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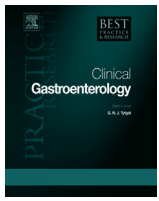

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Influence of fermented milk products, prebiotics and probiotics on microbiota composition and health

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A B S T R A C T

The gut microbiota is a highly diverse and relative stabile ecosystem increasingly recognized for its impact on human health.

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Microbiota
Mechanisms of action
Clinical evidence

The homeostasis of microbes and the host is also referred to as eubiosis. In contrast, deviation from the normal composition, defined as dysbiosis, is often associated with localized diseases such as inflammatory bowel disease or colonic cancer, but also with systemic diseases like metabolic syndrome and allergic diseases. Modulating a gut microbiota dysbiosis with nutritional concepts may contribute to improving health status, reducing diseases or disease symptoms or supporting already established treatments. The gut microbiota can be modulated by different nutritional concepts, varying from specific food ingredients to complex diets or by the ingestion of particular live microorganisms. To underpin the importance of bacteria in the gut, we describe molecular mechanisms involved in the crosstalk between gut bacteria and the human host, and review the impact of different nutritional concepts such as pre-, pro- and synbiotics on the gastrointestinal ecosystem and their potential health benefits. The aim of this review is to provide examples of potential nutritional concepts that target the gut microbiota to support human physiology and potentially health outcomes.

Introduction

Gut microbiota changes in health and disease

The human body hosts roughly ten times more microorganisms than eukaryotic human cells. These organisms are part of a complex ecosystem comprising more than 3.3 million genes and corresponding to a large spectrum of enzymatic activities leading to molecular signals and metabolites that may directly influence our health and well-being. It is clear that our diet can have a significant impact on the composition and functionality of the gut microbiota and in this way can influence our health status [1–3]. As long as recorded human history goes, a strong relation was recognized between diet and our state of health [4,5].

During the last decade the human intestinal microbiota has gained increased interest for its postulated impact on human health. Its potential implication in diseases within the gastrointestinal tract and beyond has been widely reported [6–10]. Among these, especially the immune related diseases such as allergy, inflammatory bowel disease, but also metabolic and degenerative diseases, typically increasing in industrialized societies, have been associated with altered patterns in the gut microbiota [11,12].

Even though it is difficult to demonstrate causative relationships for specific commensal bacterial species in health and disease, there is emerging evidence for certain gut microbial species being involved in disease aetiology [13–17]. In addition, in many cases, reduced microbiota diversity can be correlated to compromised health, implicating this more generic microbiota-related parameter in health and disease [18–20]. The diversity, defined as the observed number of types of species and or genes in the gut ecosystem, is generally reduced in obese individuals and IBD patients when compared to healthy controls, for example [21].

Interestingly, several microbiota transplantation studies in animals have shown that the transmission of a dysbiosed gut microbiota to their healthy counterparts is sufficient to induce the disease outcome, indicating indeed a causative relationship [22]. More recently, transplantation of a human microbiota from a lean donor to obese subjects induced an improvement of insulin-resistance confirming that microbial imbalance is not solely a secondary consequence, but can contribute to the aetiology of certain diseases [23].

In-depth genetic characterization of the microbiota has recently demonstrated that human beings can be divided in three different clusters based on their microbiota composition [24]. If distinct microbiota patterns, also referred to as enterotypes, can be recognized, one can hypothesize that based on the microbiota composition different concepts can be developed to target health benefits

for the different groups of individuals. Understanding the microbiota composition in relation to health and disease may allow targeted nutritional approaches to improve health outcomes or to reduce disease severity. Some of the possibilities to modulate the microbiota host interactions are reviewed here.

Manipulating human microbiota, the specific role of pro-, pre- and synbiotics

The human microbiota composition is the result of a bi-directional interaction between the host and its microbial consortium. Immune factors such as secretory IgA [25,26] or endogenous secretions ending up in the intestine have been shown to affect the intestinal microbiota. Besides these endogenous modulations, the microbiota composition and stability is also determined by nutrition or other factors such as antibiotics, drugs, or disease. Established high resolution tools such as 16S pyrosequencing or quantitative full metagenomic sequencing allowed a detailed assessment of the effect of different nutritional intakes on the microbiota and its gene content [27]. Non-digestible oligosaccharides are major drivers of the colonic microbiota composition by promoting the saccharolytic activity of the microbiota. As such, non-digestible oligosaccharides (NDO) have been largely used to selectively promote microbiota enrichment for lactobacilli and/or bifidobacteria and to stimulate the production of certain types of short chain fatty acids (SCFA). These NDO offer an exhaustive array of molecules with different lengths, solubility and sugar composition, and form a diverse source of substrates to alter the gut microbiota and its activities. The use of specific mixtures of NDO leading to specific changes in the gut microbiota and concomitantly confer health benefits for the host is referred to as prebiotics [28].

