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REVIEW

# Molecular dialogue between the human gut microbiota and the host: a *Lactobacillus* and *Bifidobacterium* perspective

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**Abstract** The human gut represents a highly complex ecosystem, which is densely colonized by a myriad of microorganisms that influence the physiology, immune function and health status of the host. Among the many members of the human gut microbiota, there are microorganisms that have co-evolved with their host and that are believed to exert health-promoting or probiotic effects. Probiotic bacteria isolated from the gut and other environments are commercially exploited, and although there is a growing list of health benefits provided by the consumption of such probiotics, their precise mechanisms of action have essentially remained elusive. Genomics approaches have provided exciting new opportunities for the identification of probiotic effector molecules that elicit specific responses to influence the physiology and immune function of their human host. In this review, we describe the current understanding of the intriguing relationships that exist between the human gut and key members of the gut microbiota such as bifidobacteria and lactobacilli, discussed here as prototypical groups of probiotic microorganisms.

**Keywords** Probiotics · Bifidobacteria · Lactobacilli · Gut microbiota · Host–microbe cross-talk · Genomics

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## Introduction

The human gut is colonized by a myriad of bacteria, which collectively are estimated to outnumber somatic and germinal cells by a factor of ten [1]. Advances in sequencing technologies have allowed researchers unprecedented insights in niche-specific microbiota composition, revealing that the human body harbors dozens of different bacterial species in the stomach, hundreds on the skin, and thousands within the oral cavity and the large intestine [1–3]. Gut bacteria exert a profound impact on human physiology, immunology, and nutrition. The symbiotic relationship of the gut microbiota with the human host is the consequence of a long history of various co-evolutionary processes, where neither partner is disadvantaged, and where unique metabolic activities or other benefits are provided to both partners [4]. Compositional alterations that disturb the normal balance of the gut microbiota, a phenomenon referred to as gut dysbiosis, are considered, at least in part, responsible for metabolic disorders such as obesity, as well as debilitating and life-threatening diseases like inflammatory bowel disease and colon cancer [5]. Hence, ensuring the appropriate compositional balance of the intestinal microbiota is considered to be an effective means to maintain (gut) health.

According to the Food and Agriculture Organization of the United Nations and World Health Organization (FAO)/WHO criteria, probiotic bacteria are microorganisms that are consumed as live dietary supplements and that confer one or more health benefits to the host [6, 7]. Currently, the majority of probiotic bacteria that are commercially exploited belong to two genera, *Bifidobacterium* and *Lactobacillus*, both of which are common inhabitants of the human intestine. It is suggested that probiotic bacteria beneficially affect human health through different mechanisms that are typically divided into a number of general categories, involving

strengthening of the intestinal barrier, modulation of the immune response, and antagonism of pathogens either by the production of antimicrobial compounds or through competition for mucosal binding sites [4]. Although there is suggestive evidence for each of these functional claims, the precise molecular mechanisms underlying most of these beneficial activities have essentially remained obscure.

The decoding of the genome sequence of a probiotic bacterium is arguably a prerequisite step in the discovery of the genetic basis of probiotic action of such a health-promoting bacterium. Such (comparative) probiotic genome analysis, also referred to as probiogenomics [8, 9], and its subsequent functional characterization has provided important insights into the diversity and evolution of probiotic bacteria, and has in several cases guided the unraveling of the molecular basis for a particular beneficial activity. These "omics" approaches allow the simultaneous analysis of very large numbers of genes, proteins, and/or metabolites, while the integration of genomic information with data on host gene expression in the human gut is expected to further expand our understanding of the roles of (probiotic) microbiota, microbe–microbe, and host–microbe interactions [10].

Here, we will assess how probiotic bacteria interact with the immune system of the host through the action of molecules that may also play a pivotal role in gut colonization and persistence. We will focus on the model gut probiotic bacteria that belong to *Bifidobacterium* spp. and *Lactobacillus* spp., which are phylogenetically distant relatives that each possess distinct beneficial attributes.

## The human gut microbiota

The trillions of commensal bacteria that make up the intestinal microbiota primarily belong to five microbial phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* and *Fusobacteria*, which are distributed throughout the gut in different numbers, likely as a result of varying microbial ecosystems (Fig. 1) [11]. The dominant phylum in the human adult microbiota is represented by *Firmicutes*, which in some individuals represents over 90 % of the total gut microbiota [12]. In contrast, these values are considerably different in the (human) infant intestine, where *Actinobacteria* and in particular representatives of the genus *Bifidobacterium* are numerically in the majority [12]. Furthermore, the infant gut microbiota has been shown to be much less complex than its adult equivalent in terms of total number of bacteria and encountered diversity of microbial taxa [1, 12]. Also, a relatively simplified composition (compared to the adult situation) was observed for the gut microbiota of the elderly population [13]. The diversification of microbial gut communities is influenced by several variables such as host factors (e.g., pH, bile acids, transit time, and mucus), environmental factors (e.g., nutrients and medication) and microbial factors (e.g., adhesion capability, bacterial enzymes, metabolic strategies, and bacteriocin production) [14]. Another key force that drives diversity and composition of the gut bacterial community is represented by bacteriophage [15, 16].

At birth, the human intestine is considered to be sterile; however, the gut is rapidly colonized after delivery. Natural sources of gut bacteria are represented by mother's vaginal and fecal microbiota, as well as other environmental microbes [17]. Multiple factors may thus be responsible for the differences observed between the gut microbiota of infants, such as the delivery mode, local environment, type of infant feeding (breast-fed or formula-fed), gestational age, or antibiotic treatment [18, 19]. The delivery mode plays an important role in shaping the neonatal microbiota: for instance, *Lactobacillus* spp. dominate the gut microbiota of infants delivered vaginally as compared infants delivered by caesarean section [20, 21]. This is presumed to be a consequence of *Lactobacillus* spp. being among the dominant inhabitants of the urogenital ecosystem of healthy women [22, 23]. Colonization of the infant gut by *L. rhamnosus* and *L. gasseri* was shown to have a sinusoidal wave trend reaching 45 % proportional abundance in neonates up to 6 months of life. *L. rhamnosus*, *L. paracasei*, *L. fermentum*, *L. gasseri* and, though at lower frequencies, *L. plantarum*, *L. delbrueckii* and *L. reuteri*, were among the most commonly isolated bacteria in fecal samples obtained from Swedish infants during the first year of life [24]. Besides the abundance of lactobacilli, it is usually the bifidobacteria that dominate the intestinal microbiota of breast-fed full-term infants, while formula-fed, full-term infants also harbor bacteroides, clostridia, enterobacteria, and streptococci [25].

Another intriguing source of bacteria in this context is human milk, which has been proposed to act as an inoculum for breast-feeding infants, thus contributing to the establishment of a bifidobacteria-abundant infant gut microbiota [26]. Recently, comparative and functional genomic investigations have highlighted that certain human milk oligosaccharides (HMOs) play a crucial role in the development of the human infant gut microbiota [27]. Genomic analyses of bifidobacterial species that are typically associated with the infant gut microbiota, like *Bifidobacterium longum* ssp. *infantis* and *Bifidobacterium bifidum*, have revealed the existence of a gene set that encodes enzymes dedicated to the metabolism of HMOs [28, 29], which highlights the genetic specialization and presumed co-evolution of these bacteria to the infant gut and associated diet (see below).

