Bifidobacterium bifidum PRL2010: scientific support for its use as an effective probiotic in the infant gut

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ABSTRACT

Many bifidobacterial strains are currently exploited as probiotic, or health-promoting, bacteria by the food and pharmaceutical industries. However, the molecular mechanisms responsible for these beneficial effects on human health are generally poorly investigated and not properly understood. Therefore, the health-promoting activities of probiotic bacteria must be clinically investigated in order to satisfy regulatory agencies and to address public demands for effective and scientifically supported probiotic therapies. Here, we describe the current situation regarding *Bifidobacterium bifidum* strain PRL2010, and discuss evidence supporting its use as an effective probiotic for infants and expectant mothers.

Introduction

Probiotics are defined by the FAO/WHO as 'live microorganisms that when supplemented in adequate amounts confer a health benefit on the host'. Bifidobacteria are commonly used as probiotics in many food products, such as yoghurt, milk, infant formula and cheese, and in dietary supplements [1]. The probiotic concept was first recognized by Metchnikoff in 1908 when he realized that the consumption of some fermented foods had beneficial effects on human health [2]. Many publications have since corroborated the ability of some microbes to exert positive effects on host health, for instance by modulating the immune system, reducing cholesterol, alleviating acute gastro-enteritis, producing short-chain fatty acids (SCFAs), mitigating constipation, and reducing allergy symptoms, lactose intolerance and intestinal inflammation [3].

Probiotic bacteria have been commercially exploited by the food and pharma industries for several decades in the form of a vast array of functional food products and nutraceuticals [4].

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However, probiotics should be used very carefully, particularly in light of the numerous different microbes in the human gut (i.e., the gut microbiota) and the highly complex interactions between them, which may affect the metabolism, physiology and immune system of the host. The intestinal microbiota and its host enjoy a highly interactive and dynamic relationship that depends on the bacterial species present, as well as the diet, host genetics and various environmental factors.

Probiotics and the gut microbiota

A healthy host exists in a state of equilibrium with its gut microbiota. The microbiota contributes to host immunity, to homeostasis at the intestinal mucosa and to metabolism, while the host provides it with a constant and convenient habitat, while also keeping microbial numbers in check. This host–microbiota equilibrium may be disturbed when a pathogen enters the host intestine and interacts with both the host and the microbiota. If the host and indigenous microbiota withstand the invading pathogen, balance is restored quickly with consequent recovery of the intestinal mucosa. However in extreme cases, disruption of intestinal homeostasis as a result of disturbance of the host–microbiota balance may cause host death. If the pathogen acquires a niche for itself among the indigenous gut bacteria, it will eventually create a new equilibrium between the host and the microbiota with consequent chronic presence of the pathogen [5].

There are also temporary disturbances with long-term consequences for the gut microbiota although the pathogen has been removed.

The infant gut is thought to be sterile at birth with bacterial colonization occurring immediately after vaginal delivery. Bifidobacteria are the dominant bacteria of the human intestine during infancy [6, 7] and influence the establishment of a core set of human microbiota components that affect the physiology and the development of the innate immune system [8]. Thus, there is a critical time window after birth during which bifidobacteria-based probiotic therapy is a very promising approach to influence the current and future health of the host.

Bifidobacterium bifidum PRL2010 as a new probiotic strain for infants

Bifidobacterium bifidum was the first bifidobacterial species to be characterized at the beginning of the last century by Tissier [9], who isolated it from stool samples of a breast-fed infant. An increasing number of bifidobacterial species have since been identified and characterized, with B. bifidum, B. breve and B. longum subsp. longum in particular shown to be dominant in the infant gut [6] and thought to be vertically transmitted from the mother to the infant [6, 10]. In 2010, B. bifidum strain PRL2010 was isolated from the fecal sample of a healthy 3-month-old Italian breast-fed infant. Many scientific reports on this strain have described its ecology, physiology and genetic features, in addition to its cross-talk activities with its host and other members of the infant gut microbiota [11–28].

The ecological role of PRL2010 within the infant gut microbiota

Bifidobacteria are an early microbial colonizer of the infant gut [8]. The human gut at birth is thought to be essentially sterile but is then colonized by microorganisms, mainly bifidobacteria, which are vertically transmitted from the mother by direct mother–baby contact at birth and through breastfeeding [8]. However, following a caesarean delivery or bottle feeding, the newborn intestine may be rapidly colonized by environmental microorganisms including pathogens which can have long-term health effects on the host. A probiotic intervention at this stage of life may be crucial to prevent the establishment of a dysbiotic microbiota with its associated negative health implications.