Elie Metchnikoff, who is considered as the founding father of the probiotics concept (concept defined below), first made reference to the properties of fermented milk (containing lactic acid bacteria) that the native long living Bulgarian populations used and linked it to increased well-being. He described the responsible microorganisms and their effects in his book, 'The Prolongation of Life', setting the base for other studies on positive effects of bacteria [29].

Later on, finding evidence of the early colonization of the GIT of infants with a new bacterium he then named *Escherichia coli*, Theodor Escherich started recommending the use of the bacteria in digestion afflictions when he discovered it in 1885 [30]. The discovery of *Bifidobacteria* in the microbiota of human milk – fed infants by Henri Tissier at the Pasteur Institute led to the recommendation to administer bifidobacteria to infants with diarrhoea already in the 1950s [31,32].

Nowadays the most common probiotic products contain bifidobacteria and/or lactobacilli, but also include other lactic acid bacteria such as lactococci and streptococci. Other promising probiotic strains include organisms of the genera *Bacillus*, *Enterococcus*, *Escherichia*, *Propionibacterium*, and the yeast genus *Saccharomyces*. Probiotics have to be isolated and characterized as pure microorganisms, and single strains or combinations of strains must be tested in appropriate human trials to give specific and measurable health benefits.

Well characterized probiotic strains for *in vivo* survival, anti-microbial and immune properties appear to provide a relevant tool for the specific modulation of the human gut microbiota. Manipulating the microbiota with probiotics can therefore be complementary to the application of prebiotic supplementation. The combination of both pre- and probiotics, also referred to as synbiotics, constitutes another nutritional tool to modulate the microbiota. Below we have reviewed literature on the health benefits of micro-organisms that are expected to exert their benefits in the complex and dynamic environment of the gut including the residing microbiota.

Microbiota and probiotics

Clinical evidence for probiotic use

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the consumer' (World Health Organization) [33]. Advantages of using probiotics are various: among them, lowering risk and therapy support for gastrointestinal diseases [34,35], enhancing the immune responses and maintaining uro-genital health are

particularly relevant for the health care system [36]. In addition, probiotics have been demonstrated to have an adjuvant effect on vaccination [37–41], acting both locally [42] and at organism scale [43].

In present times, even if the importance of probiotics is recognized in the multitude of clinical trials and other functionally based studies, the interaction between probiotics, diet and host remains only partially understood due to the niche's complexity [44,45]. The relationship between intestinal microbiota and the host has drawn both scientific and industrial interest to unravel molecular mechanisms of action [46]. Developments of this field could, in due course, support a more disease targeted and/or personalized therapeutic or prophylactic application of bacterial strains, with strong mechanistic and scientific support [47]. In view of personalized nutrition, we would need to adapt probiotics quantity and characteristics to host-responses and therefore increase treatment effectiveness. Examination of molecular mechanisms of action of probiotics has been described in several recent extensive reviews [46,48–55].

There is an abundance of clinical evidence regarding the use of probiotics in health or disease that is hard to interpret and summarize because of the heterogeneity of methods and results. The Cochrane Collaboration, a rigorous data analysis organization, has taken up the task, and published topic-organized results in several reviews over the last 5 years. Their findings regarding significance of results and safety (comprising studies until December 2011) are summarized in Table 1.

The Cochrane reviews highlight that probiotic effects are strain specific and cannot be extrapolated even at the species level. The relevance of strain specificity is supported by a recent meta-study on all gastroenterological related diseases. It summarizes 74 studies from 1970 until 2012, concluding that six of the eight diseases: pouchitis, infectious diarrhoea, irritable bowel syndrome (IBS), helicobacter pylori infection, *Clostridium difficile* infection and antibiotic associated diarrhoea showed positive effects while traveller's diarrhoea did not show significant improvement from probiotics. For necrotizing enterocolitis, probiotics were only effective for infants with a birth weight lower than 1500 g. The probiotic species and strains that were reported with a positive effect were VSL#3 (a mixture of several lactic acid bacteria and Bifidobacteria), Lactobacillus rhamnosus GG (LGG), *Saccharomyces boulardii*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Clostridium butyricum*,

Table 1
The Cochrane Collaboration meta-analysis of probiotic effects in gastroenterology conditions.