Elements of the gut microbiota not only play an important role in the fermentation of indigestible complex plant polysaccharides and host-produced glycans (such as mucin) but are also believed to provide protection against pathogenic

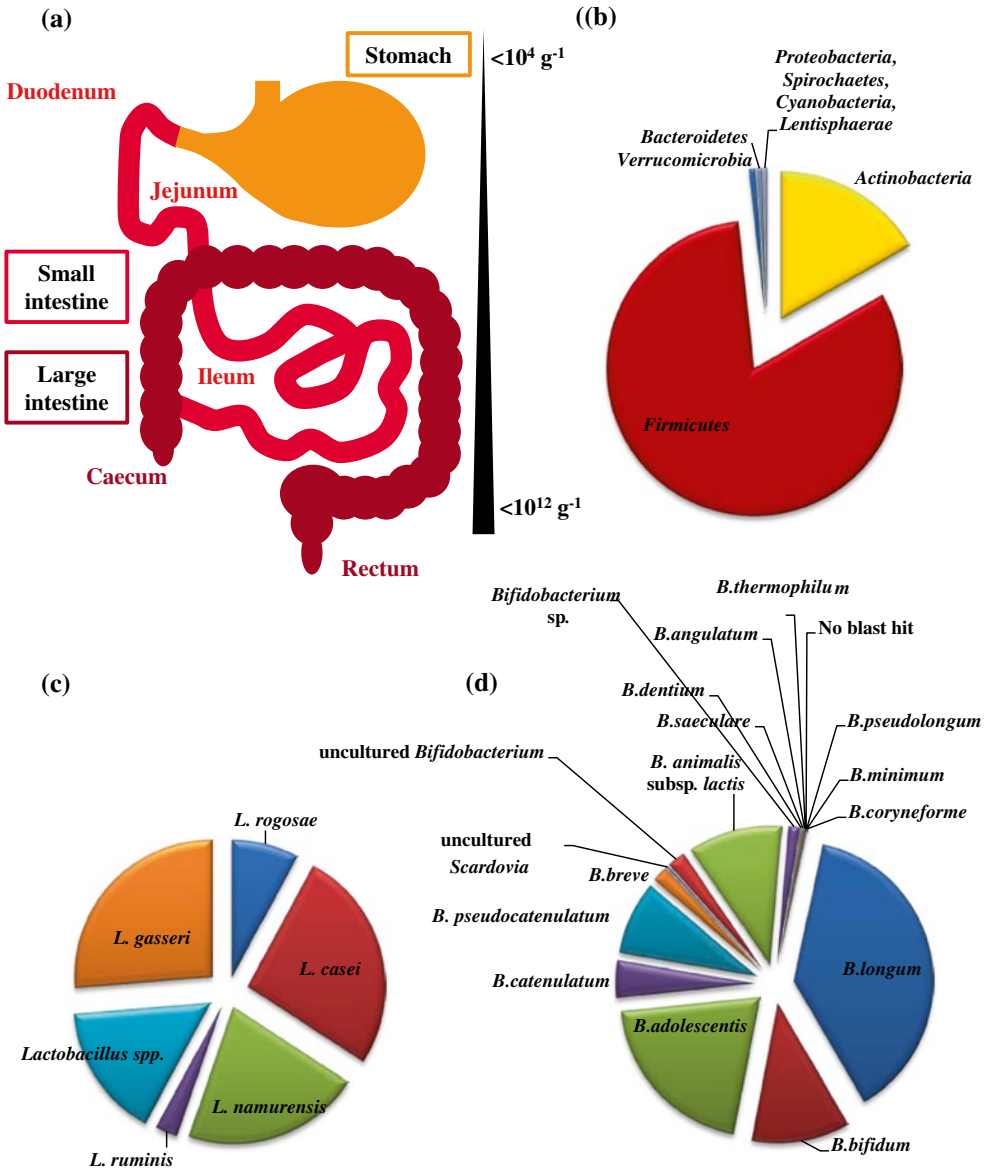


**Fig. 1** Schematic representation of the human gastrointestinal tract showing its different compartments and the relative abundance of bacteria (**a**). **b** The relative abundance of the main microbial phyla detected in the adult fecal samples [12]. **c** The aggregate microbiota composition of the genus *Lactobacillus* as determined from adult fecal sample analysis [12]. **d** The aggregate microbiota composition of the genus *Bifidobacterium* as determined from adult fecal sample analysis [12]

microorganisms. In addition, elements of the gut microbiota are thought to be required for the proper development of the host's immune system [30]. There is evidence that the gut microbiota exerts a key role in inducing IgA production [31, 32], as well as maintaining the homeostasis of several T cell populations in different human body compartments including the gut, including regulatory T cells (Treg), and T helper 1 ($T_H1$) and 17 ($T_H17$) cells [33]. It has, for example, been reported that in vitro lactobacilli can modulate the host immune response suppressing inflammation by inducing $CD4^+CD25^+Foxp3^+ROR\gamma t^-$ Treg cells [34, 35].

It has recently been demonstrated that commensal bacteria of the human gut produce molecules that mediate healthy immune responses and protect the host from inflammatory disease [36]. In this context, Mazmanian et al. [36] suggested that the human genome does not encode all functions required for immunological development, but, rather, that mammals depend on critical gene/metabolic products of their gut microbiota [36]. This has, for example, led to the discovery of key microbial gut molecules such as capsular polysaccharides that support and maintain immune functions of the human host (see below).

Additional metabolic functions of the gut microbiota include synthesis of vitamins (see review [37]), and bile acid biotransformation [38]. The major short-chain fatty acids produced by elements of the gut microbiota are acetate, butyrate, and propionate, with many other, yet minor, microbial metabolic end products such as lactate, ethanol, succinate, valerate, caproate, isobutyrate, 2-methylbutyrate, and isovalerate [39]. One of the most important

effects of these metabolites is their trophic effect on the intestinal epithelium [40]. In particular, butyrate is the preferred energy source for epithelial cells [41]. Furthermore, such metabolites have been described to have effects well beyond their place of production, as they have been reported to affect brain function [42, 43].

## The human gut microbiota and diseases

Several studies have investigated possible relationships between gut microbiota composition and various diseases, such as necrotizing enterocolitis [44], type I and type II diabetes [45, 46], irritable bowel syndrome (IBD) [47, 48], atopic diseases and allergy [49], and colon cancer [50]. Most of these diseases have been associated with dysbiosis, which is a status in which the microbiota behaves abnormally as a consequence of an alteration of its composition, a change in its metabolic activity, and/or a shift in the local distribution of bacterial communities. However, the difficulty in accurately delineating a true core microbiota makes the concept of "dysbiosis" very hard to define.

Several factors may be responsible for microbial alterations of the gastrointestinal ecosystem, including antibiotic treatment, physical injuries or psychological stresses, radiation, altered peristalsis, and dietary shifts [51]. Among the diseases whose aetiology is linked to gut dysbiosis we will discuss: (1) autoimmune diseases, (2) IBD, and (3) allergy.

### Autoimmune diseases

Autoimmune diseases occur when the body's immune system attacks and destroys its own (healthy) cells and tissues as in the case of type I diabetes, celiac disease, inflammatory bowel diseases, and allergic asthma. Although most of these diseases have unknown causes, it has been suggested that dysbiosis may be an underlying cause [14]. Celiac disease is an inflammation of the small intestine, which is triggered by the storage proteins of wheat, barley, and rye. Analysis of the fecal microbiota of celiac patients showed a markedly numerical reduction in *Bifidobacterium* species, *Clostridium histolyticum*, *C. lituseburense*, and *Faecalibacterium prausnitzii*, coupled to increased proportions of *Bacteroidetes*/*Prevotella* [52, 53].

Type 1 diabetes, which is characterized by insulin deficiency caused by immune-mediated destruction of pancreatic beta cells, is thought to be triggered by environmental factors in genetically susceptible individuals. However, recent data suggest that alteration of the gut microbiota in rats is associated with progression of type 1 diabetes. Furthermore, a microbiota survey of diabetes-prone rats versus diabetes-resistant rats revealed a higher abundance of *Lactobacillus* and *Bifidobacterium* in diabetes-resistant rats [54]. These observations are supported in studies where *L. casei* Shirota has been shown to exert a suppressive effect on experimental models of immune disorders, such as arthritis, type I diabetes [55], murine lupus [56], and chronic IBD [57]. However, the molecular mechanisms responsible for such therapeutic effects are still unknown.

