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Foreword

Functional Foods Forum (FFF) of University of Turku has reached an age of 10 years. The time has been full of interdisciplinary collaboration starting from food and health and terminology in the languages departments, continuing with clinical interventions studies in collaboration with the Turku University Hospital and other clinical centers, cancer research and studies on genes affecting our sensory work, and characterization of new probiotics and prebiotics as well as nutrition, flavor profiling and transfer of knowledge to SMEs. Ten years is not a long time, but through the keen collaboration and diligent work of the staff members of FFF the institute has reached an internationally recognized status.

This symposium on probiotics and prebiotics marks the 10th anniversary of the FFF. The multidisciplinary research program has involved uncovering new probiotics and prebiotics for specific uses, assessing the safety and tolerance, characterizing the properties of probiotics and prebiotics, improving the process stability of probiotics and participation in clinical intervention studies focusing on probiotic and prebiotic health effects, and most recently novel work on the nutrition economic aspects of probiotics and prebiotics. The work has resulted in collaboration over the different areas of science from languages to economics and basic microbiology and nutrition to pediatrics.

This symposium has been organized to highlight some of the areas where research has progressed during the past 10 years, to present work of some of our collaborators, and to establish a basis for future work in developing directions for new projects and collaboration.

On behalf of all of us at FFF, I would like to thank all the participants for joining forces with us for the celebration; and I hope that we shall continue to have the privilege of working with you in the future as well.

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Enhancing probiotic stability in industrial processes

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Background: Manufacture of probiotic products involves industrial processes that reduce the viability of the strains. This loss of viability constitutes an economic burden for manufacturers, compromising the efficacy of the product and preventing the inclusion of probiotics in many product categories. Different strategies have been used to improve probiotic stability during industrial processes. These include technological approaches, such as the modification of production parameters or the reformulation of products, as well as microbiological approaches focused on the strain intrinsic resistance. Among the later, both selection of natural strains with the desired properties and stress-adaptation of strains have been widely used.

Conclusion: During recent years, the knowledge acquired on the molecular basis of stress-tolerance of probiotics has increased our understanding on their responses to industrial stresses. This knowledge on stress-response may nowadays be used for the selection of the best strains and industrial conditions in terms of probiotic stability in the final product.

Keywords: probiotics; Lactobacillus; Bifidobacterium; stability; viability

During the last years, there has been an increasing commercial interest in the inclusion of probiotic strains in different products. However, most of the currently used probiotics are fastidious microorganisms, nutritionally demanding and very sensitive to environmental conditions, and often the product manufacture and storage involves processes that reduce the viability of the strains. This constitutes an economic burden for manufacturers and compromises the efficacy of the probiotic product, limiting the inclusion of probiotics in many product categories. In addition, stability, not only in terms of viability but also in terms of metabolic and functional activity, is needed to maintain the desired sensorial attributes and to provide the claimed health benefit during the whole shelf-life of the product. The application of different strategies to enhance probiotic stability and functionality has been the subject of several recent reviews (1–3).

Many different conditions present during the manufacture and storage of the product may affect the stability of probiotics; these include, among others, temperature, pH, water activity (a_w), oxygen content or the presence of chemicals, and other microorganisms (1). These factors, starting from the strain production process to the storage conditions of the final product, may have a profound effect on the stability and properties of probiotics. Moreover, stability in the product might not be enough, and it is also important that, following consumption, probiotics have to remain viable at sufficient levels during the gastrointestinal tract (GIT) transit.

Industrial processes may be modified to enhance the stability of the strains. To this regard, the selection of the best suited culture medium composition and cell-protectants may positively influence strain survival (4, 5). The chemical composition of the product may also play a role in stability, the presence of antimicrobial compounds and certain food additives being detrimental. Most probiotic strains are obligate anaerobes or facultative anaerobic microorganisms and, therefore, oxygen content is also relevant. In this case, the solutions adopted have been either to reduce oxygen permeation into the food or introducing oxygen scavengers for reducing its redox potential (6). Cells can also be physically protected by means of microencapsulation, which has been reported to improve stability of strains and to confer tolerance to GIT conditions (7).

Enhancing stress tolerance of probiotic strains

The intrinsic stress-tolerance of the strains seems to be a critical factor in the overall resistance to manufacture and storage of probiotic products. Therefore, apart to the different technological solutions, enhancing the strain...
intrinsic tolerance is of great industrial interest. Different approaches can be used to this end (3), which can be clustered into three main categories: selection of naturally occurring strains, stress adaptation of naturally occurring strains, and genetic modification of the strains. The first two approaches use already existing diversity and genetic potential, whilst the last one would imply genetic manipulation leading to a genetically modified organism (GMO).

Selection of the best suited naturally occurring strains
Different strains show large differences in their ability to cope with different manufacturing and storage conditions. Therefore, the initial screening and selection of those naturally occurring strains showing better properties constitutes a primary target for enhancing stability in probiotic products. Among the different probiotic products, yoghurts and fermented milks are the best established in the market. Sensitivity of probiotics to the typical environmental factors present in fermented milks (such as oxygen and acidic pH) is variable. A frequent phenomenon that affects the stability of probiotics in these products is the so-called postacidification or production of acid by the starter strains during refrigerated storage. An option to minimize this phenomenon is the selection of strains lacking the ability to postacidify (8). Another example is the tolerance to oxygen; aerobic conditions are present during the process of manufacture and storage of probiotic products, and thus aerotolerance is a desirable trait for industrial strains. To this regard, the species *Bifidobacterium animalis* subsp. *lactis* is more resistant to stress factors, including oxygen, than other *Bifidobacterium* species being the most frequently used species (9). It has also been suggested that exopolysaccharide (EPS)-producing strains may show better tolerance to stress (10), and, therefore, EPS-producing strains could be initially selected.