Published	Disease	Effect of probiotics	Reference
Jul-08	Active Crohn's disease	Insufficient evidence	[169]
Jul-08	Clostridium difficile-associated colitis in adults	Insufficient evidence	[16]
Oct-08	Active ulcerative colitis	Limited evidence that probiotics may reduce disease activity; not enough evidence to recommend the use of probiotics for the treatment of active UC	[170]
Jan-09	Allergic disease and food hypersensitivity	Insufficient evidence	[171]
Jun-10	Pouchitis	Oral probiotic therapy with VSL#3 appears to be effective for acute and/or chronic pouchitis	[172]
Nov-10	Persistent diarrhoea in children	Few trials with small number of participants for a clear effect, probiotics shorten the duration of diarrhoea and reduce the stool frequency on day 5.	[173]
Dec-10	Acute infectious diarrhoea	Significantly shortened duration of diarrhoea and reduced stool frequency compared to controls, no adverse effects	[174]
Mar-11	Necrotizing enterocolitis in preterm infants	Use of probiotics reduces the occurrence of NEC and death in premature infants born less than 1500 g	[175]
Nov-11	Prevention of paediatric antibiotic-associated diarrhoea (AAD)	Some probiotic strains are effective for preventing AAD	[176]
Dec-11	Maintenance of remission in ulcerative colitis	No definite conclusion, probiotics were as ineffective as drug therapy	[177]

Enterococcus faecum, *Lactobacillus plantarium*, *Bifidobacterium lactis* and *Lactobacillus acidophilus* combined with *Bifidobacterium infantis* [56]. While clinical studies propose prospective benefits for probiotics in a variety of gastrointestinal, pancreatic and liver diseases, as well as systemic disorders like obesity [57–60] and allergy [61–63] that may have gastrointestinal symptoms, the most the most convincing evidence to date remains in the areas of infection, allergy and irritable bowel syndrome (IBS).

The mechanisms by which probiotics bacteria promote health remain speculative but appear to be a combination of direct interaction with the host and indirect effects by modulation of the GI-tract microbiota. Below we have summarized several mechanisms by which microbes directly affect health and discussed the potential of ingested microorganisms to confer health benefits by affecting the gut microbiota composition and activities.

Microbiota modulation by probiotics: molecular mechanisms of action

A unique advantage of probiotic therapy is that these living organisms are their own delivery system and potentially bring a broad repertoire of anti-pathogenic and anti-inflammatory potential into play. Possible mechanisms of action may include: (1) enhancing the natural barrier function of the normal intestinal mucosa, (2) modulation of the immune system (3), antagonism of pathogens and (4) production of enzymatic activities and/or beneficial metabolites for the host [64]. Direct host–bacteria cross-talk is reported in both clinical and pre-clinical studies. While clinical studies provide mainly insights into symptoms alleviation and therapeutic and/or prophylactic efficacy, the real basis of the health-beneficial effect can only be obtained at the molecular level. Mechanistic insights have the potential to be further developed into therapeutic concepts. The main sites of reported probiotic action are the mucosal interface with its immune component, the small intestine and the colon, mechanisms characteristic for each site being further developed here.

Impact of probiotics on the gut barrier

The mucus layer, the epithelial lining of the mucosal tissues as well as the immune cells, present at sub-epithelial level, are all part of the mucosal barrier. Thus, modulation at all these levels can positively affect barrier robustness and thereby influence disease state(s). Notably, several local phenomena that are dependent on each other have been reported: an increase in gut permeability [65,66], higher mucosal inflammation [67,68] and changes in the mucus structure and quantity [69]. At a cellular level, epithelial cells are at the centre stage of the barrier effect, receiving molecular signals from the gut lumen, exchanging signals with the underlying immune cells but also communicating with the entire organism by means of circulating signalling molecules. The gut barrier plays a crucial role in the pathogenesis of numerous gastrointestinal diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), coeliac disease and infectious enterocolitis [70–73]. Therefore selecting probiotic strains that can promote the gut barrier appears to be a relevant strategy with broad impact on different types of disease.

Several studies using Caco-2 intestinal cells and mice showed that *L. rhamnosus GG* (LGG) or the probiotic mix VSL#3 could interact directly with intestinal epithelial cells and maintain the integrity of the epithelial barrier. LGG persistence capacity in the GIT was linked to its *in vivo* expression of pili containing a mucus binding domain [74,75]. In addition, LGG and its soluble factors (p75 and p40) were shown to prevent epithelial cell apoptosis *in vitro* through activating anti-apoptotic Akt and suppression of NF-κB. An additional effect observed in the study was that LGG enhances mucin secretion by epithelial cells [76], an outcome that was also observed for the Gram negative probiotic strain *E. coli* Nissle *in vitro* [77]. These effects can potentially contribute to pathogen exclusion and maintenance of homeostasis if reproducible *in vivo*. In addition, it shows that probiotic strains affect the same tissue – in this case the epithelium – by different pathways, all contributing to the preservation of the barrier effect.

