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Bifidobacteria and Their Health-Promoting Effects

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ABSTRACT Bifidobacteria are members of the intestinal microbiota of mammals and other animals, and some strains are able to exert health-promoting effects. The genus *Bifidobacterium* belongs to the *Actinobacteria* phylum. *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* constitute the most abundant phyla in the human intestinal microbiota, *Firmicutes* and *Bacteroidetes* being predominant in adults, and *Actinobacteria* in breast-fed infants, where bifidobacteria can reach levels higher than 90% of the total bacterial population. They are among the first microbial colonizers of the intestines of newborns, and play key roles in the development of their physiology, including maturation of the immune system and use of dietary components. Indeed, some nutrients, such as human milk oligosaccharides, are important drivers of bifidobacterial development. Some *Bifidobacterium* strains are considered probiotic microorganisms because of their beneficial effects, and they have been included as bioactive ingredients in functional foods, mainly dairy products, as well as in food supplements and pharma products, alone, or together with, other microbes or microbial substrates. Well-documented scientific evidence of their activities is currently available for bifidobacteria-containing preparations in some intestinal and extraintestinal pathologies. In this review, we focus on the role of bifidobacteria as members of the human intestinal microbiota and their use as probiotics in the prevention and treatment of disease.

THE *BIFIDOBACTERIUM* GENUS

The genus *Bifidobacterium* is included within the phylum *Actinobacteria*, class *Actinobacteria* (high G+C Gram-positive bacteria), order *Bifidobacteriales*, and family *Bifidobacteriaceae*. Currently, this genus contains more than 50 species, including several subspecies; this number rises every year. From a metabolic point of view, the more typical trait of this genus is the catabolism of monosaccharides. Bifidobacteria use a particular route for monosaccharide degradation, the so-called fructose 6-phosphate pathway, or bifid shunt. The fructose 6-phosphate phosphoketolase (Xfp) is the main enzyme of this path. Xfp possesses a dual-substrate specificity on fructose 6-phosphate or xylulose 5-phosphate. The end metabolites of the pathway are acetate, lactate, and ethanol (1). Xfp activity on fructose 6-phosphate is the most common phenotypic test for bifidobacteria, and for many years it has been the main taxonomic test to identify this genus, since this activity is present in members of the family *Bifidobacteriaceae*, but it is not present in other Gram-positive intestinal bacteria. However, currently, DNA-sequencing-based analyses are the standard techniques for identification and typing of bifidobacteria.

The species belonging to the genus *Bifidobacterium* share high rRNA 16S sequence similarity, constituting a coherent phylogenetic unit. During the past few years,

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genome sequencing has contributed significantly to clarify the phylogenetic relationships among the different *Bifidobacterium* species. In 2002, the first bifidobacterial genome, from a strain of *Bifidobacterium longum*, was published (2). Since then, the number of publicly available bifidobacterial genomes has steadily increased, with more than 50 complete genome sequences available nowadays. In this regard, comparative genomics of the different species has shed light on the phylogeny and the evolutionary adaptation of this genus (3, 4). A recent phylogenetic analysis of bifidobacteria, based on a robust reconstruction of the phylogeny of members of this genus based on 48 genome sequences, has shown that there are seven phylogenetic groups within the genus: *Bifidobacterium adolescentis* group, *Bifidobacterium asteroides* group, *Bifidobacterium boum* group, *Bifidobacterium longum* group, *Bifidobacterium bifidum* group, *Bifidobacterium pseudolongum* group, and *Bifidobacterium pullorum* group (3). These groups partially correlate with the ecological niches from which the representative species were isolated, members of the *B. asteroides* group being the common inhabitants of the microbiota of insects, and those of the *B. pullorum* group being characteristic of birds. In relation to this, members of the *B. pseudolongum* group (especially *Bifidobacterium animalis* subsp. *lactis* strains), *B. longum* group (*Bifidobacterium breve* and *B. longum* strains), *B. bifidum* group (*B. bifidum* strains), and *B. adolescentis* group (*Bifidobacterium catenulatum*, *Bifidobacterium pseudocatenulatum*, and *B. adolescentis* strains) are often found in the human intestinal microbiota, and most probiotic bifidobacteria belong to these species.

BIFIDOBACTERIA AS MEMBERS OF THE HUMAN INTESTINAL MICROBIOTA

Bifidobacterium Species Evolution with Age, Distribution in the Bowel, and Interindividual Variability

Bifidobacteria are among the dominant bacterial populations in the gastrointestinal tract (GIT) of humans. Among the bifidobacterial species described so far, *B. catenulatum*, *B. pseudocatenulatum*, *B. adolescentis*, *B. longum*, *B. breve*, *B. bifidum*, *B. animalis*, and *Bifidobacterium dentium* are commonly detected in feces of healthy subjects (5, 6). The last two species are not rigorously considered to be autochthonous to the human bowel, being detected in fecal samples but not in mucosa-associated samples. In fact, *B. animalis* subsp. *lactis* is frequently applied in probiotic dairy products and food supplements, and its presence in feces possibly reflects a dietary origin. However, *B. dentium* has been described as residing mainly in the human oral cavity, and several studies link this species to the development of caries (7, 8).

Although bifidobacterial intersubject variability clearly exists, it seems that there are differences between fecal- and mucosa-associated *Bifidobacterium* species, with *B. longum* and *B. pseudocatenulatum* typically being isolated from both mucosa and fecal samples and *B. bifidum* being more related to feces (5, 6). In contrast, a modest diversification of bifidobacterial populations was observed between different intestinal regions within the same individual (6).

Studies of the biodiversity of the human mucosa-associated bifidobacteria by culture-independent techniques have revealed no previously identified bifidobacterial sequences that may represent novel bifidobacterial species (9). By using different approaches and techniques, it has been observed that bifidobacteria numbers and diversity decrease with age, although the fact that particular bifidobacterial types are more related with the elderly still remains obscure (10–12). That bifidobacteria achieve large concentrations during the first few months after birth is more clearly established, as explained in detail below. After weaning, bifidobacterial numbers gradually decrease and other members of the gut microbiota like *Bacteroides* and *Eubacterium* become predominant. Although it has been estimated that the bifidobacterial load in adults is close to 4% of the total fecal microbiota (13), experimental biases in PCR-based culture-independent techniques may be accounting for this modest contribution. A recent work in Japan reported higher abundancies of bifidobacteria for Japanese people (14).