B. bifidum PRL2010 was shown to exert an altruistic syn-trophic effect to the benefit of other healthy bifidobacterial members of the gut microbiota [25]. In vivo murine trials involving PRL2010 demonstrated that, in contrast to other bifidobacterial strains such as B. longum subsp. infantis ATCC15697 (another member of the infant gut microbiota), PRL2010 has cross-feeding properties that support the growth of other bifidobacteria, and a high interaction index (which indicates the level of microbe–microbe interaction elicited by a bacterial strain) [25]. The cross-feeding features of PRL2010 were further evaluated in in vitro tests exploring how co-cultivation of PRL2010 cells with other bifidobacterial gut isolates belonging to different bifidobacterial species enhanced the growth abilities of the latter compared with growth yields achieved when these strains were cultivated on their own. This ability of PRL2010 was observed when it was cultivated on host glycans such as mucin and human milk oligosaccharides (HMOs) [12, 13], and on plant-derived carbohydrates such as starch and xylan [24]. It has been shown that PRL2010 releases simple carbohydrates from these complex glycans which then become accessible to other members of the (bifido)bacterial community. In contrast, the latter bacteria are not able to directly metabolize these complex carbohydrates by themselves.

PRL2010 cells in the gut of mice have been shown to provoke expansion of the murine gut glycobiome, the overall genetic arsenal involved in the metabolism of carbohydrates, towards enzymatic degradation of plant-derived glycans and host-derived sugars [25]. Interestingly, B. bifidum PRL2010 influences the murine gut microbiota by enhancing the microbial groups involved in the production of SCFAs such as butyrate and propionate [25].

Another intriguing activity of PRL2010 in relation to its interaction with other members of the human gut microbiota (i.e., microbe–microbe cross-talk) is its ability to produce protein appendages, known as sortase-dependent pili, particularly when it is in its natural ecological niche of the mammalian gut [15, 22]. Notably, these extracellular structures, in addition to having a key role in host interaction (see below), also appear to be pivotal in modulating physical contacts with other bifidobacterial cells and human gut commensals by contributing to cell aggregation [21].

Genetic adaptation of B. bifidum PRL2010 to the human gut

The human gut mucosa is covered by a thick mucin layer which provides protection against injury and prevents penetration by intracellular pathogens. Mucin may also exert a

prebiotic effect by stimulating the growth of some members of the autochthonous gut microbiota [26]. The degradation of mucin, which decreases the thickness of the mucin layer and consequently reduces the protective barrier covering the intestinal mucosa, although undesirable, may have evolved as a 'host–settler mechanism'. In fact, mucin production in the human gut generally only begins several months after birth, reaching full production at about 1 year [29]. Interestingly, mucin metabolism by some human gut commensals, including *B. bifidum* (see below), may actually trigger the secretion of additional colonic mucin, thus restoring or even enhancing the thickness of the mucus layer covering the mucosa, thereby reinforcing the epithelial barrier function, which is important especially in those with irritable bowel syndrome [30]. *B. bifidum* are currently the only bifidobacteria known to metabolize and actually grow on mucins [27, 31]. Decoding of the *B. bifidum* PRL2010 genome together with proteome and transcriptome analyses allowed precise mapping of the genetic determinants responsible for the breakdown of these host glycans [27].

Recently, an extensive genomic survey of many different *B. bifidum* strains identified the so-called truly unique genes (TUGs) of the *B. bifidum* taxon (i.e., the genes found exclusively in members of this species) [32]. Interestingly, the identified *B. bifidum* TUGs included all genes involved in the breakdown of host glycans [32]. According to the Carbohydrate Active Enzymes (CAZy) system, the PRL2010 chromosome is predicted to encode members of two carbohydrate-binding module (CBM) families, CBM32 and CBM51, which bind to carbohydrate residues in the mucin core structure [33]. Interestingly, in bifidobacteria the genetic information corresponding to predicted CBM32 and CBM51 members is only detected in *B. bifidum* genomes [26].

HMOs are chemically similar host glycans, which are efficiently metabolized by *B. bifidum* PRL2010 (manuscript in preparation) [27].

In addition, detailed characterization of the genetic arsenal of *B. bifidum* PRL2010 responsible for the uptake of carbohydrates revealed a straightforward carbohydrate transport system (when compared with other bifidobacteria), which may facilitate efficient colonization and survival in the infant gut [20].

The genetic adaptation of PRL2010 to the human gut is also demonstrated by the ability of PRL2010 to adhere to human cell lines such as Caco2 and HT29 monolayers [16]. Interestingly, the adhesion index of PRL2010 cells attaching to these human monolayers is much higher than that of other (commercially exploited) bifidobacterial strains such as *Bifi-*

dobacterium animalis subsp. *lactis* BB-12 [16]. The molecular mechanism underpinning this ability of strain PRL2010 has been elucidated: sortase-dependent pili produced by the strain modulate adhesion to human enterocytes through the extracellular matrix (ECM), which is believed to instigate cross-talk with the host [15, 34]. The production of these extracellular structures seems to be particularly increased when *B. bifidum* PRL2010 cells occupy their natural ecological niche (i.e., the human gut) or when this environment is simulated under in vitro conditions, for example by cultivating PRL2010 in the presence of complex carbohydrates commonly found in the human large intestine [15, 21]. The pili encoded by *B. bifidum* PRL2010 were also shown to modulate the innate immunity of the host [15, 18]. These pili are pivotal to the developmental programming of the host immune system during infancy; they seem to help activate the host immune system and thus trigger barriers against possible bacterial infection.