In the clinical context, administration of *Lactobacillus plantarum* in the small intestine of healthy subjects induced structural changes in epithelial tight junctions, resulting in increased tight junction specific proteins occludin and zonula occludens-1. The results were reproduced in an *in vitro* model for

human intestinal epithelium – the Caco2 cell line – and this significantly protected against chemically induced tight junction damage [66]. Since loss of tight junction integrity and the resulting increased intestinal permeability to macromolecules are associated with several diseases such as IBD, IBS and coeliac disease, the data obtained with the *L. plantarum* strain provide relevant information towards an intervention in the corresponding subjects [78].

In order to better characterize how probiotics strains affect the mucosal barrier, van Baarlen et al looked at in vivo duodenal mucosal transcriptional responses of healthy adults after several probiotics interventions with strains from the species *L. plantarum*, *L. acidophilus*, *L. casei*, and *L. rhamnosus* [79,80]. The study shows that different treatments/strains induce differential gene-regulatory networks and pathways in the human mucosa. For instance, mucosal responses to *L. acidophilus* included up-regulation of IL-1β, an activator of NF-κB signalling cascade, which may drive the transcription of genes involved in lymphogenesis and B-cell maturation, thus contributing to enhancement of barrier function. *L. rhamnosus* consumption led to differential expression of genes involved in wound repair and healing, angiogenesis, IFN response, calcium signalling and ion homeostasis, relevant for the vascularization/nourishment of epithelial cells [80]. The observed changes in transcriptional networks display similarity with responses obtained with bioactive molecules and drugs, which may point to possible novel application areas for probiotics in either therapeutic or prophylactic nutritional regimes, aiming to strengthen the mucosal barrier.

Direct impact of probiotics on the immune system

Microbiota has been suggested as one of the main actors in the initiation or maintenance of GIT related immune diseases like atopic diseases and food allergy. Several consistent studies showed specific commensal bacterial species to exert a central role in inducing sIgA production [81] as well as in maintaining the homeostasis of several T cell populations like regulatory T cells (Treg), T helper 1(TH1) and 17 (TH17) [51,82,83].

Regarding probiotics strains, multiple studies have investigated their impact on sIgA. As an example, in the clinical setting, *L. rhamnosus HN001* has been shown to modulate intestinal immunity *in vivo* by increasing the levels of sIgA and other immunoglobulin secreting cells in the intestinal mucosa. However the exact mechanism by which the bacterium promote sIgA remains speculative [84–86]. *L. rhamnosus* strain GG has been shown to improve the rotavirus-specific IgA response in children with rotavirus induced diarrhoea [87].

The impact of probiotics on macrophages and dendritic cells has been shown *in vivo* for some strains such as *L. casei* Shirota and *L. rhamnosus* Lr23. Both strains trigger formation of regulatory dendritic cells and stimulate macrophages to produce TNF-α [88]. These responses are potential mechanisms by which these strains may fine-tune local immune system components and thus lowering the chances for allergy.

When looking at the molecular evidence, one of the most documented bacterial effectors on the host immune system concerns a commensal bacterial polysaccharide (PSA) from *Bacteroides fragilis* [20]. Mazmanian et al established that intestinal dendritic cells appear to be vital to these effects. They are presenting PSA to CD4+ T cells and induce naive T-cell differentiation towards TH1 and Treg cells that sustain the production of appropriate cytokine profiles in the host tissues of germ-free animals [89]. In connection to that, it was speculated that changes in the bacterial cell-wall but also single surface protein structures will influence probiotic effectiveness. The idea is supported by an *in vitro* study on *L. plantarum* cell wall components. Bron and colleagues report a link between the type of teichoic acids functional groups a cell expresses on its surface and recognition by the immune system. By the production of wall teichoic acids (WTA) bacteria can shield relevant molecules on the surface and modulate the host immune response by affecting the secretion of inflammatory cytokines by dendritic cells [90]. The discovery of specific immune system effectors offers interesting perspectives that can either steer the identification of food-grade species harbouring similar properties and capable of modulating the immune system in a similar way, or may stimulate the application of purified effectors as novel bioactive ingredients.

Direct cell to cell contact can skew immune responses as well, and can involve intermediary roles of receptor-mediated immune-recognition. Pattern recognition receptors (PRRs) are a family of receptors responsible for the detection of 'microbe associated molecular patterns' (MAMPs) or host

derived 'damage associated molecular patterns' (DAMPs) which induce innate immune signalling [91]. A large part of the cellular immune system of the host is located below the epithelium of the gut. It is continuously challenged with signals coming from the lumen. In normal healthy conditions the mix of molecules coming from the diet and the commensal bacteria is promoting homeostasis. In the case of pathogen overload, the body senses the threat by measuring the quantity and structures of the MAMPs it receives and produces a response. This early response can be manipulated with the use of bacterial strains. As an example of such interaction, *Bifidobacterium* and *Lactobacillus* strains were shown to influence immune responses of circulating immune cells (peripheral blood mononuclear cells or PBMCs) *in vitro*. When challenged with either molecules coming from pathogens or whole pathogenic microorganisms such as *C. albicans*, PBMCs pre-treated with probiotics s respond with different cytokine profiles that are skewed towards tolerance [92]. This may have a therapeutic potential, as dysregulation of the innate pathogen recognition system was linked to an increase in IBD symptoms [93].