Bifidobacteria Acquisition and Development in Infancy

Although bifidobacteria can be detected in the feces of adults, they form a relatively small proportion of the total bacterial community. However, they are numerous in the feces during the first year of life and are among the pioneers of the bacterial succession that occurs in the large bowel of babies when the gut microbiota begins to be established. Indeed, bifidobacteria are numerically dominant members of the intestinal microbial communities by the age of 3 to 4 months (15, 16). Bifidobacterial members are probably enriched in the bowel of the suckling infants because of the variety of oligosaccharides present in human milk. Additionally, human milk is a source of living bifidobacteria for the infant gut (17, 18). In fact it has been reported that breast-fed

infants generally harbor a more complex and numerous *Bifidobacterium* microbiota than formula-fed infants (19, 20). Human colostrum and milk contain high concentrations of human milk oligosaccharides (HMOs). Some of these are recalcitrant to digestion by the infant and thus pass to the large bowel where they can be foraged by gut bacteria. HMOs are structurally diverse and composed of several monosaccharides (glucose, galactose, *N*-acetylglucosamine, fucose, or sialic acid), and they mainly consist of a lactose core linked to units (n = 0 to 15) of lacto-*N*-biose (type I) or to *N*-acetyllactosamine (type II) (21). Bifidobacteria are among the best described gut bacteria with ability to utilize HMOs. Several species possess glycosyl hydrolases that cleave specific linkages within the HMO molecules, the best characterized being those synthesized by *B. bifidum*, which, together with *B. longum* subsp. *infantis* and *B. breve* are the most abundant species in breast-fed neonates (15, 22). Thus the ability of these species to utilize these otherwise indigestible carbohydrates explains their abundance in the breast-fed infant.

Genomic data suggest that bifidobacteria may possess particular adaptation traits to explain this ecological specialization. For example, genome analysis of *B. longum* subsp. *infantis* ATCC15697 has shown that it is an archetypical human milk-utilizing bacterium, because its genome features genes encoding enzymes involved in the breakdown of HMOs (23). *B. bifidum* is predicted to possess lacto-*N*-biosidase activity, which allows an efficient catabolism of HMOs (24). These two species are specialized toward HMO utilization, although they compete for HMOs using different strategies; *B. bifidum* has an array of several membrane-associated glycosyl hydrolases, whereas *B. longum* subsp. *infantis* is more efficient in the import and intracellular breakdown of HMOs (25). Indeed, it seems that strains from the latter species seem to have a similar HMO utilization pattern, while *B. bifidum* strains are more diverse, and some of them are not able to use fucosylated or sialylated HMOs (26). Similarly, the HMO utilization profile of *B. breve* is also variable depending on the strain, but contrary to *B. bifidum*, some strains consume HMOs decorated with fucosyl or sialic acid residues. In any case, the capability of *B. breve* to use these milk oligosaccharides also explains its high presence in the feces of breast-fed babies (27).

In contrast, the genomes of enteric bifidobacteria residing in the intestine of adult humans, such as *B. adolescentis*, do not appear to harbor genes related to the utilization of human milk components, and, instead, they contain a large arsenal of genes dedicated to the metabolism of complex carbohydrates commonly found in the adult-type diet (e.g., starch and starch-derived carbohydrates) (28–31). Until now there have been no clear relationships between type of diet (Western, Asian, Mediterranean) and the enrichment in the gut of particular *Bifidobacterium* species, but differences have been reported between different human groups and countries (14, 32).

A recent bifidobacterial diversity study based on sequence analysis of PCR amplicons of the 16S rRNA gene from infant stools from different geographical origins reinforced the notion of bifidobacteria as being a predominant component of the infant gut microbiota, which may undoubtedly influence the development of the immune system and physiology of the infant (33).

Correlations with Other Members of the Microbiota and Cross Talk Interactions

Bifidobacterium-mediated health benefits are the result of a complex dynamic interplay established among bifidobacteria, other members of the gut microbiota, and the human host. These intricate correlation patterns have not yet been fully deciphered at a molecular level; thus, efforts are currently being pursued to understand the metabolic fluxes within the gut ecosystem discerning the microbiota-host cross talk in health and disease. This will establish the basis for host health modulation through microbiome-targeted approaches, in a more precise, secure, and controlled manner (34).

Some of the first evidence of bifidobacterial capability to interact with other gut bacteria was reported in works pointing toward the existence of a correlation between reduced bifidobacterial presence within the gastrointestinal tract and the overrepresentation of enteropathogens and disease risk (35, 36). Accordingly, among the bifidobacterial proposed benefits, inhibition of enteropathogens and reduction of rotavirus infection (37) are some of their best established outcomes. Numerous *in vitro* studies have demonstrated that bifidobacteria can inhibit pathogens through the production of organic acids (38), antibacterial peptides (39), quorum-sensing inhibitors (40, 41), or immune stimulation (42) among other mechanisms, providing molecular clues of their capacity to prevent certain infections.

Another fact pointing toward the existence of a critical bifidobacteria-gut microbiota-host cross talk has been provided by the observation that microbiota establishment in early infancy seems to follow an orchestrated and organized pattern of bacterial populations succession (43, 44). The first gut colonizers, among which bifidobacteria represent a dominant group, con-

tribute to reduce the environment and produce metabolites that enable other bacterial populations to stably colonize the gut later on (45). This supports the idea that strong bacterial correlations shape the gut microbiota establishment, stabilization, and evolution (44). Indeed, the fact that HMOs are preferentially metabolized by *B. longum* and *B. bifidum* species, which are the most abundant in the breast-fed infant gut microbiota, supports the existence of critical molecular interaction microbiota-host-dietary components, conditioning bifidobacterial presence in the intestine (46). Furthermore, it is worth mentioning that significant mutualistic effects have been described between bifidobacteria and other intestinal bacteria. In this regard, using colonized germ-free mouse models, it has been shown that *Bacteroides thetaiotaomicron* is able to expand its capacity to utilize polysaccharides in the presence of *B. longum*, suggesting that resident gut symbionts are able to adapt their substrate utilization in response to bifidobacteria (47).

The microbial populations within the gut microbiota coexist in a delicate balance that can be affected by perturbations such as those imposed by antibiotic treatments, enteropathogen challenges, or dietary compounds, e.g., nondigestible carbohydrates (48). Although these perturbations affect the gut microbiota and can have negative consequences on host health, their dependence on environmental factors offers the possibility of modulating gut microbiota composition through various approaches. *In vitro* and *in vivo* studies have shown that modulating bifidobacterial levels through probiotic or prebiotic supplementation can change the overall composition and metabolism of the gut microbiota (49–53). For instance, supplementation with a *B. longum* strain augmented production of pymelate, butyrate, and biotin in a human-gut-derived microbiota mouse model (53). These effects were suggested to be mediated through yet to be deciphered cross talk mechanisms with the human-gut-derived microbiota. However, based on the evidence provided, the authors of this work hypothesized that the increase in biotin production was due to the coexistence of *B. longum* and *Bacteroides caccae*. Moreover, *B. longum* supplementation also correlated with the reduced presence of *Enterobacteriaceae* (54) and augmented representation of *Eubacterium rectale*, supporting a bifidobacterial effect on quantity and functionality of other gut microbiota members (53).

