The indigenous microbiota and its potential to exhibit probiotic properties

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Sylvie Miquel, Rebeca Martin, Muriel Thomas, Luis G. Bermudez-Humaran and Philippe Langella

Abstract

Humans harbour a different microbiota depending on the tissue considered. Most of the microorganisms are contained in the gastrointestinal tract (GIT) and this gut microbiota represents approximately 10^{14} cells that correspond to the highest bacterial density for any ecosystem. Our microbiota represents a huge diversity in term of species and functions. A healthy gut microbiota is composed of a well-balanced community of three permanent residents termed symbionts (with beneficial effects), commensals (no effect), and pathobionts (potentially induce pathologies under certain situations), but no pathogens. The term dysbiosis (microbial imbalance) has been related to many different kinds of pathologies although it is not clear whether the imbalance of such a microbiota is a cause or a consequence of the illness. Nowadays, the challenge of linking microbiota to human health and disease is being tackled by different research teams around the world with the aim to investigate the implication of potential beneficial bacteria that could be decreased in the studied microbiota of patients. From this perspective, it could be interesting to use them as potential probiotics to try to resolve dysbioses.

Our indigenous microbiota: definition and composition

Few years ago, microorganisms of interest were essentially human pathogenic bacteria, but today, more and more studies aimed to characterize the interactions between commensal microbial and human physiology (Weinstock, 2012). The term microbiota refers to the microorganisms population present in a specific environment (soil, ocean, bowel, skin, vagina, etc.) including bacteria, viruses, archaea, protozoans and fungi. Humans could be considered as 'meta-organisms' constituted of 10-fold more microorganisms than human cells (Neish, 2009). Humans harbour different microbiota depending on tissues considered; we can thus distinguish the cutaneous, the oral, the intestinal, the vaginal, and the airway microbiota. Nowadays, the area of metagenomics has given a new dimension to the analysis of microbiota based on classic microbiological techniques of the bacterial culture. In fact, a complete characterization of the microbiota was difficult before the use of high-throughput sequencing and the emergence of 'omics' approaches (metagenomics, metatranscriptomics and metabolomics) (Neish, 2009). This approach has also allowed to define the term 'microbiome' referring to the genetic material of the catalogue of microbial taxa associated with humans (Ursell *et al.*, 2012). Being frequently confused with the term microbiota, the microbiome was first defined by Joshua Lederberg in 2001 (J. Lederberg and McCray, 2001) and is sometimes referred as our 'second genome' consisting of 150-fold more genes than the human genome itself (Bruls and Weissenbach, 2011; Qin *et al.*, 2010). Today, the availability of the reference gene catalogues obtained from MetaHIT project (Qin *et al.*, 2010) and the American Human Microbiome Project (Consortium, 2012) has allowed to evaluate richness of the human gut microbiome. The human microbiota (microbiome by extension), can be considered as a additional human organ from a physiological standpoint with essential roles as an organ (Baquero and Nombela, 2012).

Depending on the considered tissues, the bacterial communities of a same individual have a very different composition (Weinstock, 2012). Most of the microorganisms are contained in the gastrointestinal tract (GIT) and represents approximately 10^{14} cells that correspond to the most important bacterial density for an ecosystem (Backhed *et al.*, 2005; Gill *et al.*, 2006). These microorganisms are for the greater part, around 70%, extremely oxygen sensitive (EOS) bacteria located in the colon (Ley *et al.*, 2006). Firmicutes (the *Clostridum leptum* and *Clostridum coccoides* groups) and Bacteroidetes are the two dominant phyla, representing ~ 90% of the microbiota, and the third most dominant group is Actinobacteria with only ~ 3% (Tap *et al.*, 2009). In the vagina, lactobacilli are the dominant bacteria (70% of the population) even if it could include some microorganisms also present in the GIT (Doderlein, 1982; Martin *et al.*, 2008b). There is a huge diversity in the bacterial species which constitute the intestinal microbiota: approximately 160 species of different bacteria are present within every individual (Qin *et al.*, 2010). Besides, there is variation in bacterial populations within a specific microbiota depending of the micro-localization. For instance, along the GIT, bacterial populations differ from the upper to the lower part of the

tract but also between intestinal mucosa and the luminal content (Eckburg *et al.*, 2005; Swidsinski *et al.*, 2008b). Furthermore, even if at the level of phyla, the composition is relatively similar from one individual to another, an important interpersonal variability was observed at the level of species (Eckburg *et al.*, 2005). In spite of this relative heterogeneity, through faecal metagenomic analysis, it is possible to distinguish three main robust clusters named 'Enterotypes' in the gut microbiome, which are driven by species composition. Each of these three enterotypes are identifiable by the variation in the levels of one of three genera *Bacteroides*, *Prevotella* and *Ruminococcus* (Arumugam *et al.*, 2011). Their abundance and proportions vary between individuals and is associated with long-term dietary habits (Wu *et al.*, 2011).

Our microbiota represents a huge diversity in term of species and functions. It will be interesting to understand how we could study their composition to better understand how they interact with the host. It fact, we will see that this 'organ' participate to the human health. Medicine has developed organ-based specialties such as nephrology, hepatology, cardiology or pneumology. Perhaps, we can envisage 'microbiomology' as a future specialty of or a branch of clinical microbiology, devoted to the study of the physiology, pathology, diagnostics, therapy and prevention of alterations of the community structure of the microbiome (Baquero and Nombela, 2012).

How to study the microbiota

Nowadays, the most common microbiological approaches to study the human microbiota are divided depending on the need or not of the culture of the bacterium object of the study. The possibility to study bacteria without culturing them is a great success in the Microbiology as it has been one of the most important inflexion points in this science.