The point has been made on the importance of baseline heterogeneity of human subjects when interpreting their response to probiotics treatment [94]. Inter-subject variation has been consolidated based on *in vivo* duodenal transcriptomics studies showing that despite conserved response patterns upon probiotic consumption can be observed, the baseline mucosal-molecular state of individual humans is considerably different and the conserved responses to probiotics may correlate with physiological perceivable consequences in only a susceptible subpopulation [46,48,80]. Although molecular responses to probiotics appear to have a significant level of conservation between individuals, the baseline variation of these same individuals may explain the distinction between responders and non-responders in probiotic trials [95], this leads to the suggestion that future use of probiotics could benefit from selection of suitable bacterial strains for administration to specific subgroups of individuals, or subsets of patient cohorts, that are stratified on basis of molecular-diagnostics [46,48,96].

Overall, the influence of probiotics on immune markers is extensively documented on basis of *in vitro* models, and several clinical trials have confirmed these observations [97–100]. Because of baseline heterogeneity of human subjects their responses to probiotic intervention and concomitant modulation of the commensal microbiota further explorative work is required to decipher these interactions and eventually optimize the clinical outcomes of probiotic intervention studies [96].

Potential health promoting metabolites produced by probiotics

On top of the molecular interactions between bacterial molecules and immune response, probiotics can exert beneficial effects through the production of bacterial metabolites. Metabolic functions of the microbiota that positively influences the host include pH changes [101], production of vitamins [102], fatty acids [103] and bile acid transformation [104], some relevant cases being discussed hereafter.

Indrio and colleagues assessed the metabolic activity of the microbiota by measuring infant fecal pH and showed similar results between infants who were human milk fed or fed with a formula fermented by BbC50 and ST065, a profile that differed from the formula without added probiotics [101]. A diet with a lower pH was correlated with protection from pathogenic gastric and pulmonary challenge in rabbits [105]. In infants, the microbiota is not mature, and the concentration and diversity of bacterial groups may not be sufficient to oppose colonization by a newly introduced member [106]. Therefore, increased protection from pathogens for infants that cannot be human milk fed is a relevant benefit.

While pH changes can result from normal growth of most lactic acid bacteria, also a variety of members of the commensal bacteria are capable of producing vitamins or degrading bile salts. These commensal microbiota characteristics are good examples of the mutualistic relationship of the mammalian host with its microbiota, especially since humans (and many other mammals) cannot synthesize many hydrosoluble vitamins. Among vitamins of the B complex, folic acid was shown to lower colon cancer risk [107,108]. Folate biosynthesis by the colonic microbiota was shown to be performed by several *Bifidobacterium* strains *in vitro* (e.g. *Bifidobacterium bifidum* and *Bifidobacterium longum subsp. infantis*) [109] and also *Streptococcus thermophilus* [110], *Bacillus subtilis* and *E. coli* [111]. The finding was confirmed *in vivo* when administration of high-folate producing strains increased fecal levels of folate in

humans, which is especially useful at this level for the homeostasis of mucosal enterocytes of the colon [108,112]. Exploiting the capacity of the microbiota to deliver vitamins may represent a more natural way of vitamin supplementation, compared to chemically synthesized vitamins, and provides additional health-benefits to fermented products while not affecting production costs [102].

As an important part of the diet, the dietary carbohydrates are known to influence microbial metabolism in the intestine. Dietary glycan degradation results mainly in the formation of short chain fatty acids and gases. Major bacterial fermentation products are acetate, propionate and butyrate, and their production tends to lower the colonic pH, also influencing the luminal capacity for pathogen antagonism. These weak acids influence the microbial composition and directly affect host health, with butyrate being the preferred energy source for colonocytes. Certain bacterial species in the colon are nourished through cross-feeding, using either the breakdown products of complex carbohydrate degradation or fermentation products such as lactic acid for growth [103].

Highly linked to metabolization of dietary lipids and providing an essential role in energy harvest, bile acids are synthesized from cholesterol in the liver and further metabolized by the gut microbiota into secondary bile acids. It has recently been proposed that the gut microbiota influences the host by modulating bile acid synthesis. The microbiota is capable of changing the bile acid pool composition in the small intestine by altering the host expression profile of genes involved in bile acid synthesis, conjugation, and reabsorption [104]. This is a clear example of commensal host–microbiota adaptation, as bile salts serve both a metabolic role and as a (secondary) bacterial signal to the host for microbiota status/luminal content composition.