Bifidobacterial molecules, such as the exopolysaccharides present in the outer cell-surface layer, have been proven to be capable of modulating gut microbiota in *in vitro* fecal batch cultures (55) and *in vivo* mice trials (56), thus providing a molecular basis for gut microbiota-bifidobacteria cross talk. In fact, *in vitro* studies have shown that *Bacteroides fragilis* and *Faecalibacterium prausnitzii* modify their metabolism upon growth in the presence of bifidobacterial exopolysaccharides (57–59).

On the other hand, numerous studies have demonstrated the capability of prebiotics to promote bifidobacterial presence within the microbiota, correlating with other changes in the overall microbiota composition and metabolism. Thus, bifidobacterial promotion through prebiotics, including inulin, arabinoxylans, galactooligosaccharides, and fructooligosaccharides, also correlated with greater *Lactobacillus-Bifidobacterium* to *Enterobacteriaceae* ratio, and modulated short-chain fatty acid production (52, 60–62). In fact, most *in vitro* and *in vivo* evidence on bifidobacteria cross talk with other gut microbiota members has been obtained through analysis of prebiotic metabolism. Cross-feeding mechanisms between *B. longum* NCC2705 and *E. rectale* ATCC 33656 were found to be the basis of the bifidogenic and butyrogenic effects of arabinoxylan oligosaccharides (63). Similarly, recent works have provided evidence to understand the cross-feeding mechanisms between *Bifidobacterium* and *Bacteroides* species (59, 64) and *Bifidobacterium* and *F. prausnitzii* (58), respectively, which would help understand butyrogenic activity of coculture fermentations (65). These results also contribute to clarify bacterial interactions within the gut during prebiotic fermentations.

Some other works have also studied the potential cross talk mechanisms between bifidobacterial strains. Ruiz and colleagues analyzed through a proteomic approach the interaction between a *B. longum* and a *B. breve* strain (66), evidencing a significant effect on the production of carbohydrate utilization enzymes. Indeed, a more recent work proved the existence of cross-feeding mechanisms between *B. bifidum* PRL2010, a strain specialized in extracellular breakdown of HMOs, and *B. breve* UCC2003 (67). This latter strain is unable to utilize sialic acid as the sole carbon source *in vitro*, although it can grow at the expense of the residues that *B. bifidum* PRL2010 cleaves from mucins (67). In fact, a detailed analysis on glycoside utilization capabilities within the genus *Bifidobacterium* highlighted that particular species are specialized toward the utilization of specific carbohydrates, therefore suggesting that bifidobacterial species might cooperate for carbohydrate utilization within the gut (48). These facts support the use of mixtures of probiotic strains, which might provide a synergistic effect, improving their capability to exert the

desired effects on the gut microbiota and, concomitantly, on host health (68).

It is also worth highlighting that some bifidobacterial colonization traits are modulated by intestinal factors, including the presence of other microorganisms. For instance, Yuan and colleagues (69) found, using an *in vivo* model, that exposure to intestinal environment induces production of a series of proteins which are not expressed upon growth *in vitro* in *B. longum*, like the chologlycine hydrolase. Furthermore, transcription of the bifidobacterial gene clusters required for exopolysaccharide production, a molecule essential for its intestinal colonization ability (42), is strongly upregulated by intestinal factors, as evidenced following growth on fecal-based media (70). Similar observations were made regarding the bifidobacterial pilae structures. The Tad pilus-encoding cluster, which was reported to be essential for *B. breve* UCC2003 colonization of the mouse intestine, was only expressed in the mouse intestine, but not when grown under laboratory conditions (71). Whereas the specific triggering factors of these bifidobacterial traits remain to be determined, it is reasonable to hypothesize that microbial-derived molecules or metabolites, through yet to be deciphered cross talk mechanisms, may be key signaling factors. In fact, in *B. bifidum* PRL2010, pilus expression occurred *in vitro*, but it was strongly upregulated following coculture with other bifidobacterial and *Lactobacillus* strains (72).

Further evidence of the existence of a bifidobacterial-gut microbiota cross talk has been provided by the fact that individuals with different gut microbiota composition appear to respond differently to *Bifidobacterium* supplementation (73). Although the molecular mechanisms of the cross talk behind this different behavior are far from being understood yet, their comprehension would greatly help to design probiotic-based therapies that can be functional even in those subpopulations currently classified as "nonresponders" on clinical trials (74).

BIFIDOBACTERIA AS PROBIOTICS

In a healthy state of intestinal "eubiosis" there is a population of naturally occurring microbiota that helps to keep our homeostasis by maintaining or adjusting physiological processes to counteract changes. The equilibrium can be broken when internal or external factors alter this microbial community, leading to a state of "dysbiosis," often resulting in a health problem (75). A clear example of an imbalance in the intestinal microbiota is the consequence of the use of antibiotics to treat infections, which are needed to eradicate pathogens, but they also disrupt the symbionts, both mutualists and commensals, inhabiting our gut (76). This fact, together with the increased resistance to antibiotics reported in recent years, leads to the interest in the application of beneficial microorganisms, or probiotics, to help in the recovery of infections through the restoration of the intestinal homeostasis. The first international consensus definition of probiotics was proposed in 2001 by a group of experts, joined by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) (77); it has been recently accepted, with a minor grammatical correction by a Scientific Committee of the International Scientific Association for Probiotics and Prebiotics (ISAAP) (78) as follows: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host."