Classical microbiological approaches

The first analyses of the human microbiota have been developed thanks to culture dependent methodologies (Moore and Holdeman, 1974). For instance, Finegold and co-workers in the early 1970s have already studied the effect of diet on the human gut microbiota using this culture method (Finegold *et al.*, 1974). The protocols of identification were based on microscopic observations and pure cultures (Lagier *et al.*, 2012) joint to phenotypic identification methods. The Gram staining (Biswas *et al.*, 1970) allowed describing a wide number of human ecosystems, such as the faeces population and the vaginal microbiota, and the use of biochemical systems, such as the Analytical Profile Index (API) galleries, is still a method of reference nowadays. These first data were very useful, but as it is known that only 1% of the bacteria can be easily grown *in vitro* (Vartoukian *et al.*, 2010), new approaches have been developed to avoid methodological bias due to culture restrictions.

The main problem of culture dependent methods is that they are focused on aerobic bacteria whereas a wide range of bacteria belonging to the human microbiota prefer micro-aerophilic conditions (Lagier *et al.*, 2012). Furthermore, many microorganisms need special growth conditions, such as the EOS bacteria, that render their culture difficult and their detection even complicated (Miquel *et al.*, 2013). Others have never been grown in culture and may require specific, yet unknown, growth conditions impairing their identification by culture dependent methods (Martin *et al.*, 2014b). For example, the mucolytic bacteria *Akkermansia muciniphila* was isolated by dilution of faeces in anaerobic medium containing gastric mucin as the sole carbon and nitrogen source (Derrien *et al.*, 2004). Consequently, the major populations isolated were those bacteria that grow quickly in aerobic conditions in classical rich nutrient media (Hugenholtz, 2002). In addition, the use of phenotypic identification methods is time consuming and expensive (Seng *et al.*, 2009).

Can we consider that culture is an old method with a limited future? Our feeling is that the use of culture dependent methodologies is still required to achieve complete knowledge about bacterial physiology. We are sure that innovative methods have to be developed to optimize the growth of fastidious bacteria.

'Omics' approaches

Until the 1990s, knowledge of microbiota was limited because bacteriological culture was the only technique available to describe its composition. For example, only a small portion (estimated at < 30%) of the gut microbiota has been cultured to date (Fraher *et al.*, 2012). This is because culture-based approaches, even if being extremely helpful to understand the physiological potential of isolated microorganisms, do not essentially provide complete information on the composition of microbial communities (Orphan *et al.*, 2000). In the 1990s, concomitantly with the expansion of molecular tools, new culture independent methods significantly improved the knowledge about microbiota (Zoetendal *et al.*, 2006). These techniques were mainly based on divergences of the sequence of the small subunit ribosomal RNA (16S rRNA) giving quantitative and qualitative information about the microbiota through time and space (Fraher *et al.*, 2012). Examples of these techniques are denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), fluorescence *in situ* hybridization (FISH), DNA microarrays, and next-generation sequencing of the 16S rRNA gene or its amplicons (Fraher *et al.*, 2012).

To study the human microbiota, it is imperative to understand the role of microbes in the host ecosystem and, for this, it is crucial to probe their genetic potential, expression, and ecological status (Lamendella *et al.*, 2012). To achieve this new concept of microbiota as a collection of dynamic ecological communities, new techniques have been developed. These meta-omic or 'systems biology' approaches offer a complementary support to the classical microbiology and are based on high-throughput methods (Del Chierico *et al.*, 2012). The more relevant are those that study the genome, metabolome, transcriptome and proteome of the human microbiota (metagenomics, metabolomics, transcriptomics and proteomics, respectively), although new fields of research show up frequently as the applications of these high-throughput methods are huge. The term metagenomic was originally coined for the shotgun characterization of total DNA and now is also used to name the study of marker genes such as the 16S rRNA gene (Ursell *et al.*). Its objective is to determine the microbiome

which is the catalogue of microbial taxa associated with humans and their genetic material (Ursell *et al.*). Metabolomics is the study of the set of metabolites synthesized by a biological system (Fiehn, 2002). Transcriptomic refers originally to the identification of the critical messenger RNAs (Kiechle and Holland-Staley, 2003), nowadays the transcriptome is considered the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA molecule produced in one or a population of cells, although most of the interest is in mRNA (Pinto *et al.*, 2011). Proteomics is defined as a large-scale study of proteins, in particular their functions and structures (Ghafourian *et al.*, 2013) being the entire set of proteins expressed by the genome.

All these powerful tools provide information on community diversity and structure (Gans *et al.*, 2005) even if some bias linked to sample extraction and preparation, data acquisition and data mining, may limit some interpretation. So for us, our next challenge is to combine omics methods with culture approaches in order to better understand the functions and the physiological relevance of bacterial communities. The main purpose is to benefit of an integrated view of our physiology involving bacterial and eukaryotic cell communities.

The microbiota: our best companion

The microbial diversity of the GIT results from a co-evolution between the microbial communities and their hosts which represents its ecological niche. For a long time considered as commensal, from the Latin: cum ('with') and mesa ('table'), which means the sharing of the meal, the term mutualism is preferred today to speak about the host-microbiota relation. Indeed, commensalism is a relation between individuals of two species in which one species obtains food or other benefits from the other without either harming or benefiting the latter. Commensalism is then a non-parasitic utilization of a live species by another. However, in the case of the host-intestinal microbiota relation, we speak about a symbiotic mutualism relation because both partners developed an intimate and long-term association with mutual profit (Fig. 12.1). In fact, there is a co-evolution between the human and its microbiota with an acquisition by the microbiota of functionalities

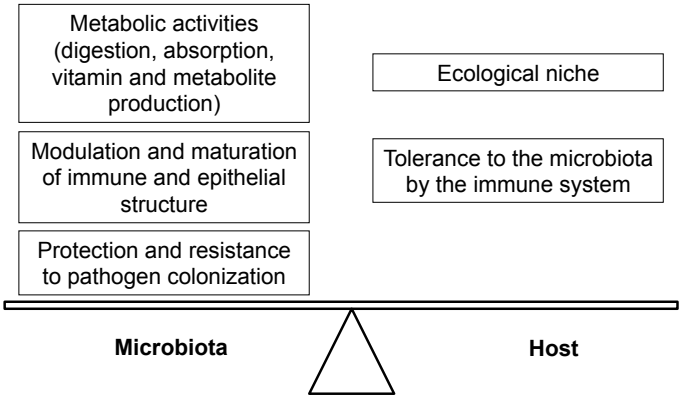

Figure 12.1 Symbiotic mutualism relation forged during the evolution between the host and its microbiota. (Adapted from Claire Cherbuy; http://mediatheque.inra.fr/media/detail/246161/private).