An additional benefit probiotics can have on gut metabolism is degradation of lactose (the major sugar present in milk) in into D-glucose and D-galactose – the so-called lactase activity [113]. *Streptococcus thermophilus* produces a β-galactosidase (lactase) in the intestinal tract of mice and its presence correlates with a local reduction of the lactose content [114]. This shows that bacteria must be alive in order to help with lactose digestion. The resulting benefit is highly relevant for lactose intolerant patients alleviating abdominal pain, diarrhoea, and flatulence and was observed also after consumption of yoghurt [114].

Analysis of host–microbe interactions can thus contribute to the understanding of metabolic activities of single or mixes of probiotics and facilitate the development of dietary interventions for metabolically linked disorders.

Probiotics health benefits associated to microbiota modulation

As seen above, potential health benefits of probiotics appear to depend on direct effects of probiotic strains by means of secreted cell components, metabolic effects and cell to cell interactions. The impact of probiotics strains on the human GIT microbiota seems to rely on changes in the microbial network interactions while quantitative changes appear to be moderate and poorly documented. The intricacy of the niche, the unavailability of tools that go deep enough with the analysis or the management of data with many confounding variables but especially the fact that no current consensus exists about what is a healthy microbiota makes it hard to gather this type of evidence in humans.

Probiotic bacteria, generally ingested at a level of 10^{8-9} cells, reach the colon in an amount based on survival rate in stomach and small intestine. The impact of ingested probiotics on the colonic environment is essentially attributed to the fecal persistence of the ingested strains. They colonize the gut temporarily and disappear once the consumption stops. *Lactobacillus* or *Bifidobacterium* probiotic strains can be recovered at a level ranging from 10^7 to 10^9 cells per gram corresponding to less than 0.1% of the fecal microbiota [36,115].

Modulation of commensal microbiota by transiting probiotics can be expected due to antimicrobial compounds with broad spectrum such as reuterin [116] or plantaricins [117] or indirectly through modulation of the immune system or gut barrier function. The production of lactate during the transit may affect specifically the microbiota by promoting lactate users such as *Roseburia intestinalis* [118] or *Eubacterium halii* [119]. These commensals, as mentioned above, will produce different types of SCFA by secondary fermentation of lactate and other primary fermentation metabolites. The impact on the microbiota of probiotics can be leveraged through food matrix fermentation process. For instance, it has been shown that it is possible to steer *S. thermophilus* to

degrade lactose and generate galacto-oligosaccharides (GOS) [120]. GOS accumulates as kinetic intermediates of lactose hydrolysis and are hydrolysed by β-galactosidases only when lactose conversion approaches 100% [121]. The main impact of fermented products is thought to come from cellular components of bacteria, metabolites and degradation of milk proteins during the fermentation process. On top of GOS, other beneficial metabolites, such as acetic acid and lactic acid result from this fermentation process [122].

Overall, few studies have reported slight changes in the fecal microbiota associated with probiotics ingestion in humans [123–128]. One of the obvious example concerns the use of probiotics in the prevention of antibiotic-induced diarrhoea and acute infectious diarrhoea [129,130]. Meta-analyses and randomized controlled trials (RCTs) substantiate the use of organisms like *Saccharomyces boulardii* and *Lactobacillus rhamnosus GG* in the prevention of antibiotic-associated diarrhoea (AAD) that are frequently caused by outgrowth of *C. difficile*. Antibiotherapy is associated with a significant alteration of the microbiota and probiotic interventions were observed to positively affect the recovery towards a normal microbiota following the termination of treatment by preventing outgrowth of opportunistic pathogens like *C. difficile*. Similarly other gut related disease such as IBS or colics that have been associated with microbiota dysbiosis [131–133]. VSL3 has also been shown, in a different study, to significantly improve gut comfort of IBS subjects [134].

A recent intervention looking at a probiotic effects on overall microbiota composition focused on patients with diarrhoea-dominant IBS (IBS-D) treated with a probiotic mixture of *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, *Bifidobacterium breve*, *B. lactis*, *B. longum* and *Streptococcus thermophilus*. Interestingly, fecal microbiota profiling showed a more similar microbial composition in probiotics-treated patients than that of the placebo group and patient's relief of symptoms correlated with uniformization of fecal microbiota profiles. This study is one of the first to suggest that microbial community composition is more stable during the period of probiotics treatment and that it positively correlates with improvement of disease symptoms [135].

A similar effect of stabilization of local ecology of the gut was observed after daily supplementation of the diet of infants at high risk for asthma development with of *L. rhamnosus GG* (LGG) from birth until 6 months of age. The global microbiota analysis associated LGG abundance with a distinct community composition characterized by a higher diversity, also linking it to a reduced incidence of the allergic symptoms [136].