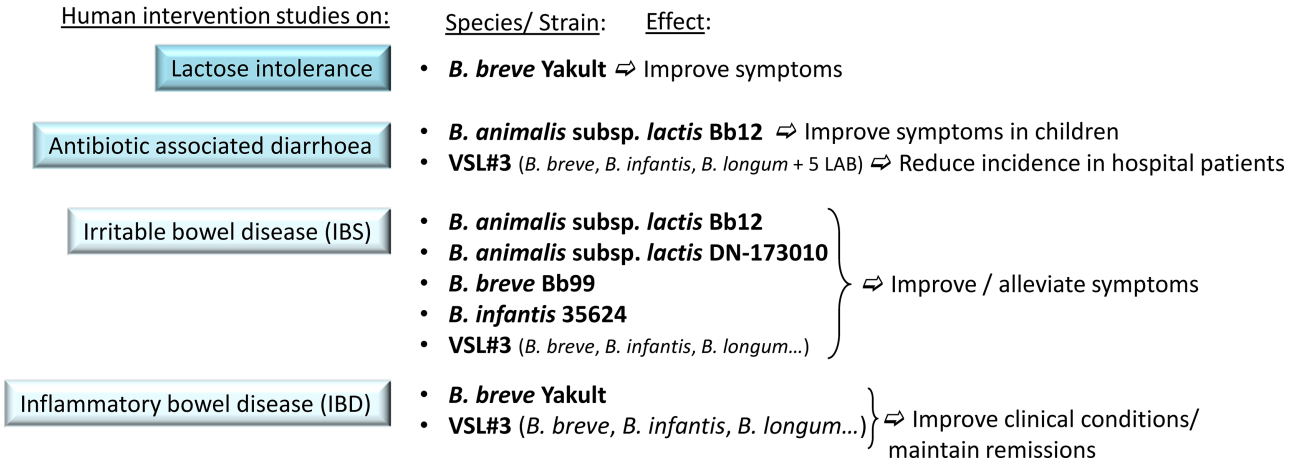
The first observations about the occurrence of certain bacteria in "normal" feces in relation to the intestinal physiology and health were established at the beginning of the last century. In 1900, the pediatrician H. Tissier found anaerobic bacteria, with a bifurcated ("bifid") shape, that were abundant in the feces of breast-fed babies and he named them *Bacillus bifidus*; afterward, he proposed the use of this bacterium for the treatment of intestinal infections (79). In the same decade, E. More reported the presence of an acid-tolerant, pleomorphic bacterium, which he named *Bacillus acidophilus*, inhabiting the intestine of infants who subsist entirely on mother's milk (80). Both authors disputed the first assertion that these bacteria constitute the dominant "flora" of the breast-fed infant (81). Simultaneously, at the beginning of the 1900s, E. Metchnikoff postulated that the long life expectancy of Bulgarian peasants was due to the higher consumption of fermented dairy products. These early observations could be considered as the starting point to link the possible benefits of intestinal bacteria and certain foods to human health; later on, after a long evolution of the definition of probiotic based on scientific research (collected in reference 82), the scientific community reached the consensus definition indicated above, which is nowadays widely accepted.

Currently, the genera most commonly used as probiotics to maintain a healthy intestinal function in humans are *Lactobacillus* and *Bifidobacterium*. Some specific species of bifidobacteria, as well as of lactobacilli, have the GRAS (Generally Recognized As Safe) status, given by the FDA. In addition, some of them, based on a long history of safe consumption in different

foods, have obtained the QPS (Qualified Presumption of Safety) mark, given by the European Food Safety Authority (EFSA); the last revision of the QPS list maintains the species *B. adolescentis*, *B. animalis*, *B. bifidum*, *B. breve*, and *B. longum* as safe biological agents intentionally added to food or feed (83). However, the probiotic efficacy of bifidobacteria showing positive effects on gastrointestinal functions after human intervention trials has only been studied for a few strains, normally supported by multinational food companies, most of them belonging to the species *B. animalis* subsp. *lactis, B. breve, B. longum*, and *B. bifidum* (75). In many cases, there is insufficient scientific evidence to support the positive effects reported for the strains, since clear biomarkers for bifidobacterial efficacy are, as yet, not identified (Fig. 1). This is the case of some studies reporting an improvement or alleviation of symptoms related to different inflammatory bowel diseases, such as ulcerative colitis (84, 85) or irritable bowel syndrome (86, 87). In addition, most bifidobacterial strains were tested in combination with other microorganisms, typically lactic acid bacteria (LAB), or with prebiotic carbohydrates, making it difficult to prove the probiotic effect of a single strain (88). Also, the vehicle used for probiotic delivery could play a relevant role. In this regard, it has been demonstrated, *in vivo*, that *B. animalis* subsp. *lactis* HN019 changes its effect on the (mice) host when the strain is given in a fermented dairy format in comparison with the unfermented milk (89).

The driving force of the global probiotic market was conducted by dairy companies to launch now well-known products during the past 20 years (90), although other non-dairy-based products have been introduced in the market as well (91). Dairy products, and specifically fermented milks and yogurts, constitute a good matrix for the delivery of bifidobacteria (92). Although (cow) milk is a rich source of nutrients for microbial growth, these are not always bioavailable; in the case of bifidobacteria, some amino acids could be limiting because of the weak proteolytic activity reported for this genus (93), thus limiting the growth in milk and milk-based matrices used to manufacture dairy products. In spite of this, it has been reported that some strains are able to grow in milk and dairy products (94–95); even more, *B. bifidum*, when growing in kefir, increased the expression of genes involved in the host-bacteria interaction, such as pili, thus helping the persistence of the bifidobacteria later on in the gut (96). Besides, it is worth mentioning that breast milk is the most suitable medium to support a high population of bifidobacteria, probably because of the high concentration of human milk oligosaccharides (26). Nevertheless, the dairy matrix is a good environment to improve bifidobacterial survival in food, allowing the delivery of the probiotic in a metabolically active state (97). The preparation of bifidobacterial suspensions in skimmed milk increased significantly their viability under simulated gastrointestinal transit with human stomach and duodenal fluids (98). The protein network of caseins could act as a protectant for the bacteria during the gastrointestinal transit. In this regard, we have visualized, by confocal scanning laser microscopy, strains of *B. animalis* subsp. *lactis* growing inside the pores enclosed by the casein network formed after fermentation by the bifidobacteria (Fig. 2). In comparison with milk, the use of fermented products containing dairy starters together with bifidobacteria could also lead to an increase in the functional benefit of probiotic foods (89, 99). As an example, the

FIGURE 1 Positive effects of some *Bifidobacterium* strains on gastrointestinal functions studied by means of human intervention studies.