absent in human genome. In fact, the intestinal microbiota, by its enzymatic activities, is a complementary metabolic repertoire of the human digestive system. The intestinal bacteria present beneficial properties for the host in terms of synthesis of molecules, degradation of substrates and detoxification. For example, we could quote the acquisition of genes for degradation of the plant polysaccharides and other complex sugars, non-digestible by human enzymes (Ley *et al.*, 2008). Their degradation by bacterial fermentation in short-chain fatty acids (SCFA) constitutes an important source of energy for colonocytes (Macfarlane and Macfarlane, 2011). Moreover, the intestinal bacteria synthesize vitamins, amino acids and are involved in the metabolism of bile salts (Vyas and Ranganathan, 2012). The intestinal microbiota also participates in absorptive capacities of nutriments by the GIT by a direct action on its physiology. For example, the SCFA are not only involved in the energy metabolism but also present properties of regulation of the physiology of the host (differentiation, cellular growth, etc.) (Neish, 2009). A recent publication using gnotobiotic models showed that the colon evolved in parallel with the microbiota composition inducing a homeostasis of the two maturated partners (Tomas *et al.*, 2013). In the same way, during the post natal period, bacteria associated with the mucosa could regulate angiogenesis increasing the absorptive capacity of the intestine (Stappenbeck *et al.*, 2002). Thus, the microbiota supports human nutrition by two main mechanisms: increase of the availability in energy substrates for the host and regulating intestinal absorption capacity.

Besides being a nutritive supply, the microbiota also participates in the protection of the host. Indeed, the organism defences at the level of the GIT have the delicate task to distinguish commensal microbiota from pathogenic agents. The mechanisms of the intestinal immune system allowing its tolerance to the microbiota remains poorly known (Littman and Pamer, 2011). To get a glimpse of an answer, it is necessary to better understand the recognition systems of microorganisms developed by the intestinal epithelial cells. Particularly, specific receptors to Microbial-associated molecular patterns (MAMPS) exist, the pattern recognition receptors (PRRs) (Wells *et al.*, 2011). Briefly, the activation of these receptors by commensal bacteria leads to the activation of the innate immunity system and is essential to maintain intestinal homeostasis and the protection of the epithelium against pathogenic bacteria (Lundin *et al.*, 2008; Rakoff-Nahoum and Medzhitov, 2008; Rakoff-Nahoum *et al.*, 2004). So, the microbiota limits the inflammation of the intestinal epithelium and plays a major role in the development of the immune system (Macdonald and Monteleone, 2005). To illustrate the hypothesis, the best example is the use of germ free mice that present an immature immune system at the GIT level and the conventionalisation of these mice (reconstitution of a normal microbiota) restored the immunity of this mucosa (Umesaki *et al.*, 1995). Furthermore, at birth, the immune system is still immature and it was shown that contact with bifidobacteria influences its postnatal development via the production of IgA and an insufficient exposure to these microorganisms could lead to inappropriate immune responses later in the childhood and could induce asthma or allergies (Sjogren *et al.*, 2009).

Thus, the microbiota participates to the protection of the host by supporting the development of the immune system, but commensal bacteria act also directly as a 'microbiological barrier' against pathogens. Indeed, it was shown that germ free mice are more sensitive to intestinal pathogens (Vollaard and Clasener, 1994). This protective capacity of the microbiota could be explained by different mechanisms: a competition with pathogenic bacteria for the access to nutriments and/or for a contact with the epithelium, the production of bactericidal or bacteriostatic molecules and, a modification of the physiology of the epithelium through production of mucus or modulation of epithelial cell tight junctions (Martin *et al.*, 2014b; Yu *et al.*, 2012). For instance, two major commensal bacteria *B. thetaiotaomicron* and *F. prausnitzii* could directly modulate the intestinal mucus barrier by their complementary metabolism by modifying goblet cells and mucin glycosylation (Wrzosek *et al.*, 2013). Moreover, commensal bacteria were able to induce maturation of intestinal barrier function through (i) modulation of the expression of tight junctions ~~(claudin 3)~~ that regulate paracellular permeability to maintain separation between tissue compartments by sealing the intercellular space, and (ii) intracellular permeability regulated by toll-like receptor signalling (Jakaitis and Denning, 2014).

It is now clear that the normal intestinal microbiota could influence numerous physiological aspects in the healthy host through the establishment of cross-species homeostatic regulation well detailed in the review of Sommer and Bäckhed (2013). Thus, the microbiota composition and its relation with the gut have resulted from the dynamics of selection and competition (Angelakis *et al.*, 2012). For instance, the bacterial microbiome of the inhabitants of Burkina-Faso was more adapted to the degradation of cellulose suggesting an adaptation to Burkina-Faso's high-fibre diet (De Filippo *et al.*, 2010); One could suggest that our microbiota shelters unknown bacteria adapted to specific functions and represents a real richness for future research concerning human health. Our microbiota takes an important part in human health and an imbalance of its composition could participate to the development of many diseases.