Recently, other extracellular structures produced by *B. bifidum* PRL2010 cells such as teichoic acids, which may promote interactions between this strain and the host, have been identified and characterized [28]. However, these data are based solely on comparative genome analyses and further in vivo validation of their possible interaction with the host is needed.

B. bifidum PRL2010 and the host immune response

Strains belonging to the *B. bifidum* species have crucial roles in the evolution and maturation of the immune system of the host, which is still immature at birth [35]. It was demonstrated that *B. bifidum* strains, including PRL2010, in contrast to other bifidobacterial species, provoke significantly increased production of cytokine IL-17 [27, 35]. The host response triggered by *B. bifidum* PRL2010 cells was investigated using a high-throughput gene expression approach, an in vitro cell line model and a murine model [18]. The overall host response driven by PRL2010 cells can be summarized as a local pro-inflammatory response that appears to modulate the immune system. Simultaneously, PRL2010 cells attenuate the pro-inflammatory response by down-regulating some chemokines such as the heat shock proteins, while up-regulating defensin and tight junction genes [18]. Furthermore, results from ELISA assays show that exposure to *B. bifidum* PRL2010 activates the synthesis of IL-6 and IL-8 cytokines, presumably through NF-κb activation [18]. The effects of PRL2010 on mucosal integrity have recently

been examined in an in vivo murine model of ulcerative colitis. Pre-treatment with PRL2010 cells provoked increased expression of tight junction-encoding genes, which was associated with a marked reduction in all colitis-associated histological parameters [18].

Other probiotic features of *B. bifidum* PRL2010 cells

B. bifidum PRL2010 cells were able to survive passage through the gastric barrier as well as resist bile salt and pancreatic juice exposure [17], which again suggests that this strain is genetically adapted to the environment of the human gut and thus belongs to the autochthonous human gut microbiota.

Additionally, the ability of PRL2010 cells to closely adhere to epithelial cells has been shown to interfere with colonization by enteric pathogens such as *Escherichia coli* and *Cronobacter sakazakii* [16]. The proposed mechanisms of this beneficial action of *B. bifidum* PRL2010 is pathogen displacement due to the high level of attachment of PRL2010 cells to the host mucosa. Interestingly, prophylactic administration of *B. bifidum* PRL2010 cells to pregnant mothers or to infants may help to combat infection by *C. sakazakii*, which can cause necrotizing enterocolitis, and also limit bloodstream and central nervous system infections in infants [36]. Finally, *B. bifidum* PRL2010 cells also reduce cholesterol levels in synthetic growth media. This phenomenon was confirmed by in vivo trials using a murine model treated with PRL2010, where this strain appeared to assimilate cholesterol as well as convert cholesterol to coprostanol [23]. All together, these data suggest that *B. bifidum* PRL2010 has hypocholesterolaemic activity, which may occur by a direct action of PRL2010 and/or be due to modulation of members of the gut microbiota such as *Collinsella* spp., which have previously been shown to be positively correlated with total cholesterol [37].

Conclusions

Various members of the *B. bifidum* species have been reported to exert health benefits upon their human host, including antibacterial activity against pathogens such as *Helicobacter pylori* [38, 39], reduction of apoptosis in the intestinal epithelium of infants with necrotizing enterocolitis [40], modulation of the host immune system [41, 42], and alleviation of inflammation associated with chronic gut dysfunction [43, 44]. However, none of these benefits have yet been dem-

onstrated in the infant gut, although it is hoped that *B. bifidum* species can prime the immune system, enhance the mucus layer and modulate the establishment of microbiota homeostasis. Nonetheless, these beneficial bacteria need to reach and persist in the gut in a viable form. Some *B. bifidum* probiotic strains, including mmibb 75 [43, 45], BGN4 [46] and *B. bifidum* PRL2010, can survive gastrointestinal challenge [16] to colonize the intestine and affect resident microbial communities. Human probiotic bacteria can also compete against and displace pathogens. Notably, in vitro trials involving *B. bifidum* PRL2010 cells show clear inhibition of the adhesion of pathogenic bacteria such as *E. coli* and *C. sakazakii* [16], which are frequently implicated in severe gastrointestinal disease in infants.