Adaptation of naturally occurring strains
Probiotic strains can be adapted to better tolerate stressful conditions; however, their adaptation capability is limited by their genomic complement. Lactic acid bacteria have adapted to nutrient-rich environments through evolution. During this process, they have lost many genes, which resulted in small size genomes. This phenomenon, know as *reductive evolution* (11), has limited the genomic potential of these microorganisms. Nevertheless, there is old evidence that selected strains can adapt to stress (12) and strain adaptation has been widely applied. Three main approaches have been used to this end: stress pretreatments, mutagenesis, and selective pressure. Of these, the last two involve changes in the genetic content of the strain, whilst the first one is limited to physiological changes.

Stress pretreatments
It has been repeatedly shown that subjecting the strains to sublethal stress before exposing them to the harsh conditions found during elaboration processes influences the stability of the microorganisms during product manufacture and storage. To this regard, acidity and heat are some of the main limiting factors affecting strain survival and it has been shown that preexposure to stress improves the subsequent survival under acidic conditions (13) and after heat-shock (14).

Mutagenesis
Random mutagenesis induced by UV light or chemicals has been commonly used in microbiology to obtain strains with altered characteristics or to study different microbial processes. This approach has been successfully used in probiotics research for, among others, increasing the stability of *B. animalis* ssp. *lactis* in low pH products (15). This approach can also be used to improve the stability of the product in terms of sensorial attributes, for instance, metabolic activity of bifidobacteria during manufacture or storage of food is often not desirable, since the production of large amounts of acetic acid may result in undesirable flavors. However, the lack of metabolic activity would compromise bifidobacterial stability. Recently, new strains of bifidobacteria producing low amounts of acetic acid have been obtained by UV mutagenesis; these strains would make possible the elaboration of stable and organoleptically acceptable products based exclusively in bifidobacteria (16).

Selective pressure
Resistant derivatives may be obtained by exposing sensitive strains to a selective pressure (stress factor). Very often, these derivatives present a stable phenotype and cross-resistance to other stresses, which is advantageous in terms of stability in industrial processes. This approach has been used, in both lactobacilli and bifidobacteria, to obtain derivative strains with improved heat (17, du Toit et al., unpublished observations), oxygen (18), or acid (19) tolerance. These adapted strains represent a promising option for the development of stable probiotic products. Some recent studies show that the addition of stress-resistant probiotics do not promote remarkable changes in the behavior of starters during manufacture, nor any detrimental effect on the sensory properties of fermented milks (20).

In general, the use of these stress-resistant strains can be useful for improving stability in industrial processes; however, care should be taken as the stress adaptation may alter other properties of the strain (21).

Genetic modification of the strains
An alternative for increasing stability is the use of genetic engineering. However, especially in Europe, GMOs are
not well accepted by consumers. Basically there are two different approaches than can be followed: (1) modify the expression/production of genes already present on the microorganism (homologous expression) and (2) introducing genes from other microbial species (heterologous expression). Examples of both alternatives do exist, for example, overexpression of a chaperone in Lactobacillus paracasei was found to increase the strain stability (22), whilst the heterologous expression of the betaine uptake system (BetL) from listeria into Lactobacillus salivarius was found to increase tolerance to acid and high osmolar conditions (23).

Future perspectives
As stated above, different strategies have been used for improving stability of probiotic products. However, very often this has been empirically done, without paying much attention to the mechanisms responsible for stress tolerance. During recent years, with the development of ‘omic’ techniques, the molecular basis of stress-tolerance has been extensively studied (24). Future research in this field must take advantage of the enormous amount of data generated, mainly from proteomic and transcriptomic studies, on stress-response to develop fast and easy methods for the selection of the strains/industrial conditions more suitable in terms of probiotic stability in the final product.

It is also especially important to take into account that the technology applied to improve probiotic stability may modify the functionality of the product. It has been reported that manufacturing processes and matrix may affect the functionality of the strains (25, 26). This underlines the need for stability also on terms of functional properties to ensure that probiotics confer the expected health benefit.

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Fructophilic lactic acid bacteria inhabit fructose-rich niches in nature

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Fructophilic lactic acid bacteria (FLAB) are a special group of lactic acid bacteria (LAB), which prefer fructose but not glucose as growth substrate. They are found in fructose-rich niches, e.g. flowers, fruits, and fermented foods made from fruits. Quite recently, they were found in the gastrointestinal tracts of animals consuming fructose, which were bumblebees, tropical fruit flies, and *Camponotus* ants. These suggest that all natural sources that are rich in fructose are possible their habitats. *Fructobacillus* spp., formerly classified as *Leuconostoc* spp., are representatives of these microorganisms, and *Lactobacillus kunkeei* has also been classified as FLAB. They share several unique biochemical characteristics, which have not been found in LAB inhabited in other niches. FLAB grow well on fructose but very poor on glucose. These organisms grow well on glucose only when external electron accepters, e.g. pyruvate or oxygen, are available. LAB have been shown to have specific evolution to adapt to their niches and have several niche-specific characteristics. FLAB must have fructophilic evolution during adaptation to fructose-rich niches. FLAB are unique food-related LAB, suggesting a great potential for future food and feed applications.