Diseases of microbiota: a break in the homeostasis

Since Robert Koch and Ilya Mechnikov were awarded two Nobel Prizes in physiology and medicine in the 1900s for their discoveries linking microbes and human health, several determinants of host–microbe interactions, including spatial (e.g. skin, vagina, and gut), temporal (e.g. birth and senescence), and environmental (e.g. diet, antibiotics treatments), have been partially unravelled (Cani and Delzenne, 2011). For instance, a mature vaginal microbiota is established only in early adolescence after the hormonal changes typical of this period (Yamamoto *et al.*, 2009). Concerning intestinal microbiota, it varies all along the life, with the particularity to increase its population number and diversity during the first year and decline upon ageing (Claesson *et al.*, 2012; Sekirov *et al.*, 2010). In adults the importance of the microbiome has been highlighted by the microbial 'abnormalities' found in pathological conditions and particularly a disruption of profile or biodiversity. In fact, many diseases are characterized by a dysbiosis, in other words, a microbial imbalance (Round and Mazmanian, 2009). A healthy gut microbiota is composed of a well-balanced community of three permanent residents termed symbionts (with beneficial effects), commensals (no effect), and pathobionts (potentially induce pathologies under certain *situations*) but no pathogens (Koboziev *et al.*, 2013). The term dysbiosis has been related to many different kinds of pathologies although it is not clear whether the imbalance of microbiota is a cause or a consequence of the illness (Martin *et al.*, 2013). In this way, 'The hygiene hypothesis ', based on the observation of a positive correlation between chronic diseases ~~and~~ hygiene and socioeconomic status (Strachan, 1989), could be explained by the creation of dysbioses limiting the regulation of the immunity by the microbiota (von Mutius, 2007).This hypothesis proposes that a lack of early microbial stimulation in industrialized countries results in an aberrant immune response to innocuous antigens later in life (Wills-Karp *et al.*, 2001). In parallel, the 'microbiota hypothesis' claims that the gut microbiota dysbiosis, due to antibiotic use and dietary changes, can disrupt the mechanisms of mucosal immunological tolerance (Noverr and Huffnagle, 2005). Recent epidemiological and clinical data (Droste *et al.*, 2000; Hoskin-Parr *et al.*), as well as experimental data obtained in mouse models (Noverr *et al.*, 2004; Olszak *et al.*), support this latter theory. Nowadays, the challenge of linking microbiome to human health and disease is being tackled 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 (Martin *et al.*, 2014b).

Impact of intestinal dysbiosis

The GIT is the major site of interaction between environmental microorganisms and host tissues. A high biodiversity of the gut microbiota is associated with a healthy status, while low biodiversity is linked to pathological conditions (Manichanh *et al.*, 2006). More than 30 diseases are associated with intestinal microbiota (dysbiosis) and the list is continuously increased. The pathogenesis of several autoimmune, atherosclerosis or rheumatoid arthritis, and chronic inflammatory diseases could be mentioned (Koboziev *et al.*, 2013). Among these diseases we could distinguish three important classes: metabolic syndromes, intestinal diseases and more prudently psychological syndromes (Fig. 12.2). The common point between all of these patients is that they are suffering of intestinal disorders with different severities. The link between psychological syndrome and gut dysbiosis is a very recent hypothesis present in the literature. It is clear that gut microbiome plays a crucial role in the bidirectional gut–brain axis that integrates the gut and central nervous system activities (Wang and Kasper, 2013). Indeed, it has been shown that the niche-specific microbiome, prominently the gut microbiome, has the capacity to affect both local and distal sites within the host. In fact, intestinal dysbiosis is involved in metabolic syndromes such as diabetes, obesity, liver disease…etc. Interestingly, colonization of germ-free mice with microbiota obtained from obese but not

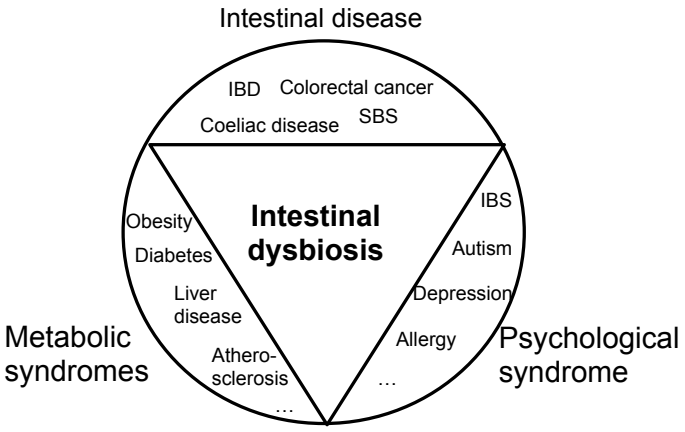

Figure 12.2 Disease associated with intestinal dysbiosis. IBD, inflammatory bowel disease; SBS, short bowel syndrome; IBS, irritable bowel syndrome.

lean mice results in the generation of mice with a significantly greater amount of total body fat (Turnbaugh *et al.*, 2006). Similar results were obtained in non-alcoholic fatty liver disease, the hepatic manifestation of metabolic syndrome (Le Roy *et al.*, 2012), suggesting that these kinds of diseases are transmissible via microbiota. Moreover, a disorder of the crosstalk between innate immune system and intestinal microbiota may promote the development of the metabolic syndrome (Cani and Delzenne, 2011). For instance, a recent study shows that mice genetically deficient in Toll-like receptor 5 (TLR5), a component of the innate immune system that is expressed in the gut mucosa and recognizes pathogen-associated molecular patterns (PAMPs) that are expressed on infectious microbes, develop hallmark features of metabolic syndrome (hyperlipidaemia, hypertension, insulin resistance, and increased adiposity) (Vijay-Kumar *et al.*, 2010).