### Gut microbiota in inflammatory bowel disease (IBD)

The initiating and perpetuating stimuli for immune dysregulation in IBD are not fully explained by genetic predisposition, since the number of known IBD-promoting gene mutations, such as CARD15, largely exceeds the clinical prevalence of the disease [58]. In addition, animal models have demonstrated that clinically identical mucosal inflammation can be initiated by different genetic immune defects [59]. These findings indicate that other modifying factors must be present, and suggest that changes in the luminal environment are crucial to the pathogenesis of IBD. Furthermore, the finding that experimental colitis does not develop in germ-free animals, while introduction of a single bacterial species can induce mucosal inflammation in animal models, coupled to the observation that diversion of the fecal stream is an effective treatment of active Crohn's disease (CD), further implicates gut bacteria in the pathogenesis of IBD [60, 61]. As mentioned above, dysbiosis is considered the main cause of development of inflammatory diseases. Marked shifts in the luminal bowel microbiota, including loss of population diversity leading to a predominance of (pathogenic) bacterial species, such as *Clostridium difficile*, *Bacteroides vulgatus* and *Escherichia coli*, have been observed before relapse in IBD [62, 63]. Reduced bacterial diversity has been demonstrated in mucosal biopsies of patients with active IBD, with loss of commensal species such as *Clostridium leptum*, *Eubacterium* and bifidobacteria [64, 65]. Recent analysis of the fecal microbiota of patients suffering from IBD revealed an under-representation of the *Firmicutes* phylum, in particular the species *Faecalibacterium prausnitzii* [66].

Although inappropriate reaction by the innate immune system to intestinal bacteria has been implicated in the pathogenesis of IBD, it has also been demonstrated that the products of bacterial activity, such as butyrate, have a regulatory effect on inflammation in IBD [40, 67, 68].

Toki et al. [69] reported that the probiotic *L. casei* strain Shirota elicits anti-inflammatory (and anti-tumor) properties, which are linked to a cell envelope-associated polysaccharide–peptidoglycan complex, capable of inhibiting IL-6 production in LPS-stimulated lamina propria mononuclear cells isolated from IBD mice.

The probiotic mixture named VSL#3 is used as a supplement in the treatment of microbiota dysbiosis and a growing body of evidence reveals its ability to reduce colitis

scores in IL10-deficient mice [70], to suppress ileitis [71] and colitis [72] in murine models, to prevent colorectal cancer in a DSS-mice model [73], and to ameliorate liver dysfunction [74].

Allergic disorders and human gut microbiota

The prevalence of allergic disorders has been steadily increasing in Western societies, and such conditions now comprise the most common chronic disease of childhood. There is an increase in the prevalence of atopic diseases [75], which represents a related group of conditions including allergic rhinoconjunctivitis, asthma, and atopic eczema. These are frequently associated with the generation of T helper ($T_H$) cell 2-type cytokines, including IL-4, IL-5, and IL-3, which promote IgE production. The $T_H2$ skewed immune type may be balanced by cytokines secreted by $T_H1$, $T_H3$, and T regulatory cells, partially as a result of stimulation by the gut microbiota [76]. The establishment of the gut microbiota provides an initial and impressive source of stimuli to the host. The route for the first allergic responses frequently arises from the gastrointestinal tract, and food allergy is a common problem in infants with atopic eczema. Aberrant barrier functions in the gut mucosa lead to increased antigen transfer across the mucosal barrier with transfer routes being altered, thereby evoking aberrant immune responses and release of proinflammatory cytokines with further impairment of the barrier functions. Such an enhanced inflammation state in turn leads to increases in intestinal permeability and results in a vicious circle of self-promoting allergenic responses and a more permanent dysregulation of the immune responses to ubiquitous antigens in (genetically) susceptible individuals.

The "hygiene hypothesis" proposes that the increased prevalence of allergic disorders in developed countries is due to a reduced early exposure to infectious agents that may alter the immune response and the immunoregulatory compartment, which may then affect the composition of the gut microbiota [77]. An alternative or derivative hypothesis is based on the changes in the intestinal microbiota that are due to increased antibiotic treatments and an altered infant diet [78]. In both cases, the regulatory mechanisms controlling the $T_H2$ responses that are commonly associated with allergic disorders do not seem to function properly. However, it is still unclear to what extent the dysbiosis in the early stages of life can be attributed to antibiotic treatment and/or by human genetics.

Several studies have described the microbiota composition of infants who develop allergic disorders [79–81]. Bifidobacteria are predominant in the intestinal microbiota of a normal, healthy infant, with high numbers of *Bifidobacterium breve*, *B. bifidum* and *B. longum*, whereas bifidobacterial species such as *B. adolescentis* and *B. pseudocatenulatum* are more characteristic of an adult-type intestinal microbiota [82].

## Recovery of the gut microbiota after perturbation

As described above, alteration of gut homeostasis might be the aetiological cause of a disease. The modification from homeostasis to a disease state can occur in different ways, including physical insult to the gut mucosa, disruption of the autochthonous microbiota through the use of antimicrobials (e.g., antibiotics), and virulence factors (for review, see [83]).

Restoration of the indigenous microbiota is expected to be driven by the action of several species, and is possibly subject to substantial microbial re-organization following the initial disruption of the microbiota. The mechanisms involved in the re-organization of the normal microbiota include co-aggregation, biosurfactant production, bacteriocin, and hydrogen peroxide biosynthesis, and competitive exclusion and modification of the diet composition (for review, see [83]). Co-aggregation of non-pathogens and pathogens interferes with the capability of pathogenic bacteria to infect the host; whereas biosurfactant biosynthesis helps to prevent the adhesion of pathogens to mucosal surfaces and, in the case of vaginal lactobacilli, the production of biosurfactants consisting of a mixture of lipids, proteins, and carbohydrates assist in the displacement of the uropathogenic *E. coli*, *Enterococcus faecalis* and *Gardnerella vaginalis* [84, 85]. Bacteriocin production is considered a key process in restoring homeostasis. Bacteriocins are small molecules synthetized by bacterial ribosomes that interfere with cell wall biosynthesis and that are capable of inhibiting growth of certain (Gram-positive) bacteria [86]. Bacteriocins are typically active against a narrow range of bacteria that are phylogenetically closely related to the producing organism, and they kill these cells by pore formation and leakage of cell contents [87]. There is good evidence that bacteriocin production is important for probiotic effects in the oral cavity, and indirect evidence for the gut environment. A recombinant *Streptococcus mutans* strain making abnormally high levels of mutacin, but lacking lactate dehydrogenase, out-competed other *S. mutans* strains to cause long term mono-colonization, yet did not cause caries [88]. In the gut environment, evidence for modulation of the microbiota is mostly indirect, from animal model experiments. For example, bacteriocin Abp118, synthetized by *L. salivarius* UCC118 [89], was shown to be important for host protection against *Listeria monocytogenes* infection in mice [90]. In a recent study of bacteriocin effect upon the total murine intestinal microbiota, we found that an Abp118-producing strain caused a relative increase in *Bacteroidetes* and *Proteobacteria*, and a decrease in *Actinobacteria*, as compared with an isogenic, non-bacteriocin-producing control,

in a diet-induced obesity model [91]. As noted below, we also detected activity of the Abp118-producing *L. salivarius* UCC118 against Gram-negative bacteria in the porcine gut, while this antagonistic effect was lacking in pigs that harbored the genetically modified knock-out strain. Although many probiotic lactobacilli produce bacteriocins (e.g., *L. casei*, *L. acidophilus*), others apparently do not (e.g., *L. rhamnosus* LGG does not appear to, though it has the required genes). Furthermore, the ethical barriers to administering genetically modified micro-organisms to humans mean that bacteriocin-producing/non-producing wild-type/knock-out comparisons have not yet been performed in humans, to our knowledge. This is a significant impediment, because such a comparison would provide definitive proof for the importance of bacteriocin production to exert a probiotic effect.