Keywords: Fructophilic lactic acid bacteria; fructose-rich niches; *Fructobacillus*; *Lactobacillus kunkeei*; adaptation electron acceptor
conditions over anaerobic conditions for their growth. The organisms cannot grow under anaerobic conditions if available carbon source is glucose and thus possibly colonize the intestinal tract of vegetarian subjects.

Biochemical characteristics
Fructophilic lactic acid bacteria have very unique biochemical characteristics when compared to other LAB. FLAB grow very well on fructose, but growth is very poor on glucose (Fig. 2), although glucose is the most general substrate for almost all LAB. FLAB produce mannitol from fructose, and they grow well on glucose in the presence of pyruvate or under aerobic conditions (Fig. 2). These results mean that FLAB need electron acceptor for glucose metabolism, and pyruvate and oxygen are used as electron acceptor. These organisms can grow well on agar medium containing glucose as sole carbon source under aerobic conditions but hardly grow under anaerobic conditions (1, 12). These characteristics easily distinguish FLAB from other LAB. Facultatively FLAB species, L. florum, grows well on fructose and on glucose in the presence of electron acceptor as same as other FLAB. However, this organism is differentiated from FLAB based on its growth characteristics on glucose without electron acceptor, meaning that L. florum can grow on glucose but at delayed growth rate. Based on the carbohydrate fermentation test, FLAB can metabolize only a limited number of carbohydrates (1). They are only two to five. Most of the Fructobacillus species can metabolize only fructose, glucose, and mannitol. Metabolism of fructose was done within 1 day, and that of glucose took 2-4 days (1).

All FLAB produce gas from glucose, indicating that they are obligately heterofermentative LAB. However, FLAB are differentiated from other obligately heterofermentative LAB by production of acetate instead of ethanol (1, 5, 7). Obligately heterofermentative LAB normally use phosphoketolase pathway for glucose

Fig. 1. Phylogenetic relationship of FLAB (shown in bold) and phylogenetically related LAB.

Fig. 2. Growth characteristics of Fructobacillus tropaeoli F214-1T on fructose (●), on glucose (▲), on glucose in the presence of pyruvate (◆), and on glucose under aerobic conditions (■).
metabolism. Acetyl phosphate arisen from cleaving of xylulose-5-phosphate by phosphoketolase is converted to ethanol via acetyl-CoA and acetaldehyde in the pathway. AdhE protein coded on adhE gene is needed for the conversion (19). However, Fructobacillus spp. do not have adhE gene (unpublished data). Because of this absence, Fructobacillus spp. produce acetic acid instead of ethanol. Conversion of acetyl phosphate to ethanol is an important step to oxidize NADH to NAD, for which NADH is produced in upper stream of the phosphoketolase pathway. Thus, the missing step leads to shortage of NADH for glucose metabolism in the pathway. Therefore, FLAB would need electron acceptors for glucose metabolism. This finding correlates well with their growth characteristics.

Niche-specific characteristics of LAB
Lactic acid bacteria can be found in various niches, and several studies suggested that LAB have adapted to their niches for their lives (20, 21). Diary LAB, e.g. Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus helveticus, Streptococcus thermophilus, prefer lactose over glucose as growth substrate and are well known to have proteolytic activity (22). Proteolysis is a very important characteristics for dairy LAB as free amino acids are scarce in milk. Gastrointestinal LAB are usually bile tolerant and produce various bacteriocins to compete with other bacteria (23). As FLAB inhabit in fructose-rich niches, they might have lost ability to metabolize glucose during their adaptation to their niches. Genome sequencing of FLAB might clear the evidences of their evolution.

Fructophilic lactic acid bacteria are unique food-related LAB, as described above, suggesting a great potential for future food and feed applications.

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Probiotic viability – does it matter?

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Probiotics are viable by definition, and viability of probiotics is often considered to be a prerequisite for the health benefits. Indeed, the overwhelming majority of clinical studies in the field have been performed with viable probiotics. However, it has also been speculated that some of the mechanisms behind the probiotic health effects may not be dependent on the viability of the cells and, therefore, is also possible that also non-viable probiotics could have some health benefits. The efficacy of non-viable probiotics has been assessed in a limited number of studies, with varying success. While it is clear that viable probiotics are more effective than non-viable probiotics and that, in many cases, viability is indeed a prerequisite for the health benefit, there are also some cases where it appears that non-viable probiotics could also have beneficial effects on human health.

Keywords: probiotics; non-viable probiotics; viability; efficacy; mechanisms

Viability is an inherent property of probiotics since the current definition of probiotics, issued by the Joint FAO/WHO Working Group (1), defines that probiotics are ‘live microorganisms which, when administered in adequate amounts, confer a health benefit on the host’. Therefore, by definition, viability is an essential requirement for probiotics. This does not necessarily implicate that viability is an essential requirement for the health benefits conferred by probiotics or their derivatives. As indicated later, there may be situations in which the health benefits of probiotics do not necessarily depend on the viability status of the cells – despite that it is widely acknowledged that, in general, viable probiotics are more effective than non-viable probiotics and that the health effects of viable probiotics have been explored to far greater extent than the potential health effects of non-viable probiotics. Research (and reviews) on this topic are hampered by lack of satisfactory terminology. No proper term or definition exists for the non-viable forms of probiotics. Terms such as non-viable probiotics and inactivated probiotics have been used, but these terms are self-contradictory since the word ‘probiotic’ as such indicates viability. In this discussion, the term non-viable probiotics is used in the lack of better terminology.