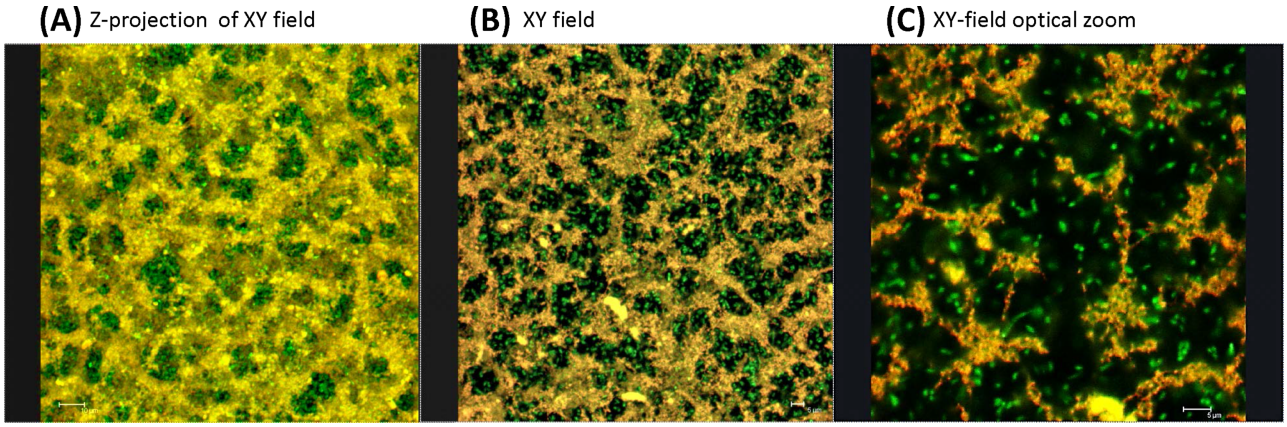


FIGURE 2 Visualization of *B. animalis* subsp. *lactis* growth in skimmed milk by using confocal scanner laser microcopy. The staining method was previously reported by Ruas-Madiedo and Zoon (167); in short, two dyes, rhodamine B (which dyes proteins) and acridine orange (which dyes nucleic acids), were added to the milk at final concentration of 0.001 and 0.002%, respectively. Afterward, stained milk was inoculated (5%) and carefully placed into high-optical-quality plastic μ-Slides (Ibidi GmbH) for direct confocal laser scanning microscopy analysis. The microplates were incubated at 37°C until they reached a pH of ≤4.5, and the confocal microscope Ultra-Spectral Leica TCS AOBS SP2 (Leica Microsystems GmbH, located in the University of Oviedo facilities) was used. Bacteria dyed with acridine orange were visualized with the laser 488 nm ion argon/krypton (green), and proteins (mainly caseins) dyed with rhodamine B were visualized with the laser 543 nm He/Ne (red) but also with the laser 488 nm. Thus, after image treatment, the bacteria are visualized in green and the casein matrix in yellow (combination red and green). The oil immersion objective 63×/1.40 combined with an amplification zoom of 1.58 was directly used (×100 magnification). Microphotographs: **(A)** a Z-projection (thickness about 10 μm) of 10 slides of an XY-field (bar, 10 μm); **(B)** a slide of an XY-field (bar, 10 μm); **(C)** an optical zoom of a region inside the XY-field showed in B (bar, 5 μm).

milk fermented by *B. bifidum* MF 20/5 has a strong angiotensin-converting enzyme (ACE) inhibitory activity due to the release of a novel ACE-inhibitory peptide (LVYPFP) from milk protein, thus giving an added functional property to the fermented product (100).

Finally, as previously stated, scientific evidence proving the efficacy of bifidobacteria has been obtained for a few strains/species; given that the beneficial effect, survival, and capability of colonization or persistence in the colon are highly dependent on the strain, the correct identification of the species/strains included in any type of food or food formulation is of pivotal relevance, and is still an issue that must be resolved in the probiotic market. Indeed, in a recent study performed with 16 probiotic products, using DNA-based methods as well as culturing techniques, only one matched its bifidobacterial label; thus, most of them differ from the ingredient list (101). Therefore, to keep the confidence of patients and consumers in probiotic products intended for use in clinical applications, or for specific human populations, more effort must be made in the correct labeling of the strains used to support the proposed claims (77).

BIFIDOBACTERIA FOR PREVENTION AND TREATMENT OF DISEASE

Scientific and medical communities are becoming more conscious about the impact of the composition of the intestinal microbiota in human health. In this sense, there are several publications demonstrating how some imbalanced microbiota populations, or particular dysbiosis, are related to a wide variety of illnesses and abnormal physiological situations, including those associated with intestinal and immunological disorders like allergy, irritable bowel syndrome, inflammatory bowel disease, obesity, metabolic syndrome, systemic lupus erythematosus, etc. (102). Interestingly, alterations in the commensal gut microbiota appear to also be related to some diseases progressing with extraintestinal manifestations, such as psoriasis, rheumatoid arthritis, or mental illnesses (103). In this regard, it has been proposed that the use of bacteriotherapy, mainly through the administration of probiotics, often as an adjuvant to medical treatments, could be helpful for the recovery of a healthy state in the framework of all these pathologies.

Bifidobacterium is one of the main genera of commensal bacteria present in the human GIT and its presence has been related to health benefits in several studies (83). Each *Bifidobacterium* species appeared to elicit different immune effects on the host, noteworthy being the ability of *B. bifidum* to expand the T-regulatory response, which may be relevant for its use in chronic inflammatory diseases (104). In this regard, supplementation of the gut microbiota obtained from a cohort of systemic lupus erythematosus patients with a *B. bifidum* strain partially corrected the altered immune response characteristic of lupus, using a dendritic cell/naive T-cell model (105). The positive effects that bifidobacteria could exert on human health have been extensively reviewed during the past few years (83, 106, 107). Because of the potential impact on human health and the GRAS, QPS status of some of the species of this genus, several strains have undergone clinical studies and are currently being used as probiotics in human nutrition. Beneficial effects resulting from the consumption of bifidobacteria on human health have mainly been associated with the prevention and treatment of intestinal diseases and immunological disorders (Tables 1 and Table 2). In this section, we will focus on the effectivity of bifidobacteria in clinical trials, and therefore neither animal models nor *in vitro* or *ex vivo* studies will be considered.

Antibiotic-Associated Diarrhea and Other Intestinal Disorders

Regarding intestinal diseases, administration of bifidobacteria has been used to improve the symptoms of lactose intolerance, mainly using strains of the species *B. animalis* subsp. *lactis* (108), or with the probiotic mixture containing the strain *B. breve* Yakult and *Lactobacillus casei* Shirota (88). The strain *B. animalis* subsp. *lactis* BB-12 has been used in the treatment of intestinal infections; for instance, it has been demonstrated that children fed with an infant formula containing this strain displayed fewer and shorter episodes of diarrhea (109). A commercial probiotic formula containing the same strain of bifidobacteria (BB-12) together with a strain of *Streptococcus thermophilus* was used satisfactorily in a clinical trial focused on the prevention of antibiotic-associated diarrhea (AAD) in infants (110). In this sense, the commercial probiotic mixture VSL#3, which contains several strains, among which are *B. breve*, *B. infantis* (or *B. longum* subsp. *infantis*), and *B. longum*, displayed an ability to reduce the incidence of AAD (111).