Another critical mechanism of gut microbiota recovery is represented by competitive exclusion. Autochthonous members of the human gut microbiota have been postulated to be capable of effective competition with transient pathogens through the production of specific molecules such as capsular polysaccharides. An example is represented by the multiple capsular polysaccharides produced by *Bacteroides fragilis*, which are not only essential for persistence and gut colonization but also have an imuno-modulatory function, and help to exclude pathogens and restore homeostasis (see below) [92]. Nevertheless, *B. fragilis* is currently not considered to represent a probiotic bacterium, while the study carried out by Mazmanian's group was conducted using a murine model rather than a human clinical trial. A perturbed intestinal microbiome has been associated with an increasing number of gastrointestinal and non-gastrointestinal diseases including *C. difficile* infection (CDI). It has become recognized that fecal microbiota transplantation can correct the dysbiosis that characterizes chronic CDI, and effect a seemingly safe, relatively inexpensive, and rapidly effective cure in the majority of treated patients. Recent studies involving on fecal microbiota transplantation have resulted in a gut microbiota restoration rate to normal homeostasis of more than 90 % [93–96].

The microbiota-modulating activity of lactic acid bacteria may be clinically beneficial. For example, a mixture of three bacteria (*L. casei*, *L. bulgaricus*, and *Str. thermophilus*) has been shown to reduce the incidence of antibiotic-associated diarrhoea (AAD) and *C. difficile*-associated diarrhoea (CDAD) [97].

In a recent randomized clinical trial, the supplementation of *B. bifidum* MIMBb75 revealed a significant reduction of the symptoms linked to irritable bowel syndrome (IBS) [98, 99].

## Bifidobacteria as prototypical human gut commensals

Bifidobacteria were originally isolated by Tissier at the beginning of the last century from stool samples of breast-fed infants, and since then more than 38 taxa have been included in this bacterial genus (for a recent review, see [100]). From the very beginning, bifidobacteria attracted the interest of both scientists and food industry because of their perceived health-promoting activities, but they only recently have been subjected to more detailed molecular analyses through genome sequencing and functional genomics efforts [8]. These analyses have significantly expanded our understanding regarding the roles of gut-derived bifidobacteria in both microbe–microbe and host–microbe interactions, and have revealed a number of key molecules, produced by particular *Bifidobacterium* species, that promote their establishment in the human intestine. The strict co-evolution of many bifidobacterial species with the eukaryotic digestive tract has lead these microorganisms to acquire important colonization factors and metabolic abilities, which render them one of the major microbial players in gut colonization during the first stages of life. Furthermore, one may argue that such a highly developed colonization ability renders bifidobacteria very effective in colonization of the gut when this is covered by a thick microbial biofilm of other enteric commensals or pathogens.

Various sequencing projects have allowed the decoding of complete genome sequences of mainly enteric bifidobacterial species, such as *B. longum* ssp. *longum* [101, 102], *B. longum* ssp. *infantis* [28], *B. bifidum* [103], and *B. breve* [104]. Notably, the subsequent analyses of such genomic data reinforces the notion of strict genetic adaptation of these bifidobacterial taxa to the human gut and has revealed the existence of key bifidobacterial molecules that are responsible for gut colonization and survival of bifidobacteria in the human intestine. A clear example of such genomic adaptation is represented by the identification of a varied arsenal of genes encoding enzymes that are involved in the breakdown of complex carbohydrates derived from the diet (e.g., plant polysaccharides) or from the host (e.g., mucin). These carbohydrates cannot be digested by host-derived enzymes and will thus reach the large intestine in an intact form (for a recent review, see [105]). These specific metabolic properties of bifidobacteria are a clear indication as to how these microorganisms function in the human gut environment and how they positively affect human health status.

Glycans and glycoproteins produced by the host constitute a key carbon and energy source for gut bacteria, in particular in the distal colon where the availability and accessibility of simple carbohydrates is limited. Mucins represent a large polymeric network of carbohydrates that cover the intestinal mucosa, forming the main glycoprotein component of the mucus layer, and thus offering the microbiota a rich source of host-produced carbon and energy [106]. The main monosaccharide constituents of mucin-derived glycoproteins are *N*-acetylglucosamine, *N*-acetylgalactosamine, fructose, and galactose, and such glycoproteins are frequently decorated

with sialic acid and sulphate groups [107]. Analysis of the chromosomal sequence of *B. bifidum* PRL2010, a strain isolated from infant stool, revealed a competitive nutrient-utilization strategy targeting mucin-derived *O*-glycans [103]. In silico analyses of the *B. bifidum* PRL2010 chromosome, together with functional genome approaches, revealed the existence of a gene set responsible for mucin metabolism. A significant proportion of the predicted proteome of *B. bifidum* PRL2010 is involved in mucin metabolism and comprises extracellular enzymes that include putative exo-α-sialidases, as well as a predicted 1,2-α-l-fucosidase, 1,3/4-α-l-fucosidase, and a putative cell wall-anchored endo-α-*N*-acetylgalactosaminidase. This enzymatic mucin breakdown machinery is completed by four *N*-acetyl-β-hexosaminidases, and four β-galactosidases, which allows the complete hydrolysis of the glycan component of mucin into monosaccharides [103]. A similar enzymatic repertoire has been identified and characterized in other members of the *B. bifidum* species [108–112], suggesting that this is a common and perhaps a species-defining property of this taxon. Recently, another human gut microbiota member, *Akkermansia mucinophila*, was identified as an important mucin degrader [113–116]. Mucin-degrading activity as operated by enteric bacteria such as *A. mucinophila* and *B. bifidum* may stimulate the secretion of colonic mucin, which constitutes an important feature especially in patients suffering from irritable bowel syndrome [117].

Mucin degradation as observed in *B. bifidum* seems to be a specialized form of a more general genetic adaptation of bifidobacteria to the human gut, which appears to be geared towards carbohydrate metabolism, where the ability to rapidly retrieve a specific carbon source from a particular environment/diet represents an important feature that would endow a bacterium with an undisputed ecological fitness [118].

Recently, the genetic requirements for carbohydrate uptake in *B. bifidum* PRL2010 have been investigated [119] and compared to those of other enteric bifidobacterial species, such as *B. longum* ssp. *infantis*, *B. breve* and *B. longum* ssp. *longum* [120]. Notably, PRL2010 contains a relatively limited number of such genes, which is thereby restricted to the uptake of a comparatively small number of carbohydrates [119]. These results suggest an interesting genetic strategy for efficient colonization and survival of PRL2010 in its ecological niche.

Another example of adaptation of bifidobacteria to the human gut is represented by the ability of several bifidobacterial species (e.g., *B. longum* ssp. *infantis*, *B. bifidum*, and *B. breve*) to utilize HMOs present in human milk [121, 122]. The genome sequence of an infant-derived bifidobacterial strain, *B. longum* ssp. *infantis* ATCC15697, has revealed the presence of an extensive gene set, located on a 43-Kb genomic island, whose encoded enzymes are involved in HMO breakdown (fucosidases, sialidases, a β-hexosaminidase, and β-galactosidases) and internalization (e.g., extracellular solute binding proteins and permeases of ABC transporter systems) [28]. Furthermore, genome analysis of this strain revealed the presence of an operon predicted to be involved in urea metabolism, which appears to be uniquely present in the genome of strains belonging to *B. longum* ssp. *infantis* [28]. Urea represents an important source of nitrogen in human milk and thus the presence of a urease-encoding operon in a bifidobacterial strain residing in the human gut of infants might represent another key sign of adaptation of *B. longum* ssp. *infantis* to this environment [122].