Mechanisms of probiotic health effects – is viability essential?
While probiotics have been linked with different health benefits in a plethora of clinical trials with a variety of different outcomes, study populations, and probiotic ingredients, it is acknowledged that, in most cases, the exact mechanisms of the health benefits are not fully understood. Mechanistic studies have provided several plausible and possible modes of action, but in many cases, it has not been possible to identify direct and undisputed cause-effect relationships. In many cases, there are several potential mechanisms that could explain a certain clinical health benefit, and it has not been easy to exclude the other potential mechanisms in favour of a single mode of action. Perhaps, this is only natural, since a clinical health benefit may be a combined result of a number of different mechanistic effects occurring at cellular and molecular levels. The potential health efficacy of non-viable probiotics depends on whether the mechanism of the probiotic health effect itself is dependent on the viability of the cells. Given that there are multiple potential mechanisms, it is clear that this consideration should be taken case-by-case.

Adhesion to host tissues is thought to facilitate the host-microbial interactions such as the effects of microbes on the immune system of the host. Therefore, adhesion may be a key determinant for probiotic efficacy. In the gut, administered probiotics are clearly outnumbered by the resident gut microbiota. As such, this may reduce the chances of probiotics for having a major effect on the host health – however, adhesion to mucosa may change the balance in the favour of probiotics locally and temporarily. Thus, at mucosal level, probiotics may become a major member of the local microbial population and become an important effector of host-microbial interactions. This may be particularly relevant in the small intestine, where the resident microbial numbers are
smaller than in the colon. The effect of viability on adhesion is not fully understood and may be strain dependent (2). Some reports suggest that viable and non-viable lactobacilli are equally adherent to intestinal mucus (3). The adherence may be dependent on the way by which the cells have been killed; one study suggested that heat-killing and protease treatments were detrimental to the ability of probiotics to adhere to human mucus, but other means of cell killing had no effect (4). An in vivo mouse study suggested that heat-killing of lactobacilli affects the localization of the cells in the intestine; viable bacteria were reported to be located in the Peyer’s patches and lamina propria shortly after administration to mice, whereas most heat-killed bacteria were located in the lumen and were rapidly cleared (5). While adhesion to host tissues may be equally efficient between viable and non-viable bacteria, prolonged colonization in the mucosa obviously requires formation of a viable colony. Production of antimicrobial compounds is one potential mechanism of probiotic action against pathogens and clearly a property of viable bacteria only. However, in addition to in situ production in the intestine, antibacterial compounds may also be produced during manufacturing process and then used as bacterial lysates or extracted ingredients. It has also been suggested that heat-killed lactobacilli may inhibit pathogen adhesion to host tissues by competitive exclusion (6).

Reduction of gut permeability is another potential mechanism of probiotic action, which has been reported for several viable probiotics, although mainly in cell cultures or in animal models. The molecular mechanisms by which the integrity of the epithelial layer is improved are not fully understood. It is known that production of short chain fatty acids such as acetic acid improves the epithelial integrity locally. Clearly, in situ production of short chain fatty acids is a property of a viable cell only. While research assessing the efficacy of non-viable probiotics is minimal, some studies have suggested that inactivated lactobacilli (7) and cell-free supernatants of probiotics (8) may improve epithelial integrity.

Interactions between probiotics and host immune system have been investigated in numerous studies with viable probiotics, but in many cell culture studies, non-viable probiotics have also been used. Probiotic cell components associated with in vitro immunomodulatory properties include cell wall extracts (9), lipoteichoic acids (10), bacterial DNA (11, 12), and S-layer proteins (13). Some clinical studies have also suggested that non-viable probiotics can modulate human immune system, e.g. by enhancing salivary IgA production (14) and by modulating host T-cell responses (15) and gene expression (16). Limited number of in vitro and animal studies have directly compared the effects of viable and inactivated probiotics on innate immunity, and in many cases, these have been found to be equally effective (17–19). A study by Gill and Rutherfurd (20) suggested that viable and killed cells of Bifidobacterium lactis HNO19 were able to enhance cell phagocytic responses in mice peripheral blood cells, but only viable cells increased the phagocytic activity of peritoneal cells. In some studies, viable probiotics have proved to be more effective than non-viable probiotics (21–23). In the case of adaptive immunity, most studies comparing the two have favoured viable probiotics (5, 20, 24–26). However, one study suggested that both viable and killed Lactobacillus cells are able to modulate the phenotype and functions of human myeloid dendritic cells (27).

In conclusion, many potential mechanisms of probiotic action are clearly dependent on cell viability and activity, but there is preclinical evidence suggesting that some mechanisms associated with probiotics may not be directly dependent on cell viability. These include adhesion to host tissues and modulation of innate immune responses. However, in vivo situation may be different and viability may be an indirect determinant of the health effect, since viable probiotics may be more likely to reach the site of action in the first place and remain at the site long enough to confer a health benefit.

Clinical benefits of probiotics – is viability essential?

Probiotic microbes have been linked with a range of beneficial effects on host health. By far, most of the health efficacy documentation has been generated using viable probiotics, and there are too few data to make firm conclusions on the clinical efficacy of non-viable probiotics. Nevertheless, some studies have been carried out using different non-viable probiotics.