Most evidence available on the impact of probiotics microorganisms on the microbiota composition and functions has been obtained by using methods targeting specific bacterial genera like *Lactobacillus* and *Bifidobacteria* [137,138] while this type of nutrition may have very subtle influence on other relevant genera as well. For example, significant reduction of bacterial diversity of members of the *Clostridium* cluster IV and significant reduction in the abundance of bacteria involved in butyrate and propionate metabolism, including *Ruminococcus bromii*, *Eubacterium rectale*, *Roseburia sp.*, and *Akkermansia sp.* are markers of dysbiosis in ulcerative colitis (UC). Increased abundance of (opportunistic) pathogens including *Fusobacterium sp.*, *Peptostreptococcus sp.*, *Helicobacter sp.*, and *Campylobacter sp.* as well as *Clostridium difficile* were found to be associated with UC [139,140]. It remains to be established if particular species would need to be followed in specific patients or subpopulation groups. The use of new sequencing technologies will bring new insights in this direction.

Microbiota dysbiosis in immune-related disease such as allergy or IBD has been convincingly demonstrated in humans [141–146]. Thus a successful probiotic intervention may be associated with a targeted modulation of the microbiota to repress specific pathobionts or stimulate endogenous beneficial groups on top of direct molecular interaction with immune cells in the small intestine. Transiting probiotics are therefore not always expected to affect the global intestinal microbiota structure in a major way, but rather to directly modulate with the immune system and miscellaneous epithelial receptors all along the digestive tract. As a consequence low abundance but metabolically active bacteria can still be meaningful in microbiota modulation, by for example modulating existing microbiota interactive metabolic networks. All evidence taken together, probiotic strains that are able to combine specific and direct interaction with the host with transient impacts on the residing microbiota can elicit complex multifaceted but more optimal health benefits.

Microbiota and pre- and syn-biotics

The prebiotics concept

Modulation of the gut microbiota by applying specific non-digestible carbohydrates (NDO) has received a lot of interest since the introduction of the prebiotic concept by Gibson and Roberfroid in 1995 [147]. In 2008, the most recent definition of the prebiotic concept is formulated as a selectively fermented dietary ingredient that results in specific changes, in the composition and activity of the gastrointestinal microbiota, thus conferring benefits upon host health [148]. This definition focuses specifically on the gut ecosystem with its indigenous microbiota as the niche of action [149]. Other niches may be considered in the future with a similar concept, however the large intestine forms an ideal environment for microbial growth and fermentation of non-digestable dietary ingredients, since it has a slow transit time, readily available nutrients and a favourable pH [150].

The majority of scientific data have been obtained using food ingredients/supplements belonging to two chemical groups namely inulin-type fructans (ITF) and the galacto-oligosaccharides (GOS). These have repeatedly been demonstrated to selectively stimulate the growth of *Bifidobacteria* and, in some cases, lactobacilli leading to a significant change in gut microbiota composition. The concepts and their health effects have been extensively reviewed by Gibson and Roberfroid in 2010 [151]. Here we highlight in the major supposed health benefits as shown in human studies.

The supposed benefits of selectively promoting the growth of lactobacilli and in particular bifidobacteria are linked to the fact that these bacteria enact both a saccharolytic metabolism and relatively large proteolytic activities, leading to enhanced levels of lactic acid, acetate and lactate and reduced colonic pH [149]. These ecophysiological changes have been linked to an improved protection against potential pathogens [147], reduction of diarrhoea [152], improved digestion and absorption [153] and immunostimulation [154].

The prebiotic concept is of particular interest in early life, especially because human milk fed infants are dominated by bifidobacteria in contrast to infants fed cow's milk based standard formula. Human milk differs substantially from cow's milk, which is generally the basis for infant formulae (IF). While NDO are virtually absent from cow's milk, it represents the third most abundant fraction after lactose and lipids in human milk [155]. Use of a prebiotic mixture of short chain galacto-oligosaccharides (scGOS) and long chain fructo-oligosaccharides (lcFOS) (in proportions of 9:1) showed prevention of allergies and infections in newborns with effects lasting beyond the intervention period [156–158]. This finding underpins the importance of early microbial colonization and how it can influence a healthy development. Clinical data also revealed that supplementation with scGOS/lcFOS increased stool frequency and stool softness in both term and preterm infants, and similar to what is observed in human milk fed infants [159].