The best examples of the implication of microbiota in diseases and the importance of the homeostasis between these two partners are the development of intestinal diseases and particularly inflammatory bowel disease (IBD). IBD, including Crohn's disease (CD) and ulcerative colitis (UC), are characterized by a chronic activation of the immune system, of genetically predisposed patients in response to environmental factors and dysbiosis (De Cruz *et al.*, 2012; Lepage *et al.*, 2011). For instance, the proportion of Firmicutes, in particular the symbiotic *Faecalibacterium prausnitzii*, was found to be low in intestinal microbiota of CD patients (Sokol *et al.*, 2008) whereas there was an increased in opportunistic pathogen bacteria, in particular Adherent invasive *Escherichia coli* (AIEC) (Darfeuille-Michaud, 2002; Miquel *et al.*, 2010). A study showed that colitis (IBD like) and colonic hypersensitivity (IBS-like syndrome) can be provoked in healthy rodents by the transfer of microbiota coming from mouse suffering from colitis (Crouzet *et al.*, 2013; Garrett *et al.*, 2007). The excessive inflammation of the GIT would be particularly induced by an inappropriate stimulation of the immune system in the presence of an unbalanced commensal microbiota and its metabolites (Sartor, 2008). Dysbiosis has also been reported for numerous other intestinal patho-physiological contexts such as IBS (Rajilic-Stojanovic *et al.*, 2011), colorectal cancer (Sobhani *et al.*, 2011), short bowel syndrome (SBS) (Joly *et al.*, 2010) and coeliac disease (De Palma *et al.*, 2010).

Vaginal dysbiosis

Although dysbiosis has been considered at length in the ~~gastrointestinal tract~~, it can also occur on any exposed surface or mucous membrane such as skin (Grice *et al.*, 2008; Grice and Segre, 2011), oral cavity, airway (Charlson *et al.*, 2011; Gollwitzer and Marsland, 2014), and the vagina.

The vaginal microbiota that is mainly formed by lactobacilli, has an important role in human development, physiology, and immunity (Martin *et al.*, 2014b). Abnormal vaginal microbiota can occur because of sexually transmitted pathogens or overgrowth of resident organisms (Lamont *et al.*, 2011). The most common pathologies are bacterial vaginosis (BV), the proliferation of *Candida* sp. (mainly *C. albicans*) (candidiasis) and *Trichomonas vaginalis* (trichomoniasis) (Martin *et al.*, 2008a). The community of mutualistic vaginal bacteria, constitutes the first line of protection for the host by excluding non-indigenous microbes that may cause illness (Martin *et al.*, 2008a).

The lactobacilli exerts their beneficial role by two main mechanisms: (i) exclusion and (ii) inhibition of growth (Boris and Barbes, 2000) (Fig. 12.3). The exclusion is driven by the competition for epithelial cell receptors with urogenital pathogens, of which the most important are: group B *Streptococcus* species, *Staphylococcus aureus, Gardnerella vaginalis, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans* and *Actinomyces neuii* (Boris *et al.*, 1998; Martin *et al.*; Osset *et al.*, 2001a,b; Vielfort *et al.*, 2008; Zarate and Nader-Macias, 2006). Furthermore, the ability of some lactobacilli to co-aggregate with pathogens like *E. coli, C. albicans*, and *G. vaginalis* has been found (Boris *et al.*, 1998; Osset *et al.*, 2001a). Although the molecular mechanism involved are not completely known and may depend on the *Lactobacillus* strain involved and the pathogen inhibited, shaving and seeding treatments with proteases, lipases or periodic acid point out the proteinic, lipidic or polysaccharide nature of the adhesins and cellular receptors involved (Munoz-Provencio *et al.*, 2010; Sanchez *et al.*, 2008; Tjalsma *et al.*, 2008).

Inhibition of growth is due to the generation of antimicrobial compounds. Lactobacilli are able to produce mainly organic acids from the fermentation of sugars that contribute to the low pH of the vagina, the major factor in the inhibition of pathogen growth (Boskey *et al.*, 2001). Besides, some vaginal lactobacilli are able also to produce other antimicrobial compounds such as bacteriocins, biosurfactants and hydrogen peroxide (H_2O_2) (Velraeds *et al.*, 1998, 2000). The production of H_2O_2 seems to be the second major antimicrobial mechanisms after organic acid production. In fact, the prevalence of H_2O_2-producing strains has been correlated with lower incidence of bacterial vaginosis (BV) and vaginal infections (Eschenbach *et al.*, 1989; Hawes *et al.*, 1996). H_2O_2 exerts its bactericidal effect through generation of oxidizing metabolites such as the radical OH^- that introduces breaks in the DNA of the cell (Klebanoff and Belding, 1974). Furthermore, hydrogen peroxide is able to regulate the lactobacilli population, due to the ability of some lactobacilli to degrade and produce this molecule (Martin *et al.*) and the lysis of lysogenic lactobacilli

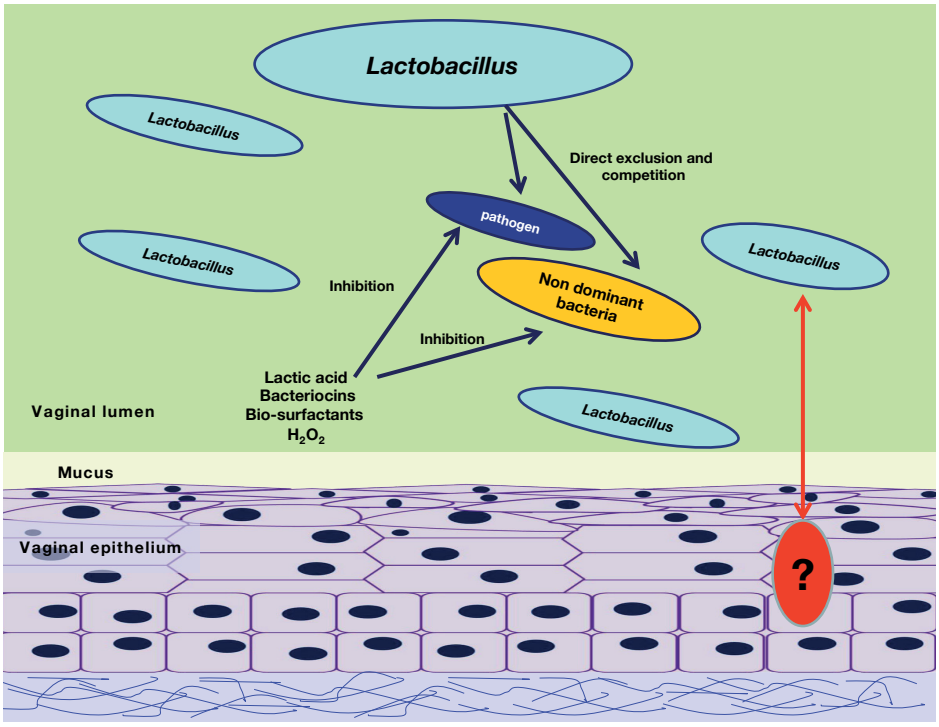

Figure 12.3 Beneficial effects of lactobacilli on the vaginal ecosystem. Lactobacilli protect the host epithelium thanks to two main mechanisms: (i) exclusion, driven by the formation of a biofilm that mask the epithelial cell receptors and (ii) inhibition of growth, due to generation of antimicrobial compounds. The direct interactions between the lactobacilli and the host are not well known. Modified from (Martin *et al.*, 2014b).