Gut health is the most important target for probiotics. Prevention and treatment of different forms of diarrhoea is one of the most successful and best documented health benefits of viable probiotics, but efficacy studies with non-viable probiotics are rare. One study suggested that a treatment with heat-killed Lactobacillus acidophilus LB was effective, even more so than a treatment with viable non-specified strain of L. acidophilus (28). One study compared viable or heat-killed Lactobacillus rhamnosus GG and found no difference in their effect on diarrhoea duration, but the study lacked a proper placebo group (29). Ouwehand and Salminen (30) have earlier concluded that both viable and non-viable probiotics may be useful for short-term treatment or prophylactic treatment of diarrhoea, but viable probiotics are necessary for an enhanced immunological response. Irritable bowel syndrome is a popular target for probiotic research, but to date, the research has focused almost exclusively on viable probiotics. However, in one clinical study, heat-inactivated cells were used as controls for viable cells (31). The administration of the viable product resulted in subjective improvement of the symptoms in 80% of the

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patients, compared to 40% in the control group, suggesting that viable probiotics may be more efficient in the treatment of irritable bowel syndrome. While no clinical data suggest that probiotics alone would be efficient in eradicating Helicobacter pylori, both viable and non-viable probiotics have been reported to increase the eradication rates of a standard anti- H. pylori regimen (32, 33). Some studies have concluded that both viable and non-viable probiotics are equally effective in the treatment of H. pylori infections (34), which others have highlighted the importance of viability (35, 36).

Improvement of lactose digestion by probiotics deserves special attention in the context of viability. Most studies comparing the efficacy of live and dead lactobacilli in improving lactose digestion have been performed using yoghurt starter cultures, not probiotics. Most of the clinical studies comparing live and pasteurized yoghurt suggest that viable cells are more effective in improving lactose digestion (37, 38). However, cell viability as such may not be the critical factor for the efficacy. In one study, it was concluded that, to improve lactose digestion, the bacteria need not to be alive, but intact cell walls are required to protect the active β-galactosidase during gastrointestinal passage; the efficacy of pasteurized bacteria was low, but the effect of bacteria killed with gamma irradiation was similar to the effect of viable bacteria (39).

Prevention and treatment of allergic disease has been a popular target of probiotic research, and some studies have also included non-viable probiotics. In one small trial comparing viable and heat-inactivated L. rhamnosus GG in the management of infant atopic eczema and cow’s milk allergy, the latter were associated with increased gastrointestinal symptoms (40). Moreover, one study reported fewer subjective allergy symptoms in adults consuming yoghurt containing viable bacteria compared to subjects consuming heat-inactivated yoghurt (41). On the other hand, certain reports have suggested that both viable and non-viable probiotics may be useful in the treatment of allergic rhinitis (42, 43). It is possible that probiotic viability is more important in the management of eczema compared to the management of allergic rhinitis.

Efficacy of probiotics in prevention and supportive treatment of cancer is challenging and far from elucidated. Nevertheless, some early reports have suggested that heat-killed Lactobacillus casei Shirota could be useful in the treatment of carcinoma of the uterine cervix (44, 45) and secondary to lung cancer (46). In one preclinical study, viable L. casei was found to be more effective than heat-killed L. casei in the prevention of secondary tumours in preimmunized mice (47). On the other hand, heat-killed lactic acid bacteria are more effective than viable bacteria in the binding of aflatoxin, a potent dietary carcinogen (48).

Conclusions

Viability is an inherent property of probiotics, since the current definition of probiotics includes a requirement of viability. The definition of probiotics also includes a requirement of a health benefit. Probiotic viability has traditionally been thought to be a prerequisite for a health benefit. Indeed, in most cases, viable probiotics have proven to be more effective than inactivated probiotic products. Most importantly, the overwhelming majority of the clinical health efficacy research has been carried out with viable probiotics. Nevertheless, depending on the mechanism of action, there may be situations in which the health effects of probiotics are not dependent on the viability status of the cells, and there are some clinical reports suggesting efficacy of products containing inactivated probiotics. The research focussing on the importance of viability of probiotics is further complicated because – in a manner similar to other microbes – the viability of probiotics is not a simple on/off situation. For example, during storage in fermented probiotic products, part of the microbial population may become ‘dormant’, while other parts of the population may be already dead or still fully active and viable (49). The relevancy of these different subpopulations on the health efficacy of probiotics is unknown. There may also be a need to redefine the concept of viable in this context as several of the gut bacteria are viable but not culturable.

Conflict of interest and funding

The author is employed by DuPont Nutrition and Health, a manufacturer of probiotics.

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Kiwifruit (Actinidia deliciosa) changes intestinal microbial profile

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Background: Kiwifruit is high in pectic polysaccharides and dietary fiber. This study aimed to find out how the ingestion of kiwifruit will affect intestinal microbiota populations, namely Lactobacillus, Bacteroides, Clostridium, Bifidobacterium, and Enterococcus.

Methods: Freeze dried kiwifruit (equivalent of two fresh kiwifruits) was given to each of the six subjects daily for four days. Faecal samples were collected before, during and after kiwifruit consumption. The faecal bacteria were enumerated by qPCR and RT qPCR methods.

Results: The effect of the kiwifruit on intestinal microbiota profile varied between individuals; in general, the kiwifruit demonstrated a prebiotic effect of promoting the content of faecal lactobacilli and bifidobacteria (as compared to the baselines of the same individual before consumption) for as long as the fruit was consumed. The effect was however transient, the levels of the two bacteria returned near to that of the baselines upon cessation of consumption.

Conclusion: Kiwifruit is a prebiotic in selectively enhancing the growth of intestinal lactic acid bacteria.

Keywords: kiwifruit; polysaccharides; gastrointestinal microflora; faecal lactobacilli; faecal bifidobacteria

The human intestine has about 10^8 bacterial cells/ml of luminal content, and the composition of the gut microflora has been found to play important roles in the health and diseases of humans (1–3).

Some slow or non-digestible polysaccharides are termed prebiotics, for they selectively enhance health-promoting gastrointestinal microflora, such as bifidobacteria and lactobacilli, thus confer benefits upon host well-being and health (4, 5).