The adult microbiota is more complex in contrast to that of infants and is no longer dominated by *Bifidobacteria*. Nevertheless, many prebiotics cause a promotion of this genus in the colon of adults. More recently it was shown that ITF (inulin-type fructans) selectively changes the gut microbiota in obese women, leading to modest changes in key metabolites associated with obesity and diabetes [160]. Interestingly DeWulf et al showed ITF to not only selectively promote Bifidobacteria but also *Faecalibacterium prausnitzii*. *F. prausnitzii* is regarded as beneficial in IBD patients, due to its anti-inflammatory effects [161–163]. The increase of this species elicited by the consumption of prebiotics may be explained by increased levels of acetate produced by Bifidobacteria that may act as metabolic intermediate for this secondary-fermentor, and butyrate-producing organism. The recent revolution of 'omics'-approaches will define the human microbiota more and more precisely in relation to health and disease, and will help to understand how prebiotics can help in preventing or treating diseases associated with gut microbiota dysbiosis.

The synbiotic concept

A synbiotic is a combination of pro- and prebiotics. Current available combinations include bifidobacteria and fructooligosaccharides (FOS), *Lactobacillus* rhamnosus GG and inulin, and bifidobacteria and lactobacilli with FOS or inulin [164]. Although the field of synbiotics is just developing many applications have been proposed already. A few examples are presented below.

The combination of scGOS/lcFOS (9:1) and *B.breve M16-V* in a 12-week intervention in infants around 5 months of age showed reduced severity of atopic dermatitis in a subgroup of infants with elevated IgE levels but not in the whole study group. However at one year of age it was found that the synbiotic group showed attenuated use of asthma medication and lower prevalence of asthma-like symptoms in the whole study group at one year of age suggesting, long-term effects of the intervention early in life [165,166].

Fermented milk supplemented with 2 probiotic strains, *Bifidobacterium lactis* Bi-07 and *Lactobacillus acidophilus* NCFM, and a prebiotic, isomalto-oligosaccharide, was orally administered to healthy adults and mice, and immune as well as fecal bacteria analyses were conducted using the same culturing methods. The same effects on the composition of the intestinal microbiota were observed in man and mice: increases in fecal bifidobacteria and lactobacilli and decrease of fecal enterobacteria compared to control [167].

Although, probiotics can have complementary effect to prebiotics, the future opportunities of improved health benefits may lie in supplying the combination of both. Synbiotic concepts may therefore optimize the global efficiency of modulating the microbiota in a positive way.

Concluding remarks

Understanding the complexity of the gut microbiota composition and functionality, in relation to health will offer opportunities for directed approaches through either food or pharma to improve health in the general population. The microbiota can be used to develop diagnostic tools to characterize disease status or disease risk. Modulating the microbiota with nutritional concept or drugs to cure or prevent diseases seems a target within reach [168]. The general use of fecal transplantation strategies seems unrealistic for many applications, whereas defined and accepted food strategies seem more appropriate. The concept necessary to reach the optimal effects need to be determined and could vary from simple prebiotics or single probiotics strains to more sophisticated concepts that include complex mixtures of viable micro-organisms and/or prebiotics in synbiotic concepts. The target could be general well-being, or to modulate or correct endogenous host microbe interaction in a more specific way either in upper or lower parts of the intestine. It is clear that understanding the taxonomic composition of the microbiota is as relevant as understanding the functionalities of the microbiota, which is a reflection of the ecosystems capacity to interact with specific target pathways in the host organism. The development of high throughput molecular technologies for microbiota functionality characterization will certainly catalyse the discovery of new targets for nutrition interventions that can improve health outcomes also in a clinical setting.

Practice points

- Fermented milks, pro-, pre and synbiotic concepts are all relevant means for microbiota management.
- Intestinal microbiota manipulation is effective in the treatment and/or prevention of several intestinal diseases like antibiotic-associated diarrhoea or ulcerative colitis.
- If the desired health benefit of the ingested probiotic microorganism is associated to specific microbiota functionalities (for example lactose degradation), one may expect a continuous consumption may be required.
- The most important characteristics of a probiotic are: survival during the passage through the stomach and small intestine, direct interaction with the host by immune or metabolic pathways, colonization capacity and interaction with the resident microbiota.
- We suggest here the use of a targeted approach in the use of probiotics by selection of suitable bacterial strains with specific properties and the use of schemes for different patient subgroups.

Research agenda

- More insight is needed in the characterization of a 'normal' microbiota at a functional level.
- Screening for strains with a high protective potential is necessary.
- The mechanisms of action of single probiotic strains and combinations are essential for their use in the clinical practice.
- Clinical studies with better design and larger cohorts are necessary to support concepts fitting in the 'health by means of diet' concept.

Conflict of interest statement

All authors except Prof. Kleerebezem are employees of Danone Research Center for Specialized Nutrition and therefore declare potential conflicts of interest. Danone Research Centre for Specialized Nutrition in Wageningen essentially focuses on Baby Nutrition and Medical Nutrition products. Several disciplines (Milk Science, Immunology, Microbiology, Nutrition & Metabolism, Neurobiology) in life sciences and technology are represented in the research centre.

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