Clostridium difficile-Associated Diarrhea

In the case of the *C. difficile*-associated diarrhea (CDAD), a mixture containing a strain of *B. bifidum* and *Lactobacillus acidophilus* was efficient at preventing the proliferation of this pathogen after antibiotic therapy (112). In this sense, Goldenberg and coworkers (113) concluded, after an exhaustive meta-analysis (23 clinical trials, n = 4,213), that there is moderate evidence suggesting that probiotics are effective in the prevention of CDAD. Regarding *C. difficile* infection, the best methodology to reduce the associated diarrhea and to eradicate this pathogen is the fecal microbiota transplantation, which has shown up to 95.9% success, including recalcitrant and severe cases (114). In this therapy, the complete microbiota of a healthy donor is placed into the patient to modify their intestinal microbiota and to displace *C. difficile* (115). For this purpose, the feces of the healthy donor, in which the intestinal microbiota constitutes a very important part, are homogenized in saline buffer and administered to the patient in different ways, such as colonoscopy, endoscopy, or enema (116). Because it is likely that the feces from a healthy person contain between 1 and 4% bifidobacteria, it remains to be elucidated whether increasing the number of bifidobacteria by appropriate donor selection, or by targeted enrichment of this population before transplantation, could be useful, not only in CDAD, but also in other diseases through fecal microbiota transplantation.

Helicobacter pylori Infection

Regarding the use of bifidobacteria to avoid bacterial infections, it is worth mentioning their application in infections caused by *H. pylori*, a Gram-negative bacterium present in the stomach that is responsible for chronic ulceration, a pathology that has been linked to the development of gastric cancer. Although very successful in the beginning, antibiotic treatments decreased in effectiveness after years of antibiotherapy, which resulted in the emergence of antibiotic resistance in *H. pylori* strains. In this regard, the use of probiotics has been efficient in reducing *H. pylori* loads, or even definitively eradicating the pathogen. Li and coworkers (117) published a meta-analysis that compiled the clinical trial studies involving *H. pylori* treatment, and they showed that the use of probiotics could be as effective as pharmacological approaches. In addition, probiotics were revealed in the same analysis as the best treatment in terms of tolerance for the patient. Recently, Boltin and coworkers (118) reviewed the use of probiotics for *H. pylori*-induced ulcer disease; they concluded that the use of probiotics alone is not enough to eradicate *H. pylori*, but

TABLE 1 *Bifidobacterium* strains used as probiotics with demonstrated effectivity in humans trials

Effect	Strain(s)	Reference(s)
• Improve symptoms in lactose-intolerant patients	• *B. breve* Yakult + *Lb. casei* Shirota	88
	• *B. animalis* subsp. *lactis*	108
• Prevent antibiotic-associated diarrhea	• *B. animalis* subsp. *lactis* BB-12 + *S. thermophilus*	110
• Reduce the incidence of antibiotic-associated diarrhea	• VSL#3 (*B. breve* + *B. infantis* + *B. longum* + *S. thermophilus* + *Lb. acidophilus* + *Lb. paracasei* + *Lb. plantarum* + *Lb. delbrueckii* subsp. *bulgaricus*)	111
• Prevent *C. difficile*-associated diarrhea	• *B. bifidum* + *Lb. acidophilus* (Cultech strains)	112
• Prevent gastrointestinal infections	• *B. animalis* subsp. *lactis* BB-12	109
• Improve functional gastrointestinal symptoms in adults	• *B. animalis* subsp. *lactis* HN019	136
• Improve gastrointestinal symptoms in women	• *B. animalis* subsp. *lactis* CNCM I-2494	168
• Alleviate symptoms of IBS	• *B. animalis* subsp. *lactis* BB-12 + *Lb. rhamnosus* (GG + LC705) + *P. freudenreichii* subsp. *shermanii* JS	86
	• *B. breve* BB99 + *Lb. rhamnosus* (GG + LC705) + *P. freudenreichii* subsp. *shermanii* JS	142
	• *B. infantis* 35624	87, 169
	• *B. longum* 101 + *Lb. acidophilus* 102 + *Lactococcus lactis* 103 + *S. thermophilus* 104	143, 170
• Ameliorate symptoms of IBS in children	• VSL#3	144
• Improve symptoms of IBS	• *B. animalis* DN-173010 + *S. thermophilus* + *Lb. delbrueckii* subsp. *bulgaricus* + *Lc. lactis*	137, 138
• Improve clinical conditions of patients with UC	• *B. breve* Yakult + prebiotic GOS	171
• Reduce symptoms of patients with UC	• VSL#3	140, 141
• Reduce proinflammatory biomarkers in patients with UC	• *B. infantis* 35624	139
• Remission of UC in children	• VSL#3	84
• Maintain remission in recurrent pouchitis	• VSL#3	85, 172
• Reduce the pouchitis activity index	• VSL#3	173
• Prevent necrotizing enterocolitis in preterm infants	• *B. bifidum* NCDO 1453 + *Lb. acidophilus* NCDO1748	147
	• Infloran (*B. infantis*) + *Lb. acidophilus*	146
• Prevent necrotizing enterocolitis in neonate	• ABC Dophilus (*B. infantis* + *B. bifidus*) + *S. thermophilus*	148
• Reduce levels of *Helicobacter pylori*	• *B. bifidum* YIT4007	121
• Improve immune function in resected CRC patients	• *B. animalis* subsp. *lactis* BB-12 + *Lb. rhamnosus* GG	126
• Reduce cancer risk, improving epithelial barrier function	• *B. animalis* subsp. *lactis* BB-12 + *Lb. rhamnosus* GG + prebiotic	128
• Reduce postoperative septicemia in colectomy	• *Lb. plantarum* CGMCC1258 + *Lb acidophilus*-11 + *B. longum-88*	129
• Reduce postoperative infection complications	• *B. longum*	131
• Improve disease score in atopic dermatitis (AD) in children	• *B. animalis* subsp. *lactis* HN019 + *Lb. rhamnosus* HN001	153
• Improve clinical conditions in children with AD	• *B. animalis* subsp. *lactis* UABLA-12	154
• Improve AD and reduce IgE	• *B. bifidum* + LAB	155
	• *B. breve* M-16V + prebiotics	156
• Reduce allergic symptoms, reducing Th2 cytokines	• *B. animalis* subsp. *lactis* NCC2828	157
• Reduce pollinosis symptoms	• *B. longum* BB536	158
• Reduce sensitization in infants with mother suffering atopy	• *B. animalis* subsp. *lactis* BB-12 + *Lb. rhamnosus* GG	159
• Reduce eczema incidence	• *B. animalis* subsp. *lactis* BB-12	160
	• *B. animalis* subsp. *lactis* HN019	161
	• *B. breve* BB99 + LAB	162
	• *B. animalis* subsp. *lactis* AD011 + *B. bifidum* BGN4	163
• Prevent eczema incidence in high-risk children	• *B. animalis* subsp. *lactis* W52 + *B. bifidum* W23	164

it is suitable to improve the efficacy of antibiotic regimens for *H. pylori* eradication and prevent AAD.