Some widely consumed fruits and vegetables are high in polysaccharide content. Comparatively, the kiwifruit (Actinidia deliciosa) contains high amount of pectic polysaccharides and dietary fiber (6, 7), which has been found to improve immune system and relieve chronic constipation (8, 9). It is possible that the kiwifruit could confer prebiotic effects on intestinal microbiota.

The anaerobic bacteria, namely Clostridium, Bifidobacterium, and Bacteroides (10–12) are dominant intestinal microflora. Some species of Bacteroides were reported to produce metabolites that are carcinogenic and have the potential to aid the development of colon cancer. Other types of undesirable intestinal bacteria such as Clostridium may cause pseudomembrane colitis when in active form.

This study focused on five groups of bacteria found in the intestinal flora: Lactobacillus, Bacteroides, Bifidobacterium, Clostridium, and Enterococcus, and investigated how each of the bacteria group responds to the ingestion of kiwifruit. This would contribute to the understanding of the nutraceutical application of fruits.

Materials and methods

Human subjects

It was an open trial involving six healthy Chinese female adults aged 18–25 years. Participants were free from any chronic and recent illness that may compromise the immune system. All participants were kept free from probiotic and prebiotic products such as yogurt and cheese and fermented foods and beverages for 2 weeks before the sampling. Individual informed consents were obtained. The privacy and confidentiality of all data and information collected from trial participants were ensured both during and after the conduct of the trial.
Individuals will not be identified in any reports and publications based on the trial data.

**Kiwifruit**
The freeze dried kiwifruit was provided by Zespri International Limited, New Zealand.

**The experiment**
The six healthy subjects underwent a three-phase experimental regime. Phase 1 is the pre-experiment baseline. In phase 2, the subjects were given 32 g of the freeze dried kiwifruit dissolved in 100 ml water before breakfast. The quantity of freeze dried kiwifruit is equivalent to two fresh kiwifruits. The subjects stopped taking kiwifruit in phase 3 and this represents the washout period. The subjects were refrained from consuming fruits, fruit juices, and yogurts during the experimental period. Each of the three phases consisted of 4 days, whereby fecal samples were obtained daily.

**Faecal samples processing**
The stool samples were collected at the household level by each participant. A portion of freshly voided feces was collected into sampling tube and then suspended in RNAlater (Ambion, USA) to make a 10-fold dilution (v/w) of fecal homogenate. The fecal samples were transported to the laboratory within 12 hours. In the laboratory, the fecal samples were stored at −80°C until use.

Nucleic acids (total DNAs and RNAs) extraction was performed and the intestinal microbiota classified and enumerated by quantitative PCR (qPCR) (11, 12) and reverse transcription-PCR (RT-qPCR) (13, 14).

**Nucleic acids extraction**
DNA and RNA were extracted from the fecal samples.

**DNA extraction**
Fecal suspension (200 μl) was vortexed for 3 min with 300 mg glass beads (ca. 0.1 mm) in 300 μl Tris-SDS and 500 μl TE-saturated phenol, to disrupt the bacterial cells. In 400 μl of the supernatant, 40 μl of phenol/chloroform/isoamyl alcohol (25:24:1) was added and shacked vigorously for 45 seconds, after which the supernatant (250 μl) was mixed with 25 μl 3 M sodium acetate (pH 5.2) and 300 μl isopropanol. The mixture was centrifuged at 4°C and the supernatant decanted; 500 μl 70% ethanol was added and mixed, and again the supernatant was discarded. The DNA extract was air dried at room temperature for 30 min and stored at −80°C until use.

**RNA extraction**
In 200 μl fecal suspension, added 450 μl lysis buffer and 300 mg glass beads (ca. 0.1 mm) and then shacked vigorously for 5 min to disrupt the bacterial cells. To this, 500 μl water-saturated phenol was added and mixed, which was then heated at 60°C for 10 min (hot-phenol method). After which, 100 μl chloroform/isoamyl alcohol (24:1) was added. To 470 μl of the supernatant, 470 μl chloroform/isoamyl alcohol (24:1) was added and mixed. To 400 μl supernatant, 40 μl 3 M sodium acetate (pH 5.2) and 400 μl isopropanol was added and mixed. The supernatant was discarded, the pellet air dried at room temperature for 30 min. To the RNA extract, 0.2 ml nuclease-free water was added and mixed and stored at −80°C until use.

**Bacterial enumeration**
Fecal DNA and RNA extracts were subjected to the qPCR and RT qPCR analysis, respectively, using Applied Biosystems PRISM 7500.

qPCR analysis was performed to enumerate the following three predominant bacterial groups: Clostridium coccoides group, Bacteroides fragilis group, and Bifidobacterium. The primers, PCR conditions, and data analysis were according to that of Matsuki et al. (15).

RT-qPCR analysis was performed to enumerate the following two subgroups: Lactobacillus and Enterococcus. The primers, PCR conditions, and data analysis were according to that of Matsuda et al. (16) and Dubernet et al. (17).

**Statistical analysis**
Statistical analyses for significant differences were performed according to parametric, Student t test (MPO activity).