Further data concerning bifidobacterial adaptation to the human gut is represented by the ability of these bacteria to survive the stressful conditions that are encountered in the intestine such as exposure to an acid environment during passage through the stomach, to bile salts in the intestine, and osmotic stress as a result of dietary changes. Enteric bifidobacteria are endowed with a repertoire of molecular chaperones, bile efflux transporters, bile salt hydrolases, and ATPases to cope with these stressful conditions (for a review, see [123]). Furthermore, bifidobacteria possess various sensing systems and defence mechanisms against stress thereby allowing them to survive harsh conditions and sudden environmental changes. Microbial stress responses rely on coordinated gene expression in order to alter various cellular processes and structures (e.g., DNA metabolism, housekeeping genes, membrane composition), which act in concert to improve bacterial stress tolerance [123]. The existence of regulatory networks in the bifidobacterial genomes enables tight regulation of the expression of stress-induced molecular chaperones, and consequently allows the cell to rapidly react to various and sometimes complex environmental changes [124].

## Lactobacilli as a model probiotic group

The genus *Lactobacillus* encompasses about 145 species, belongs to the phylum *Firmicutes* [125, 126], and is recognised for its extensive phylogenetic, phenotypic, and ecological diversity [127]. Enteric lactobacilli detected in adult human fecal samples include strains of the following species: *L. acidophilus*, *L. crispatus*, *L. gasseri*, *L. plantarum*, *L. ruminis*, *L. casei*, *L. paracasei*, and *L. reuteri* [128–130], while infant fecal samples have indicated an abundance of *L. acidophilus*, *L. casei*, *L. paracasei*, and *L. salivarius* [128, 131]. Interestingly, lactobacilli have also been detected at high relative levels in the stomach and in the small intestine of humans. This predominance of lactobacilli is exemplified in a recent analysis of ileal contents from ileostomy patients employing metagenomic and HITChip approaches [132, 133]. Whole genome sequencing efforts of probiotic

lactobacilli have resulted in the complete genome sequencing of eight probiotic species including *L. acidophilus*, *L. casei*, *L. fermentum*, *L. gasseri*, *L. johnsonii*, *L. plantarum*, *L. reuteri*, and *L. salivarius* (reviewed by [8, 134]). Comparative genomics of the sequenced *Lactobacillus* strains has demonstrated that, in contrast to bifidobacteria, lactobacilli genomes show a considerable degree of auxotrophy for amino acids, which is compensated by an expanded set of peptide and amino acid transport functions [134]. An extension of this comparative analysis to include lactobacilli from intestinal, plant, or milk environments revealed functions that underscore their particular niche adaptation. In this context, typical milk-adapted lactobacilli such as *L. delbrueckii* ssp. *bulgaricus* and *L. helveticus* contain various genes that sustain their adaptation to growth on lactose, whereas genomes of other lactobacilli revealed the existence of sugar-uptake systems and mucus-binding proteins that are suggestive of a specific intestinal adaptation [135–137]. In addition, the finding that certain lactobacilli (are predicted to) encode typical intestinal enzymes such as bile salt hydrolase (BSH) is also suggestive of an ecological adaptation to the intestine [138]. For example, in silico analysis of the *L. salivarius* UCC118 genome revealed the presence of a large extra-chromosomal replicon, also known as a megaplasmid, that represents more than 10 % of the overall coding genome capacity of this strain, encoding biologically important features such bacteriocin production, a bile salt hydrolase, and components of the phosphoketolase pathway, reclassifying this organism as a facultative heterofermentative lactic acid bacterium [139].

Genome dissection of the probiotic strain *L. rhamnosus* GG demonstrated the presence of pili-encoding loci that have been shown to be pivotal in bacterial colonization and persistence within the human gut (see below). Similarly, the *L. johnsonii* NCC533 genome encodes fimbrial structures that were suggested to play a role in epithelial cell adhesion [140]. Other encoded lactobacilli molecules suggestive to be important for human gut adaptation have been discovered by the analysis of putative secretomes through in silico approaches. The prediction of the secretome of *L. plantarum* WCFS1 revealed the existence of at least 12 proteins, mainly hydrolases and transglycosylases that are predicted to be involved in the adherence to host components like collagen and mucin [141]. Similarly, putative adhesin proteins have been identified in the genome of *L. acidophilus* NCFM [142], and further studies confirmed their role in bacterial adherence to a human cell monolayer (Caco2 cells) [143].

## The intestinal mucosa and probiotic action

As mentioned above one of the main routes through which probiotic bacteria are believed to act is by means of microbially produced molecules, which can be metabolites, proteins, or polysaccharides, that modulate the host immune system. It is now recognized that the innate and adaptive immune systems in the colon are linked to other important enteric functions such as adsorption of nutrients [144]. Anatomically, the intestinal mucosa is formed by a single-cell thick layer known as the epithelium and by an underlying layer, i.e. the lamina propria, which is virtually sterile and contains various immune cells. The epithelium is largely formed by columnar cells that perform key roles in the adsorption of nutrients and protecting from the passage of "non-self" luminal components like bacteria. Furthermore, Paneth cells and goblet cells in the epithelium contribute to innate immune defenses [145] by producing a vast array of antimicrobials (e.g., defensins and lysozyme) and mucin, the latter constituting a protective layer on the epithelium by preventing a direct epithelial contact with luminal bacteria [146–148]. Adaptive immunity in the intestinal mucosa is exploited by the lymphoid tissues associated with the lamina propria of the gut [gut-associated lymphoid tissue (GALT)] formed by B cells, macrophages, dendritic cells (DCs), and T cells [145]. It is suggested that, for probiotic action, the most important classes of T cells are the T helper ($T_H$) and regulatory T cells ($T_{Reg}$) [149], where the latter regulate the production of the anti-inflammatory interleukin-10 (IL10). Macrophage cells are mainly involved in the elimination of pathogens, whereas DCs regulate both adaptive and innate immunity [150]. DCs are intimately associated with the lamina propria in an immature form that can activate the nuclear factor-kB (NF-kB) pathway, which allows the maturation and subsequent activation of DCs through exposure to so-called microbe-associated molecular patterns (MAMPs) [151].

Complex interaction between intestinal mucosa, macrophages, and DCs are responsible for the development of the intestinal immune homeostasis in response to elements of the gut microbiota [152, 153]. This process is regulated by the recognition of specific pattern recognition receptors (PRRs) recognizing MAMPs derived from bacteria, including probiotics. PRRs encompass a long list of receptors such as the Toll-like receptors (TLRs), NOD-like receptors (NRLs), and C-type lectin receptors (CLRs) [154].

## Bacterial cell wall components and probiotic effectors

Efficient probiotic molecules are believed to cover or protrude from the microbial cell, thus being exposed to the external environment of the bacterial cell [144]. Many such molecules represent MAMPs that are recognized by specific PRRs, such as TLR, NLR and RIG-I-like receptors (RLR), produced by the host mucosa [148]. Several of the constituents of these extracellular macromolecular structures have