by a H_2O_2-mediated prophage induction mechanism (Martin *et al.*, 2009; Pavlova *et al.*, 1997). Although, the presence of bacteriocins is thoroughly described in numerous bacterial species (Cotter *et al.*, 2013), only four bacteriocins have been found, to our knowledge, up to day in vaginal *Lactobacillus* strains: Lactocin 160, Salivaricin CRL 1328, L23 and recently a bacteriocin-like substance produced by *Lactobacillus fermentum* CS57 isolated from human vaginal secretions (Aroutcheva *et al.*, 2001; Martin *et al.*; Ocana *et al.*, 1999; Pascual *et al.*, 2010; Sabia *et al.*, 2014; Simoes *et al.*, 2001).

Despite all the defence mechanisms asserted by lactobacilli, sometimes the proportion of *Lactobacillus* drops under a critical level and this circumstance is used by other microorganisms that can act as opportunistic pathogens (Garcia-Rodriguez and Munoz, 1991). In general these microorganisms can be found in the normal vaginal microbiota, but in a lower abundance due to the antagonist effect of the lactobacilli. Among them, the most common aetiological agents are *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella* and *Peptostreptococcus* that can induce from an asymptomatic infection up to vaginosis (Sobel, 2000; Thorsen *et al.*, 1998). Bacterial vaginosis patients are those who fulfil the Amsel criteria: homogeneous vaginal discharge, amine (fishy) odour when potassium hydroxide solution is added to vaginal secretions (commonly called the 'whiff test'), vaginal pH greater than 4.7 and presence of clue cells (greater than 20%) upon microscopy combined with a significant diminution of Gram-positive lactobacilli replaced by Gram-negative bacteria (Amsel *et al.*, 1983). BV is the most recurrent vaginal imbalance and has been connected with a high diversity microbiota (Pavlova *et al.*, 2002) and the presence of unfamiliar bacteria such as *Mobiluncus* sp., *Atopobium* sp., *Megasphaera* sp. and *Ureaplasma urealyticum* (Doyle *et al.*, 1995; Fredricks *et al.*, 2005; Haggerty *et al.*, 2009; Hyman *et al.*, 2005; Kazor *et al.*, 2003). Thanks to metagenomic approaches some uncultivable microorganisms have also been associated with BV (Fredricks *et al.*, 2005; Lamont *et al.*, 2011; Oakley *et al.*, 2008; Pavlova *et al.*, 2002). Epidemiologically, vaginal dysbiosis such as BV has been associated with preterm birth, development of pelvic inflammatory disease and acquisition of sexually transmitted infections produced by *Neisseria gonorrhoeae*, *Chlamydia* spp. and HIV (Brotman, 2011).

It is interesting to study the characterization of dysbiosis in more detail to investigate the implication of potential beneficial bacteria that could be decreased in the studied microbiota of patients. From this perspective, it could be interesting to use them as potential probiotics to try to resolve dysbioses.

The commensal beneficial bacteria

Commensal microorganisms offer a wide range of benefits to the host. The cross-talk between the host and its microbiota is fundamental for the maintenance of the host homeostasis (Leser and Molbak, 2009). The microbiota is capable to regulate the intestinal epithelium, the presence of pathogenic bacteria and the immune responses as well as to confer nutritional benefits as discussed before (Martin *et al.*, 2013). Both host and indigenous microorganisms, have then adapted to each other to maintain the advantages that this mutualism offers (Sekirov *et al.*, 2010).

Numerous bacterial examples have been analysed in more or less detail. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii*, both prominent members of the intestinal microbiota of mice and humans, have been widely studied (Miquel *et al.*, 2013; Zocco *et al.*, 2007). *B. thetaiotaomicron* exerts several beneficial effects, such as the modulation of the expression of a large quantity of genes implicated in different aspects of host physiology (Zocco *et al.*, 2007), the modulation of the colon epithelium (Wrzosek *et al.*, 2013) and the defence of the intestinal barrier (Resta-Lenert and Barrett, 2006). Indeed, the acetate produced by *B. thetaiotamicron* seems to be key in the cell differentiation (Wrzosek *et al.*, 2013) and this bacterium prevents cytokine induced increase in permeability (Resta-Lenert and Barrett, 2006). Thanks to the analysis of its genome and proteome, the huge ability to adapt and regulate gene expression in response to a changing ecosystem has been pointed out, highlighting the high level of adaptation of this bacterium to its niche (Comstock and Coyne, 2003).

F. prausnitzii, is a major EOS component of the intestinal microbiota which represents around 5% of the bacterial population of the microbiota and 8% of Firmicutes (Eckburg *et al.*, 2005; Miquel *et al.*, 2014). Its prevalence is low in many health disorders, such as IBS, colorectal cancer, coeliac diseases, obesity, and particularly in IBD patients, suggesting its potential as an indicator of intestinal health (Miquel *et al.*, 2013; Sokol *et al.*, 2008; Swidsinski *et al.*, 2008a). Moreover, *F. prausnitzii* is a butyrate producer and has demonstrated anti-inflammatory effects *in vitro* and *in vivo* using mice colitis models making it a key member of the microbiota that may contribute to intestinal homeostasis (Martin *et al.*, 2014a; Sokol *et al.*, 2008). This bacterium also impacts physical and chemical epithelial barrier functions though modulation of tight junction and mucus layer (Carlsson *et al.*, 2013; Wrzosek *et al.*, 2013). Thus, *F. prausnitzii* is a beneficial commensal bacterium that could be a good candidate of our indigenous microbiota to elaborate new prophylactic or therapeutic applications in human health (Miquel *et al.*, 2013).