**Results and discussion**
Many fruits, seeds, and vegetables contain high polysaccharides and dietary fibers and their potential prebiotic effects have been demonstrated in in vitro studies; however, very few human trials have been conducted on these naturally occurring polysaccharides (4, 7, 18, 19). In this study involving six healthy young adults, upon consumption of two kiwifruit equivalent of freeze-dried kiwifruit powder, the fecal content of lactobacilli was found to increase significantly (p < 0.05) within 24 hours (Fig. 1A), except two subjects who did not respond (plotted independently in open symbols). Interestingly, the lactobacilli content remained at around 10⁷ and 10⁸ cells/g feces despite continuous consumption of the kiwifruit for 4 days. It is not clear if this represents the intestinal ecological niches available to lactobacilli or it is the number of lactobacilli that could be supported by the amount of kiwifruit consumed. The fecal lactobacilli content of healthy Asian reported in previous studies ranged between 10⁶ and 10⁸ cells/g feces (15). If 10⁸ lactobacilli/g feces is indeed the highest level of intestinal lactobacilli attainable, the largest impact for consumption...
of kiwifruit would be observed in lactobacilli-deficient individuals.

The prebiotic effect of kiwifruit appears to be short term. The content of lactobacilli returned to that of the baseline level rapidly after consumption of kiwifruit was stopped. Continuous consumption of the prebiotic is necessary to maintain a high level of lactobacilli.

A similar pattern of enhancement of intestinal bifidobacteria (Fig. 1B) was also observed. The variation in the bifidobacteria content among the five human subjects (except the non-respondent in open symbol) appeared to be high. Statistical significant different ($p<0.05$) between the experimental and the baseline was only detected after 4 days of kiwifruit consumption (day 8). Intestinal bifidobacteria content is sensitive to prebiotic level in the diet (4). Chinese diet is typically of low meat and high vegetable, and the various vegetables consumed by the subjects daily may contain different oligosaccharide level.

No significant different in the enterococci content was observed before and after consumption of kiwifruit, although its average number seems to be lower during the washout period (Fig. 1C).

For clostridia (Fig. 1D) and bacteroides (Fig. 1E), no significant different ($p>0.05$) was observed between the experimental and the baseline, due to the large standard deviation among the subjects. A general trend of lower cell content for the two bacteria during kiwifruit consumption (days 4–8) is nevertheless apparent.

The perturbation in their population could be due to unfavorable environment (organic acid and antibiotic production) created by the lactic acid bacteria (20).

**Conclusion**

The study implies that kiwifruit can act as a prebiotic in selectively enhancing the growth of intestinal lactic acid bacteria (lactobacilli and bifidobacteria) and causing perturbation in the population of *Clostridium* and *Bacteroides*. The extent of their prebiotic effectiveness was depending on individual. The general trend is that kiwifruit consumption enhanced the population of *Bifidobacterium* and *Lactobacillus* within 24 hours, and the effect last only during the consumption of the fruit.

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Probiotics and irritable bowel syndrome

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Background: Irritable bowel syndrome (IBS) is a major cause of abdominal discomfort and gut dysfunction worldwide. It is a poorly understood functional gastrointestinal disorder for which no effective medication is available. It is a benign condition, but its social and economic burden is significant. The symptoms consist of abdominal pain, bloating, flatulence, and irregular bowel movements. Alterations in the intestinal microbiota and mucosal inflammation may contribute to the development of IBS and probiotics could thus relieve the symptoms. This review gives an overview on the existing data on the effects of probiotics on the gastrointestinal symptoms of IBS.

Methods: A PUBMED search was made to review the relevant literature, and additional studies were obtained from the references of the selected articles.

Results: Clinical trials suggest that certain probiotics or combinations of bacteria have beneficial effects on the IBS symptoms. However the heterogeneity of studies, e.g. suboptimal study design, inadequate number of subjects, different doses and vehicles, inadequate length, make it difficult to compare the differences between probiotics and the effect may be strain-specific.

Conclusions: Though evidence is very promising, no general recommendations on the use of probiotics in IBS can be given yet. Further clinical trials and data on the mechanisms of action are needed. Probiotics are considered safe and if future scientific data is able to substantiate their efficacy in IBS, they certainly could be a treatment option in relieving the symptoms in IBS.

Keywords: IBS; probiotic; irritable bowel syndrome; gastrointestinal symptoms; lactobacilli; bifidobacteria

Irritable bowel syndrome (IBS) is the most common diagnoses in gastroenterology. It is estimated that approximately 10–20% of the adult population suffers from this syndrome (1). It is a heterogeneous functional disorder that impacts the individual’s quality of life as well as the whole society by greatly increasing medical costs. The diagnosis is based on symptoms through the Rome III criteria often following a long medical process of excluding organic diseases (2). The symptoms include abdominal pain, distension, flatulence, and irregular bowel movements (3). It is a condition for which no reliable biological, endoscopic, or radiologic finding nor effective pharmacological treatment is available.

The pathophysiology of IBS is only partly understood, but there is growing evidence of the role of imbalance of the intestinal microbiota, intestinal infections, and a dysfunctional intestinal barrier in the development of IBS and its symptoms [for review see Clarke et al. (4)]. Therefore, the therapeutic potential of probiotics has gathered a lot of interest.

Numerous clinical trials have investigated the effects of probiotics in patients with IBS, but the evidence is not yet consistent due to small number of subjects, variability in study design, heterogeneity of probiotic strain, dose and treatment duration, and patient characteristics in the clinical studies [for review, see Lee and Bak (5)]. However, some of the probiotics or their combinations have shown promising beneficial effects. The most commonly studied probiotics in IBS are lactobacilli and bifidobacteria, but findings from one strain should not be extrapolated to other strains as stressed by FAO/WHO joint expert report (6).

The aim of this summary is to give an overview based on existing evidence on the effects of probiotics in the treatment of symptoms in IBS.

Clinical trials on single probiotic strains

Summary of randomized controlled trials on the effects of probiotics in the treatment on IBS is shown in Table 1.