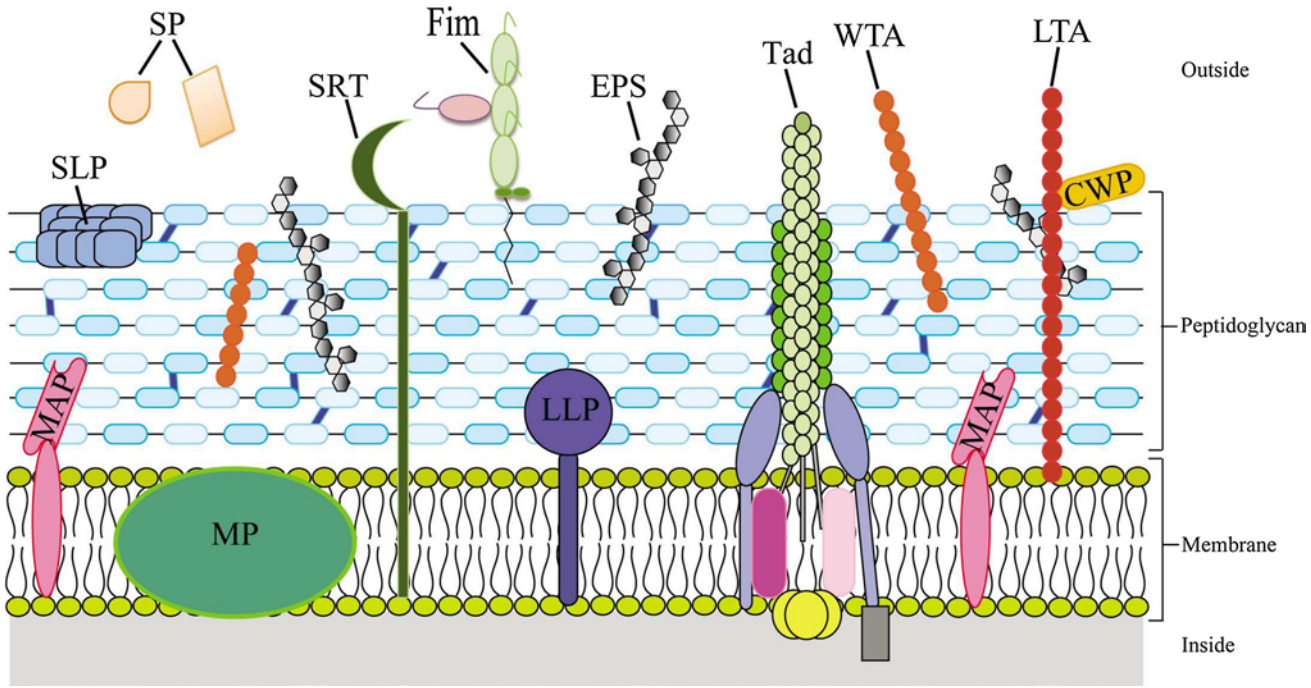


**Fig. 2** Schematic overview of the Gram-positive cell wall together with main macromolecular structures that have been implicated in host–microbe interaction. Specific components of the cell envelope are shown such as peptidoglycan layer, wall and lipoteichoic acids (*WTA* and *LTA*), exopolysaccharide (*EPS*), as well as secreted proteins (*SP*), membrane proteins (*MP*), cell wall-associated proteins (*CWP*), lipoproteins (*LPP*), membrane-associated proteins (*MAP*), surface layer proteins (*SLP*), fimbrial proteins (*Fim*), and tad proteins (*TAD*). Furthermore, the sortase-dependent assembly apparatus (*SRT*) is indicated

been proposed to be directly involved in interactions with mammalian cells and thus in promoting a positive health effect (Fig. 2).

Among these microbial extracellular molecules are: (1) peptidoglycan; (2) (lipo)teichoic acids; (3) capsular polysaccharides; (4) fimbrial/pili structures; (5) surface layer proteins; (6) bacteriocins; and (7) sortase-dependent proteins (other than sortase-dependent pili). Notably, most of these cellular structures also occur in pathogenic bacteria, where they are considered to play key roles for survival and colonization within the human body. Apparently, the genetic strategies followed by microorganisms to establish themselves within their eukaryotic host are similar despite the opposing effect on the health status of their host. It is widely accepted that interactions between various bacteria and the human host can be categorized as a continuum, ranging from symbiosis to commensalisms, through to pathogenesis, thus reinforcing the notion that the mechanisms/effector molecules used for bacterial colonization are very similar for both pathogens and probiotic bacteria.

## Peptidoglycan

Bacterial cell wall envelope components represent an important source of immune-modulatory action. For example, the cell wall constituent peptidoglycan is known to be the target for TLR2, which preferentially recognizes the diaminopimelic acid or the lysine moieties within the interpeptide bridge of peptidoglycan [155, 156]. Nevertheless, it has been claimed that the observed immune-modulatory action of peptidoglycan is due to lipoprotein contamination [157]. Alternative peptidoglycan-mediated signaling may occur by NOD-like receptors that interact with cell wall components such as the glutamyl-mesodiaminopimelic acid present in the peptidoglycan of Gram negative bacteria or through muramyl peptides of Gram-positive bacteria [158, 159]. Such immune-modulatory activities of peptidoglycan constituents have been demonstrated in the human gut commensal *L. salivarius*, which was shown by in vivo murine studies to induce local mucosal IL-10 production through the release of specific muropeptides [160]. Nevertheless, these muropeptides are not released by other gut lactobacilli, such as *L. acidophilus* NCFM, suggesting that the importance of peptidoglycan components as immune-modulators is strain-specific [160].

Peptidoglycan from *L. casei* has been reported to play a role in the inhibition of IL12p40 mRNA, leading to a reduction in secretion of IL12 and IL23, two cytokines that have been implicated in autoimmune diseases and inflammatory bowel diseases [161]. Furthermore, the probiotic strain

*L. casei* Shirota has been shown to exert anti-inflammatory properties by means of a cell wall-derived polysaccharide–peptidoglycan complex capable of down-regulating IL-6 production in LPS-stimulated lamina propria mononuclear cells [162].

Teichoic acids

Gram-positive bacteria are capable of synthesizing teichoic acids that are organized on the cell surface as lipoteichoic acid (LTA), when anchored to the cytoplasmic membrane with a glycolipid anchor, and/or wall teichoic acid (WTA), when it is covalently linked to the peptidoglycan [163]. A large body of information is available regarding the LTA produced by lactobacilli and their immune-modulatory properties in terms of induction of tumor necrosis factor (TNF) by means of a TLR2-dependent mechanism [164–166]. It has been shown that differences in LTA composition are responsible for differential immunostimulatory effects. For instance, Ryu et al. [167] demonstrated that LTA from *L. plantarum* KCTC 10887BP causes TNF-α secretion and activates NF-κB transcription to a lower degree than LTA from *S. aureus* ATCC 6538 or *B. subtilis* ATCC 6633. The amino acid composition and stereoisomers present in the polyglycerophosphate backbone of LTA are critical for its biological activity; in fact, substitution of D-Alanine with L-Alanine causes significant reduction in cytokine secretion by human PBMCs [168]. Analysis of mutations in the *dlt* operon, which is responsible for D-alanylation of LTA, have elucidated the important role of *dlt*ABCD-encoded proteins on growth rate, morphology, and biofilm formation of *L. rhamnosus* GG [169] and on gastrointestinal tract colonization by *L. reuteri* 100–23 [170]. Furthermore, a *L. plantarum* WCFS1 mutant strain that is unable to D-alanylate LTA induces less TLR2-dependent secretion of pro-inflammatory cytokines but more production of anti-inflammatory cytokines than the wild-type strain, which improves the capability of the mutant to be protective against colitis relative to that of the wild-type [164]. Similar results have been achieved for other lactobacilli, such as *L. rhamnosus* GG and *L. acidophilus* NCFM, thereby suggesting that modifications of LTA increase anti-inflammatory immune-modulation [171]. In addition, progression of cancer polyposis in mice is arrested by oral administration of an LTA-deficient *L. acidophilus* strain NCK2025, due to the ability of this bacterial strain to enhance an anti-inflammatory subset of Treg [35]. In addition, LTA containing a low D-alanine content is responsible for the beneficial effect of *L. plantarum* EP007 on visceral pain perception in response to colorectal distension (CRD) in rats [172]. Moreover, pretreatment with LTA from *L. plantarum* K8 has been shown to reduce LPS-induced TNF-α secretion in vitro and to repress endotoxic shock induced by LPS in BALB/c mice in vivo, suggesting that LTA induces tolerance in the host [173]. Furthermore, WTA in lactobacilli have been shown to induce IL-10 through a TLR2-dependent pathway [174]. Taken together, these studies validate the observation that the immunomodulatory effects of *Lactobacillus* spp. are indeed species- and strain-dependent, and that the profound range of modulatory effects on the innate immune system warrant further scientific scrutiny.