Information is very scarce regarding the molecular mechanisms supporting the claimed probiotic actions of many probiotic bifidobacterial strains currently on the market. In contrast, there are a lot of scientific data on the genetic background responsible for interactions between *B. bifidum* PRL2010 and both the host and other members of the gut microbiota (Fig. 1).

B. bifidum PRL2010 cells can be used as a probiotic strain in infants, and also as a novel probiotic for pregnant women in order to colonize the mother's gut with *B. bifidum* PRL2010 before delivery and thus guarantee the vertical transmission of an appropriate bifidobacterial community to the newborn at birth. However, this strain needs rigorous clinical testing before meaningful probiotic claims for humans can be made.

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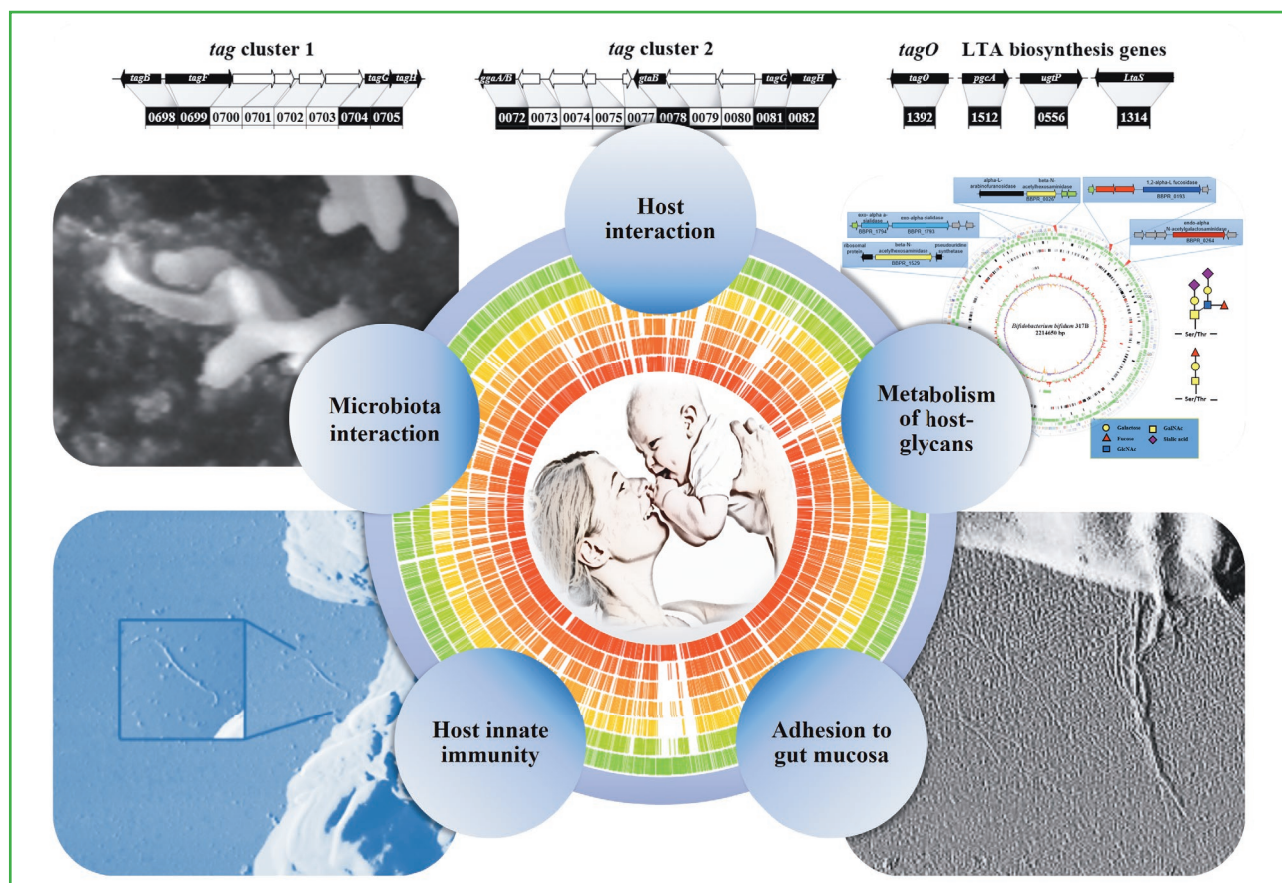
Figure 1 - The role of *Bifidobacterium bifidum* PRL2010 in the infant gut. Each circle represents a different feature of PRL2010 (potentially) involved in interactions with the host and other members of the infant gut microbiota. The cross-talk with the host and with others members of the gut microbiota may be mediated by teichoic acids as well as by the enzymes involved in the breakdown of host glycans (e.g., mucin and human milk oligosaccharides) and pili encoded by the Tad locus and Sortase-dependent pili, whose genetic locus are well characterized in the *B. bifidum* PRL2010 genome

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