The use of probiotics can be then considered as an adjuvant therapy for the eradication of *H. pylori* thanks to (i) their ability to stimulate mucin production, therefore limiting the adhesion of the pathogen to the gut surface; (ii) production of short-chain fatty acids and other antimicrobial substances that may reduce *H. pylori*

TABLE 2 Selection of meta-analyses and reviews about the effect of probiotic products containing bifidobacteria on certain diseases

Effect	Reference(s)
• Probiotics effective in prevention of CDAD	113
• Reducing loads or eradicating *H. pylori*	117
• Improve efficacy of antibiotics against *H. pylori* and AAD prevention	118
• Modulate microbiota composition reducing liver disease	134, 135
• Probiotics and gut microbiota role in IBS	145
• Probiotic benefit in eczema prevention	166
• Probiotics and colorectal cancer prevention	133

density; and (iii) protection against human pathogens due to host receptor competition and immune modulation capabilities (119). On the contrary, and based on other meta-analyses, some authors agree that probiotic supplementation does not improve the eradication rate of *H. pylori* (120). Most probiotics used in these studies are members of the genus *Lactobacillus*, but also some bifidobacteria species were tested as well, such as the strain *B. bifidum* YIT4007. This strain was able to reduce gastric mucosa alterations and other gastrointestinal symptoms due to a reduction in the levels of *H. pylori* (121). Other published works showed that the administration of a pretreatment with yogurt containing mixtures of bifidobacteria and lactobacilli improved the eradication of *H. pylori* (122, 123). A recent study reported an *H. pylori* eradication rate of 32.5% in adults after 10 days of administration of the commercial mix of probiotic VSL#3, which includes several probiotic bifidobacteria as mentioned above (118).

Colorectal Cancer

Probiotics have also been used to modify/modulate the microbiota of patients toward a healthy microbiota in those cases in which alterations of microbiota populations are associated with disease. In this context, probiotics have been used to modulate the microbiota in colorectal cancer (CRC). In CRC patients, it is known that the composition of the microbiota is one of the factors favoring the development of carcinogenic lesions, notably by the presence of genotoxic bacteria such as *Fusobacterium nucleatum* or colibactin-producing *Escherichia coli* (124, 125). Only a few clinical trials (involving bifidobacteria administration) have been performed in CRC, although there are promising results in *in vitro* and preclinical studies. As a whole, the main positive effects obtained in the clinical trials are the improvement of the gut environment by reducing the secondary effects of surgery and chemotherapy, notably at the level of the epithelial layer and involving tissue regeneration. In this sense, administration of *B. animalis* subsp. *lactis* BB-12 together with *Lactobacillus rhamnosus* GG and inulin improved the immune functions in resected CRC patients (126). Other works reported on the ability of bifidobacteria to induce fecal microbiota modifications in CRC patients (127) as well as to reduce some cancer risk factors by improving epithelial barrier function and reducing colorectal proliferation by commensal microorganisms (128). Recent studies showed that administration of bifidobacteria as part of a perioperative probiotic treatment reduced the rate of postoperative septicemia in patients undergoing a colectomy (129) and in colorectal liver metastases surgery (130). Moreover, Zhang and coworkers (131) showed that the preoperative administration of bifidobacteria in CRC patients reduced the postoperative infection complications through a mechanism involving maintenance of the intestinal microbiota populations, reduction on the numbers of *E. coli*, and restriction in bacterial translocation from the intestine to the bloodstream.

Chemotherapy Treatments

Chemotherapy treatments cause diarrhea and alter the normal function of the GIT, perturbing the proportions of populations conforming the intestinal microbiota. Alterations in the gut microbiome could be avoided with the use of probiotics before, during, and after the chemotherapy. In this sense, it is estimated that the complex consortia of microorganisms will be more efficient than a single strain when restoring the microbiota. Wada and coworkers (132) showed that the administration of *B. breve* strain Yakult improved the intestinal environment through the production of small-chain fatty acids favoring cross-feeding relationships among the microorganism communities inhabiting the gut of CRC patients receiving chemotherapy. Some of the following works are examples of clinical trials, but many others are indeed preclinical models aimed at analyzing the prevention and treatment ability of bifidobacteria in CRC. To expand this information, the reader is prompted to read an excellent review on the subject by Ambalam and coworkers (133). As has been mentioned, the use of probiotics in CRC is not a direct treatment for the disease, but an adjuvant therapy that would help patients avoid alterations in their gut microbiota. Bifidobacteria-containing probiotics are expected to help recovery from chemotherapy, while reducing the possibilities of septicemia after surgery. Because of the

advantages of probiotic consumption, several authors have proposed that an oral intake of probiotics should be included as a preoperative and postoperative treatment in CRC (133).

Liver Disease

Probiotics, including bifidobacteria, have become part of novel therapeutic approaches in hepatology, mainly because of their beneficial effect modulating the composition of the intestinal microbiota, a factor that can influence liver disease onset (134). The increasing interest in the use of probiotics for prevention and treatment of liver disease is related to the effect of gut microbiota in the pathogenesis of several liver complications including cirrhosis. However, the scientific evidence in this field is still controversial and further studies are required in order to include probiotics in liver treatments with a reasonable guarantee of success (135).

Inflammatory Bowel Disease and Irritable Bowel Syndrome

The positive effects exerted on human health by bifidobacteria and, in general, by probiotics, are related to their ability to modify the composition of the intestinal microbiota and their capability to modulate the immune response. Both parameters are altered in intestinal pathologies like irritable bowel syndrome (IBS), in certain physiological situations such as obesity, autoimmune diseases such as systemic lupus erythematosus, and chronic inflammatory diseases such as inflammatory bowel disease (IBD), including Crohn disease (CD), ulcerative colitis (UC), and pouchitis. Indeed, the prevalence rate of these noncommunicable diseases has increased significantly in developed countries during the past few decades (83). In these pathologies, *B. animalis* subsp. *lactis* HN019 has been able to improve the gastrointestinal function in adults (136), and *B. animalis* subsp. *lactis* DN171010 reduced IBS symptoms (137, 138). The beneficial effect associated with the immune modulation capability of bifidobacteria has been related to their ability to reduce systemic proinflammatory biomarkers. This is the case of the strain *B. infantis* 35624 that was able to reduce proinflammatory cytokines at gastrointestinal (mucosal immune system) and nongastrointestinal levels (systemic immune system) (139). The administration of other *Bifidobacterium* strains has also been efficient in alleviating IBS symptoms, such as a probiotic mixture containing *B. breve* BB99 (140) or the strain *B. animalis* subsp. *lactis* BB-12 (86). Another probiotic mixture that reduced IBS symptoms contains the strain *B. longum* 101, together with two strains of the genus *Lactobacillus* (141). Another example was the administration of VSL#3 for 6 weeks, which resulted in the reduction of IBS symptoms and the improvement of the quality of life in children (142). Further reading on the use of probiotics in IBS and the gut microbiota role can be obtained in the review by Distrutti and coworkers (143).