Capsular polysaccharides

Members of the genus *Bacteroides* have, furthermore, been shown to be capable of producing multiple capsular polysaccharides [175], several of which, such as polysaccharide A (PSA), possess immuno-modulatory properties [176]. These findings highlight the importance of capsular or exopolysaccharides produced by gut commensals as important mediators of gut microbiota colonization, host–microbe cross-talk, and/or immune modulation. In this context, much of the current knowledge on capsular polysaccharides has focused on pathogens, and on the role of pathogen-encoded surface polysaccharides in pathogenesis, such as biofilm formation, tissue adherence, and anti-phagocytic activity during immune evasion [177]. In the context of capsular polysaccharides as mediators of host–commensal interactions, many studies have reported on the expression of multiple capsular polysaccharides by *B. fragilis*, and on attempts to eliminate capsular-mediated protection against the host-immune system, which was shown to result in growth defects of such acapsular mutants with subsequent spontaneous phenotypic reversion [92, 175, 178, 179]. The establishment of the expression of other capsular polysaccharides restores the reduced fitness of acapsular mutants in the gut. This represents an elegant way of identifying essential functions needed for bacterial colonization during host–symbiont mutualism. Shen et al. [180] reported a peculiar mechanism of communication between the commensal *B. fragilis* and the immune system of its host involving the delivery of PSA through outer membrane vesicles. Such cross-talk enhances IL10-producing Treg cells in vitro and may protect mice from experimental colitis in vivo [36, 180, 181].

The infant gut isolate *B. breve* UCC2003 was shown to possess a genome containing a gene cluster predicted to specify the production of two different cell surface-associated exopolysaccharides (sEPSs) [104]. Notably, the alternate biosynthesis of each of these sEPSs is directed by either half of this bidirectional gene cluster, which seems to be subject to phase variation by means of an apparent promoter inversion mechanism [182]. It has been shown that sEPS produced by UCC2003 facilitates increased stress tolerance to both bile and low pH, while it was also demonstrated that sEPS production influences gut persistence, but not, at least

under the specific conditions tested, initial colonization of this strain. sEPS-producing *B. breve* UCC2003 cells elicit only a weak adaptive immune response as compared to isogenic mutants lacking this cell envelope-associated structure. Furthermore, it was established, in a murine model, that colonization of *B. breve* UCC2003 expressing sEPS, as compared to an isogenic EPS-negative derivative, reduced infection levels of the murine pathogen *Citrobacter rodentium*. These data implicate bifidobacterial sEPS in modulating microbe–host cross-talk, resulting in host-mediated immune tolerance of the commensal, while it also, in an as yet unknown manner, provides protection against a pathogen [182].

The role of capsular polysaccharides in other probiotic bacteria such as lactobacilli has also been investigated [183, 184]. Notably, in the *L. casei* strain Shirota, the capsular polysaccharide (CPS) has been shown to modulate the suppression of pro-inflammatory responses in macrophages [183]. In addition, down-regulation of CPS production in *L. rhamnosus* GG appears to be involved in adherence to intestinal epithelial cells. In contrast, up-regulation of CPS has been demonstrated to protect *L. rhamnosus* GG against intestinal innate immune factors like the antimicrobial peptide LL-37 [185]. However, difficulties related to the analysis of their complicated glycan structure have hindered the identification of their specific roles in the interaction with PRRs [186]. The levan exopolysaccharide from *L. reuteri* strain 100–23 has been shown to be required for colonization of the mouse gut and to exert an immune-stimulatory role inducing secretion of Treg (Fox3+) in the spleen [187]. As for LTA, the increasing evidence for surface polymers of lactobacilli modulating innate immune cells is an exciting development and merits more investigation.

### Pili and fimbrial structures

Non-flagellar appendages were identified in bacteria in the early 1950s, and a relatively abundance of molecular data has been collected on the functions of pili in pathogenic microorganisms (for a review, see [188]). These structures are considered crucial in the initial adhesion of pathogens to host tissues during colonization and thus represent key effector molecules in pathogenesis. However, their presence was only very recently established in lactobacilli and bifidobacteria. Human gut colonization of the probiotic *L. rhamnosus* GG has been demonstrated to be linked to two so-called sortase-dependent pili, SpaCBA and SpaEF [189, 190]. Pili of *L. rhamnosus* GG were shown to be essential for efficient adherence to a human intestinal epithelial cell line as well as biofilm formation [191]. This study also showed that an *L. rhamnosus* GG pilus-defective derivative promoted elevated mRNA levels of the chemokine IL-8 in vitro compared to the wild-type, possibly involving an interaction between LTA and TLR2.

Sortase-dependent pili have also been discovered in bifidobacteria [192]. The publicly available genomes of enteric bifidobacteria, such as *B. bifidum*, *B. longum* ssp. *longum*, *B. adolescentis* and *B. breve*, encompass one to three predicted sortase-dependent pilus gene clusters, each of which are predicted to encode one major pilin subunit plus one or two minor pilin subunits, as well as a so-called sortase specifically dedicated to covalently assemble these pilin subunits [192]. In addition, transcriptomic analyses revealed that the genes encompassing the major and minor pilin subunits of each of these sortase-dependent pilus gene clusters are transcribed as a polycistronic mRNA, and that these genes are differentially expressed depending on the available carbohydrate in the growth medium [192]. Apart from sortase-dependent pili, a member of the so-called type IVb or tight-adherence (Tad) pilus family was recently shown to be specifically expressed by *B. breve* UCC2003 under in vivo conditions in a murine [104]. Mutational analysis of the corresponding *tad* locus of UCC2003 demonstrated that this cluster is essential for efficient in vivo murine gut colonization. Notably, the *tad* locus is highly conserved among all sequenced bifidobacterial strains, which supports the notion of a ubiquitous pilus-mediated host colonization and persistence mechanism for intestinal bifidobacteria [104, 144].

### Surface layer proteins

The bacterial cell wall is decorated with cell wall-associated proteins that may be covalently anchored, e.g., N- or C-terminally anchored proteins, lipoproteins, and LPxTG-anchored proteins, or non-covalently anchored, e.g., proteins containing LysM, WxL, or SH3 domains, choline-binding domains, peptidoglycan-binding domains, and surface layer proteins, also known as S-layer proteins [134]. S-layer proteins are widely distributed in both Gram-positive and Gram-negative bacteria and are characterized as paracrystalline arrays that completely cover the cell surface [193]. Human gut lactobacilli such as *L. acidophilus* and *L. crispatus* have been shown to produce a clear S-layer, displaying typical adhesive properties [194, 195]. Notably, S-layer proteins of lactobacilli display additional roles involving competitive exclusion of intestinal pathogens as well as immune-modulatory activity through the induction of anti-inflammatory cytokines [196, 197]. Recently, the S-layer protein of *L. helveticus* MIMLh5 has been shown to mediate a stimulatory effect on innate immunity by triggering the expression of pro-inflammatory factors TNF-α and COX-2 in the human macrophage cell line U937 via recognition through TLR-2 [198].

Bacteriocins

Bacteriocins are antibacterial peptides produced by bacteria, which in the case of probiotic microorganisms has been shown to modulate pathogen growth in in vivo models [90]. Bacteriocin gene transcription in *L. salivarius* UCC118 has recently been demonstrated to be strongly up-regulated following adhesion to epithelial cells in vitro [199], suggesting that quorum sensing in the gut could have ecological aspects and biological consequences. Bacteriocin production may also affect microbiota composition in murine and porcine models [200, 201], although such effects in the latter study appear to be minor, and affecting genera and species not susceptible to the bacteriocin in vitro.

Bacteriocins produced by commensal bacteria may also possess a role in immuno-modulation. For example, the bacteriocin produced by *L. plantarum* WCFS1, plantaricin, has been shown to be pivotal in the synthesis of the anti-inflammatory cytokine IL-10 [202]. This evidence correlates nicely with publications suggesting that antimicrobial peptides impact on the immune response through changes in cytokine secretion as was shown in the porcine gut [203]. Notably, plantaricin has been shown to be expressed during WCFS1 colonization of mice, thus supporting the importance of this bacteriocin under in vivo conditions [204]. In contrast to the generally anti-inflammatory properties of plantaricin, levels of production of a *L. salivarius* bacteriocin have been shown to inversely correlate with the ability to restore transepithelial resistance in epithelial cell monolayers that had been treated with $H_2O_2$ [205].