Another *Bacteroides* species, *B. fragilis*, has been also widely studied. This species is an important anaerobic gut commensal of humans that prevents and cures intestinal inflammation in mice (Mazmanian *et al.*, 2008). *B. fragilis* NCTC 9343 presents a polysaccharide (PSA) promoting lymphoid organogenesis that increases CD4+ T-cells in germ-free rodents (Mazmanian *et al.*, 2005) and corrects the Th1/Th2 balance as has been shown in cellular and animal models (Mazmanian *et al.*, 2005; Mazmanian *et al.*, 2008). This strain corrects gut permeability, alters microbial composition and ameliorates defects in communicative, stereotypic, anxiety-like and sensorimotor behaviours by being capable to modulate some metabolite levels (Hsiao *et al.*, 2013). *Bacteroides fragilis* ATCC23745 plays a role in maintaining physiological expression of heat-shock protein (Hsp)25 and Hsp72 which are cytoprotective in colonocytes (Kojima *et al.*, 2003).

Escherichia coli Nissle 1917 (ECN) was isolated by Professor Alfred Nissle in 1917 during the First World War. This strain has been used as a probiotic for several years. Its capacities are huge. It is capable to induce the expression of the antimicrobial peptide human beta-defensin-d2 (hBD-2) in the human intestinal epithelial cell line Caco-2 *in vitro*. ECN strengthens the intestinal barrier function by inducing pro-inflammatory pathways, mainly NF-kb and AP-1 as well as MAPKs (Schlee *et al.*, 2007; Wehkamp *et al.*, 2004). This effect has been also demonstrated *in vivo*, where faecal hBD-2 peptide was increased by 78% after 3 weeks of *E. coli* Nissle 1917 administration (Mondel *et al.*, 2009). Furthermore, it is able to inhibit the invasion of various gut epithelial cells lines by adherent-invasive *Escherichia coli* (AIEC), *S. typhimurium*, *Y. enterocolitica*, *S. flexneri*, *Legionella pneumophila* and *L. monocytogenes* (Altenhoefer *et al.*, 2004; Boudeau *et al.*, 2003; Deriu *et al.*, 2013) and to restore the barrier dysfunction induced by *E. coli* (EPEC) and *Salmonella dublin in vitro* (Otte and Podolsky, 2004). Some cellular components of ECN have been proposed in cell signalling. For instance, the host cell internalization of peptidoglycan fragment of ECN leads to interaction with Noll-like receptor (NLR) 1 (Bron *et al.*, 2012) and its flagellins are recognized to interact with the TLR-5 (Hayashi *et al.*, 2001; Ogushi *et al.*, 2004; Schlee *et al.*, 2007). Another mechanism of action could be a competition for iron uptake especially to reduce *Salmonella* typhimurium intestinal colonization (Deriu *et al.*, 2013).

Akkermansia muciniphila is an abundant resident of the human intestinal tract (Derrien *et al.*, 2008) and mucus degrading specialist which correlates with health and disease (Joyce and Gahan). This bacterium modulates pathways involved in establishing homeostasis for basal metabolism and immune tolerance towards commensal microbiota (Derrien *et al.*) and has been correlated with inflammation, obesity and diabetic parameters (Collado *et al.*, 2007; Ellekilde *et al.*; Everard *et al.*; Kang *et al.*). [23]

Although the lactic acid bacteria (LAB) are not the major members of the human microbiota, due to their easy isolation and growth they have been thoroughly studied and most of the probiotic products available nowadays are based on bacteria belonging to this group. One of the main strains belonging to this group is *Lactobacillus rhamnosus* GG (LGG). LGG (ATCC 53103) was isolated in 1983 from the intestinal tract of a healthy human being by Sherwood Gorbach and Barry Goldin. LGG has shown positive effects in human patients with pouchitis, ulcerative colitis and Crohn's disease (Hormannsperger and Haller, 2010) and have been found to increase faecal IgA levels (Bakker-Zierikzee *et al.*, 2006; He *et al.*, 2005). A clinical study demonstrated that perinatal administration of this probiotic strain reduced the development of atopic eczema in children (Nermes *et al.*, 2011). This effect may be due to the anti-inflammatory properties of this probiotic bacterium. Consumption of LGG by children with atopic dermatitis has been reported to enhance the production of the anti-inflammatory cytokine IL-10 by the host (Pessi *et al.*, 2000). It has been demonstrated that two soluble factors, proteins p75 and p40, present in the culture supernatant of LGG induce the expression of Hsp in a p38- and JNK/MAPK-dependent way (Tao *et al.*, 2006). Additionally, LGG also prevents cytokine-induced apoptosis activating anti-apoptotic Akt in a phosphatidylinositol-3-kinase dependent manner and inhibiting pro-apoptotic p38/MAPK activation (Yan and Polk, 2002)(Yan *et al.*, 2007). These factors

