence of biomarkers typical of different pathologies, the suggestion to modulate our microbiota sounds logical from a therapeutic point of view. Furthermore, the inter-individual differences and physiological parameters suggest personal medicine as a future treatment.

In the future, this kind of approach (i.e., metagenomics) would identify biomarkers of well-being that correspond to a general and more balanced microbiota. In addition, metagenomics can also provide us with a better understanding of the relationship between us and our microbiome and the role of this interaction with health.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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