Sortase-dependent proteins (other than sortase-dependent pili)

Surface proteins anchored by the classical SrtA sortase proteins may also play a role in the active dialogue between bacteria and host epithelium. For instance, the disruption of the sortase gene *srtA* negatively influences adhesion to epithelial cells by *L. salivarius* UCC118 [199, 206] and *L. acidophilus* NCFM [143]. Similar results were obtained when the sortase-encoding gene of *L. casei* BL23 was deleted, causing a reduction of over 60 % in the mutant's ability to in vitro adhere to CaCO2 and HT29 cells [207]. Sortase-anchored proteins have easily recognizable cell wall-interacting motifs and domains, but a large variety of exposed functional domains [208], including proteins containing mucin-binding domains [209], one of which was shown by structural analysis to be an immunoglobulin binding domain [210].

## Examples of host–microbe interaction models

Post-genomic approaches involving global genome transcription profiling as well as metabolomics of probiotic bacteria have been used in order to highlight the impact of a specific probiotic strain on the host. Most of these studies have been carried out using animal models, though a few have been conducted directly in humans [211, 212]. Recently, a study aimed at investigating the duodenal transcriptome profiles of health subjects who consumed *L. plantarum* WCFS1 represents one of the most significant advances in this field [212]. Notably, a clear stimulation of a regulatory network was observed involving JUN and NF-kB signaling cascades. In addition, IL-6, IL-10, TLR, and T cell receptor signaling pathways were considerably modified [212]. Taken together, these results indicate that *L. plantarum* WCFS1 enhances the alertness of the mucosal immune system while preserving immune homeostasis.

In another in vivo transcriptomics study involving human subjects [212], duodenal biopsies collected in a placebo-controlled cross-over design trial including *L. rhamnosus* GG consumption by healthy subjects demonstrated a correlation between transcriptional modulation and the observed probiotic effects. Among the human genes that were shown to exhibit enhanced transcription upon colonization with *L. rhamnosus* GG cells were those that are associated with $T_H$ 1 cell development and pathways that attenuate apoptosis, cell proliferation, and epithelial integrity [212].

Recently, a metabolic profiling study was published involving germ-free mice that were inoculated with human gut microbiota as a model for dietary interventions with probiotic *L. rhamnosus* or *L. paracasei* strains and/or galacto-oligosaccharides [213]. Apart from changes in the microbiota composition, the interventions were correlated with changes in different host-metabolic pathways including those involved in lipid profiles, gluconeogenesis, and amino acid and methylamine metabolism. Supplementation with a probiotic resulted in considerably different bile acid microbiota correlation networks, which may be linked to BSH activity expressed by lactobacilli.

Other intriguing post-genomics studies aimed at evaluation of the impact of probiotics on the gut microbiota of their host have been carried out using axenic mice as a host model [214, 215]. In this context, global transcription profiling investigations on axenic mice, that were mono-associated with *B. thetaiotaomicron* and subsequently colonized by *B. longum* ssp. *longum* NCC2705, showed that the presence of NCC2705 enhanced the diversity of polysaccharides targeted for breakdown by *B. thetaiotaomicron* including mannose and xylose-containing glycans [214]. The modifications in the transcriptional profiles of genes associated with polysaccharide utilization by *B. longum* ssp. *longum* and *B. thetaiotaomicron* may imply the existence of symbiosis between these microbes, where each species possesses a complementary set of glycosyl hydrolase activities, which when combined allow both to participate in a synergistic harvest of xylose and mannose-containing

sugars. This study was also directed to explore the molecular impact, expressed in terms of transcriptome changes of members of the human microbiota, on a murine host was analyzed by studying the host epithelium mRNA response to co-colonization by *B. longum* ssp. *longum* NCC2705 and *B. thetaiotaomicron* [214]. Interestingly, the presence of *B. thetaiotaomicron* was shown to enhance the expression of tumor necrosis factor α-centered signaling in the corresponding murine host, whereas *B. longum* ssp. *longum* was shown to enhance the expression of the cytokine interferon-γ-mediated response genes and to reduce production of host antibacterial proteins such as regenerating islet-derived-3γ and pancreatitis-associated protein.

## Bioengineering of probiotic bacteria

In recent years, bioengineering has been applied to probiotics, with the development of "designer probiotics", for instance by expressing receptor-mimic structures to circumvent pathogens by blocking crucial ligand–receptor interactions [216]. In this context, a new type of bioengineered probiotic is constituted by an *Escherichia coli* strain expressing a lipopolysaccharide coupled to a Shiga toxin receptor, which is able to bind and neutralize toxins in the lumen of the intestine, limiting adhesion of pathogens to the gut mucosa [217]. Other bioengineered probiotic bacteria include genetically modified *L. jensenii* strains, which have been used to reduce the infections of HIV [218].

Nevertheless, despite the improved functionality of such bioengineered probiotics as compared to their natural counterparts, the use of recombinant bacteria in the food chain will meet with significant consumer reluctance, while they will also have to overcome regulatory requirements imposed by governmental authorities.

## Conclusions

Most probiotic products that are currently available on the market contain the first generation of probiotic bacteria that were originally selected based on technological stability or on a variety of easily measurable phenotypes, such as ability to tolerate bile salts or survive GIT passage, but where their health-promoting activities were much less well defined and understood. This lack of knowledge has promoted a large body of research that intends to discover the precise mechanisms by which probiotic bacteria influence human health, and which would also lead to the identification of valid biomarkers that could be used for screening and validation processes of the next generation of probiotic microorganisms. All these investigations have benefited from the involvement of so-called "omics" approaches including (meta) genomics and functional analyses. In addition, molecular interaction models are currently being developed, although more are required, that monitor the activation of in vivo cellular and systemic responses in murine models and in diet intervention trials through the measurement of previously validated biomarkers. Together, the verified molecular models with functional and comparative genomics-based approaches should enable selection of the most appropriate probiotic strain, or strain combinations, for a specific health-promoting activity or enhancement of strain-processing and administration regimes that optimize the established health benefit. Ultimately, this may allow the selection of specific probiotics for a particular human genotype, analogous to personalized genomic medicine efforts.

As has been described here, the process of identification of "probiotic genes" or biomarkers for beneficial properties has just started. This has already provided the first results with the identification of a number of key molecules decorating the microbial cell surface (e.g., cell wall components, pili, and capsular surface polysaccharides) that mediate host–microbe interaction in specific human gut commensal bacteria and ultimately might be important in modulating the health status of the host. The rapidly progressing field of human intestinal metagenomics, together with the high-throughput functional screening of the metagenomic libraries (e.g., metatranscriptomics and/or metaproteomics), will no doubt aid in the identification of further probiotic biomarkers.

Regular consumption of probiotic bacteria has, in an increasing number of cases, been shown to be a crucial factor in the maintenance of gut homeostasis as well as in delivering health benefits. Thus, it is essential to identify and characterize those microbial activities and products that are required for the survival in, colonization of, and interaction with the human gut.

The probiotic concept is also changing in accordance with new insights on gut microbiota composition. As a consequence, the so far simplistic view of good and bad microorganisms will evolve in a way such that activity of a consortium or alternative species will be modulated through nutritional interventions (e.g., by supplementation of prebiotics) or otherwise, in order to elicit specific health-promoting effects. The integration of various scientific disciplines to delineate the gut microbiota composition coupled to the precise characterization of human physiological profiles will be crucial in order to improve our understanding of the complex host–microbe and microbe–microbe interactions occurring at the gut mucosal sites. This process will be essential in order to provide molecular criteria to predict the susceptibility of individuals to a particular probiotic therapy, and thus guarantee a positive outcome of probiotic application.

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