are also able to modulate hydrogen peroxide induced damage in Caco-2 cells (Seth *et al.*, 2008). Other molecules produced by lactobacilli have been found to have important characteristics. For example, the analysis of the currently known genomic sequences of *Lactobacillus* strains predicts a broad group of bacteriocins active against Gram-positive bacteria such as *Lactococcus*, *Streptococcus*, *Staphylococcus*, *Listeria* and *Mycobacteria* (Altermann *et al.*, 2005; Chaillou *et al.*, 2005; Makarova *et al.*, 2006; Pridmore *et al.*, 2004). For instance, it is well known that *L. salivarius* UCC118 has the ability to protect mice against infection with *Listeria monocytogenes* thanks to the production of a Class II bacteriocin (Corr *et al.*, 2007). Several other lactobacilli strains have been tested in different *in vivo* and *in vitro* test with positive results. In this regard, *L. salivarius* Ls33 and its peptidoglycan were anti-inflammatory in a murine colitis model (Macho Fernandez *et al.*, 2011a; Macho Fernandez *et al.*, 2011b). *L. farciminis* CIP 103136 prevents stress-induced hypersensitivity, increase in colonic paracellular permeability, and colonocyte myosin light chain phosphorylation (Ait-Belgnaoui *et al.*, 2006). This antinociceptive effect occurs via inhibition of contraction of the cytoskeleton of colonic epithelial cells and the subsequent tight junction opening, and may also involve direct or indirect effects of nitric oxide produced by this strain (Ait-Belgnaoui *et al.*, 2006) or a decrease of the stress-induced activation/sensitization of sensory neurons at the spinal and supraspinal level (Ait-Belgnaoui *et al.*, 2009). Furthermore, *L. farciminis* CIP 103136 also improves TNBS-induced colitis (Lamine *et al.*, 2004a), mainly due to the nitric oxide released, the normalization of colonic microbiota, the prevention of bacterial translocation, the enhancement of barrier integrity and a decrease in the mucosal levels of IL-1β (Lamine *et al.*, 2004a,b).

Contrary to the gut environment, lactobacilli are considered as the dominant organisms of the vaginal cavity (Doderlein, 1982) being more than 70% of all microorganisms isolated (Eschenbach *et al.*, 1989; Redondo-Lopez *et al.*, 1990). In this ecosystem, the success obtained so far with the use of a cocktail containing the spermicide resistant *L. rhamnosus* GR-1 and the H_2O_2 producing *L. reuteri* RC-14 strains by themselves or associated with antimicrobial chemotherapy is well known (Anukam *et al.*, 2006; Reid *et al.*, 2003). These indigenous lactobacilli strains have beneficial effects on the host. GR-1 blocked the *in vitro* attachment of uropathogenic bacteria to human uroepithelial cells and prevented onset of urinary tract infection in murine models (Reid *et al.*, 1985) while RC-14 inhibited *Staphylococcus aureus* infection of surgical implants in rats (Gan *et al.*, 2002). Both strains, employed together, were able to interfere with the opportunistic pathogen *Candida albicans* (Kohler *et al.*, 2012; Martinez *et al.*, 2009) and to prevent or treat BV (Homayouni *et al.*, 2014; Hummelen *et al.*, 2010).

All of presented bacteria are examples of commensal bacteria with beneficial effects in human health and some of them are already used to treat patients. Our microbiota is a real richness for future trend in 'microbiomology' research and could be a source of new probiotics that could impact on the gastrointestinal, nervous, and immune systems.

Future trends: could probiotics be indigenous?

Probiotics are defined as 'live micro-organisms which, when administered in adequate amounts, confers a health benefit on the host' (~~group,~~ 2001). The use of them began to show clinical evidence of their impact on human health (Hungin *et al.*, 2013). The advent of probiotic treatments appears to be a promising 'pharmaco-nutritional' approach to reverse diseases linked to microbiota dysbiosis. Today, most micro-organisms marketed as probiotics in the food industry are lactic acid bacteria, belonging to the genera *Lactobacillus* and *Bifidobacterium* (Foligne *et al.*, 2013). Surprisingly, most of them, used for intestinal disorders management, are not currently present in our microbiota. However, the literature underscores the need for further understanding of the role of probiotics in health and disease (Klein *et al.*, 2010). This observation, associated with the rapid evolution of the knowledge of this complex ecosystem, suggests a large panel of potential new candidates that could be isolated directly from our indigenous microbiota. A good argument for this hypothesis is the recently gained renewed interest in microbiota transplantation. Proposed as a treatment for *Clostridium difficile* colitis, in a randomized controlled trial, faecal microbiota transplantation was shown to be very efficient in more than 80% of the patients leading to increased bacterial diversity similar to healthy subjects (van Nood *et al.*, 2013). Instead of using stools from healthy donors, a cultured strain mix of sufficiently characterized beneficial commensal bacteria is proposed as an alternative (Petrof *et al.*, 2013).

We speculate that newly discovered intestinal bacteria may be used for development of new micro-organisms containing products that could be novel probiotics with health claim. In fact, host physiology, gut maturation, innate and acquired immune responses and metabolism are largely influenced by the metabolic properties of the (gut) microbiota (Gaboriau-Routhiau *et al.*, 2009; Tomas *et al.*, 2013). Moreover, the activity and the composition of microbiota are modulated by external factors making microbiota a highly 'handleable' tissue in humans (De Filippo *et al.*). Interestingly, some bacteria, depleted in many intestinal disorders that displayed beneficial effects on the host, could be used to counterbalance the dysbiosis linked to certain diseases. It has been recently proposed that the anti-inflammatory *F. prausnitzii* bacterium, could have prophylactic or therapeutic applications in human health (Miquel *et al.*, 2013; Sokol *et al.*, 2008). Today, we could suggest other species as good candidates: *B. thetaiotaomicron*, *B. fragilis*, *L. farciminis*, etc. However, the description of several microbial communities of our environment (particularly thus of our gut microbiota) allowed us to identify new bacterial species that were unknown so far for their health benefit. The recent description of the intestinal metagenome (all genomes of the bacterial populations of an environment), confirmed that the richness concerning bacterial species of the human gut microbiome correlates with metabolic markers (Le Chatelier *et al.*, 2013). Therefore, besides being a reservoir of unexploited bacteria for academic research, our microbiota also present potentially beneficial metabolic capacities for human health that could be exploited industrially. In parallel, it could be speculated

that intestinal probiotics prepared from commensal bacteria may be efficient at a lower threshold than an exogenous strain since they could occupy their own ecological niches more easily.

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