In IBD, the probiotic mixture VSL#3, which contains bifidobacteria of different species, was able to reduce the UC symptoms in adults (144, 145) as well as the remission of the disease in children (84).

Necrotizing Enterocolitis

Regarding prevention of necrotizing enterocolitis (NEC) in newborns, bifidobacteria has also been assayed mainly as part of probiotic mixtures. A commercial product containing a strain of *B. infantis* was able to reduce the incidence and severity of NEC in very-low-birth-weight infants (146) and in very-low-birth-weight preterm infants (147). Moreover, another study showed that a commercial probiotic product containing strains of *B. infantis* and *B. bifidum* reduced the incidence and severity of NEC in a premature neonatal cohort (148). However, some results recently recorded in literature did not support a positive effect of bifidobacteria on NEC. For instance, a phase 3 clinical trial aimed at testing the effectiveness of the probiotic *B. breve* BBG-001 concluded that there is no evidence of benefit for very preterm infants (149).

Allergic Disease

Probiotic bifidobacteria have also been proposed for the prevention of nonintestinal diseases, such as allergic disease. The prevalence of atopic eczema, food allergy, and asthma has increased during the past decade, becoming a major public health problem, and indeed these allergic disorders are one of the most common causes of chronic illness and hospital admissions (150). Allergic diseases are characterized by an inadequate T-helper immune response balance, involving mainly an overrepresentation of the Th2 response with a concomitant inability to maintain the Th1/Th2 response balance. Moreover, allergic patients usually display a reduced number of T regulatory cells (Treg) (151). During the past 10 years several studies have tried to demonstrate the influence of the intestinal microbiota on allergic processes. It is believed that this influence could be mediated through the interaction of microorganisms with the mucosal immune system. In this sense, several studies aimed to demonstrate the beneficial effects of probiotics on the prevention and treatment of allergic disease

through *in vivo* studies with animal models and human trials (152). Regarding human trials for the treatment of allergy, administration of *Lb. rhamnosus* HN001 and *B. animalis* subsp. *lactis* HN019 improved the disease scores of atopic dermatitis (AD) in children suffering from atopic eczema (153). Similar results were obtained with the administration of a probiotic mixture containing *B. animalis* subsp. *lactis* UABLA-12 that significantly improved the clinical conditions in children with AD (154). Improvement in AD scores and reduction of IgE levels associated with eczema have also been observed in children after the administration of *B. bifidum* in combination with other lactic acid bacteria (155), as well as with the strain *B. breve* M-16V combined with a mixture of prebiotics (156). The immune modulatory effect exerted by *B. animalis* subsp. *lactis* NCC2818, which reduced allergic symptoms, was mediated by a reduction in the production of Th2 cytokines (157). Moreover, a human clinical trial performed with the strain *B. longum* BB536 showed that the intake of yogurt supplemented with this strain reduced pollinosis symptoms by modulating the Th1/Th2 balance (158). Use of probiotics to improve atopic eczema needs further investigation and clinical trials to infer recommendations for allergies, especially in adults where no evidence of significant reduction in eczema symptoms has so far been demonstrated.

Regarding the prevention of allergic disease by means of probiotic intake, there are several clinical trials with promising results. In general, the duration of the probiotic intervention, rather than prenatal versus postnatal treatment, seems to be the crucial factor determining the success of probiotics. Administration of *B. animalis* subsp. *lactis* BB-12 and *Lb. rhamnosus* GG during pregnancy and lactation resulted in a reduction of the risk of sensitization of infants whose mothers suffered from atopy (159). Moreover, the strain BB-12 administered during the pre- or postnatal period reduced the incidence of eczema in an atopic dermatitis cohort (160). Another strain able to reduce the atopic eczema incidence was *B. animalis* subsp. *lactis* HN019, administered during the pre- or postnatal periods (161). Another clinical trial combining pre- and postnatal treatments showed a reduction of eczema and IgE-associated eczema when the strain *B. breve* BB99 was administered together with other lactic acid bacteria strains (162). Similar clinical trials, but with longer treatment periods, showed reductions in eczema incidence after the administration of *B. animalis* subsp. *lactis* AD011 and *B. bifidum* BGN4 (163), and a preventive effect of the incidence of eczema in high-risk children after the administration of the strains *B. animalis* subsp. *lactis* W52 and *B. bifidum* W23 (164).

However, not all the clinical trials have been successful and, for instance, no significant differences between the placebo and probiotic groups were found regarding the incidence of atopic eczema when the strain *B. longum* BL999 was administered to Asian infants (165). The World Allergy Organization (WAO) recently published their Guidelines for Allergic Disease Prevention (GLAD-P) based on the use of probiotics (166). The WAO published recommendations about the use of probiotics in the prevention of allergy, based on scientific evidence and from the results of human trials. These guidelines concluded that currently there is no evidence supporting probiotic supplementation for reducing the risk of allergy incidence in children. However, there is likely to be a net benefit using probiotics for eczema prevention, which requires further clinical trials to increase the sample size and the reliability of the results. On the contrary, in case a family history of allergy (eczema) is identified as a risk factor for children, WAO suggests the use of probiotics for pregnant women, women who breastfeed their infants, and in those infants.

CONCLUSIONS

In summary, although positive results of the use of bifidobacteria for the treatment and prevention of different diseases are abundant in scientific literature, further work is needed to improve the solidity of the scientific evidence supporting the beneficial effects of this particular group of intestinal bacteria. It is noteworthy that the health benefits exerted by bifidobacteria seem to be strain dependent, notably at the level of immunomodulation, but they are also dependent on the genetic background of the target population (77). Therefore, and despite the key contribution of probiogenomics efforts in discovering the genetic background of probiotic bacteria (4), investment in basic research is absolutely necessary to clarify the molecular mechanism behind the probiotic action, a key point to be able to define strain-specific effects. Furthermore, it is also necessary to correctly identify not only the strains, but also the pathology and the population to be targeted with probiotic interventions. Finally, for future strain selection, it would be desirable to choose appropriate probiotic strains showing promising results *in vitro* and *in vivo*, and ideally good technological properties, in order to scale up the production of future probiotic bifidobacteria at affordable costs.

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