Bifidobacteria

Most randomized, placebo-controlled studies have suggested that Bifidobacterium have beneficial effects on IBS symptoms (7–9). In a trial with 77 IBS patients, ingestion of Bifidobacterium infantis 35624 for 8 weeks reduced
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Table 1. Randomized controlled trials of probiotics on IBS

| Probiotic                  | Number of subjects | Duration | Result                                                                                           | Reference |
|----------------------------|--------------------|----------|--------------------------------------------------------------------------------------------------|-----------|
| B. infantis 35624          | 77                 | 8 weeks  | Pain, IBS score, bowel movement difficulty ↓                                                        | 7         |
|                            | 362                | 4 weeks  | Abdominal pain, IBS score, distension, incomplete evacuation, straining, flatulence ↓            | 8         |
| B. animalis DM 173010      | 274                | 6 weeks  | Stool frequency in subjects with <3 stools/week ↑                                                  | 10        |
| B. bifidum MIMBb75         | 122                | 4 weeks  | IBS symptoms, pain, discomfort, distension, bloating, urgency, digestive disorder ↓                | 9         |
| L. plantarium 299V         | 60                 | 4 weeks  | Flatulence ↓                                                                                     | 11        |
|                            | 20                 | 4 weeks  | IBS score, abdominal pain ↓                                                                       | 13        |
|                            | 12                 | 4-4 weeks| i.e.                                                                                              | 18        |
| L. rhamnosus GG            | 24                 | 8+8 weeks|                                                                                                   | 12        |
|                            | 50 children        | 6 weeks  | Abdominal distention ↓                                                                           | 14        |
|                            | 104 children       | 4 weeks  | Treatment success ↑; abdominal pain frequency ↓                                                    | 15        |
|                            | 141 children       | 12 weeks | Treatment success ↑; abdominal pain ↓                                                              | 17        |
| L. acidophilus-SDC 2012, 2013 | 40        | 4 weeks  | Treatment success ↑; abdominal pain and discomfort ↓                                              | 16        |
| L. acidophilus             | 61                 | 2 weeks  | i.e.                                                                                              | 19        |
| L. reuteri ATCC 55730      | 54                 | 6 months |                                                                                                   | 20        |
| L. plantarium MF1298       | 16                 | 3-3 weeks| IBS sum score ↑                                                                                   | 21        |
| S. bouardi                  | 67                 | 4 weeks  | i.e.                                                                                              | 22        |
|                            | 35                 | 30 days  | i.e.                                                                                              | 23        |
| Streptococcus faecium      | 54                 | 4 weeks  | Clinical improvement ↑                                                                            | 24        |

pain, IBS score, and bowel movement difficulty (7). In another study, 362 women with all subtypes of IBS were treated with three different doses of B. infantis 35624 (10^6, 10^8, and 10^10 cfu) for 4 weeks. It was reported that only the dose of 10^8 cfu was significantly superior to placebo in reducing abdominal pain, IBS score, distension, incomplete evacuation, straining, and flatulence (8).

In a multicenter, placebo-controlled trial with 274 adults with constipation-predominant IBS, a fermented milk containing Bifidobacterium animalis DM 173010 showed beneficial effect on health-related quality of life during the 6-week study period. In addition, stool frequency increased in the subjects with less than three bowel movements per day. However, there were no other statistically significant differences in the symptoms of IBS between the placebo and probiotic groups (10).

Gugliametti et al. (9) investigated the effects of Bifidobacterium bifidum MIMBb75 on the severity of IBS in 122 patients. After the treatment for 4 weeks, probiotics reduced the global assessment of IBS symptoms, improved pain, discomfort, distension, bloating, urgency, and digestive disorder. In addition, B. bifidum MIMBb75 group showed a significant improvement in the quality of life. Overall, responder rates were 57% in the probiotic group and 21% in the placebo group (p <0.0001).

Lactobacilli

Placebo-controlled trials with partly contradictory results have investigated the effects of Lactobacillus species on IBS symptoms (11–17). Lactobacillus plantarum 299V has shown beneficial effects in two controlled trials (11, 13). Nobaek et al. (11) investigated the effects of L. plantarum 299V in 60 patients with IBS. During the 4-week treatment period, flatulence reduced rapidly and significantly in the probiotic versus the placebo group, but there were no differences in the reduction of abdominal pain between the groups. In another 4-week study, all 20 patients treated with L. plantarum 299V reported reduction of their abdominal pain as compared to 11/20 patients from a placebo group (p =0.0012). IBS symptoms were improved in 95% of patients in the L. plantarum 299V group versus 15% of patients in the placebo group (p <0.0001) (13). On the contrary, one 4-week study failed to show any beneficial effect of L. plantarum 299V (18).

Several controlled trials have investigated the effects of Lactobacillus rhamnosus GG on the symptoms of IBS in adults and in children (12, 14, 15, 17). These studies have suggested that L. rhamnosus GG is not effective in the treatment of common intestinal discomfort in adults, but it may offer some help to children suffering from IBS. Francavilla et al. (17) investigated the effect of L. rhamnosus GG in 141 children with IBS or functional pain. Compared with the baseline symptoms, L. rhamnosus GG, but not placebo, significantly reduced both the frequency (p <0.01) and severity (p <0.01) of abdominal pain. At the end of the 12-week treatment period, treatment success was achieved in 48 children in the L. rhamnosus GG group compared with 37 children in the
placebo group ($p < 0.03$), and this difference still was present at the end of follow-up without microbial treatment ($p < 0.03$). In another trial, 104 children who fulfilled the Rome II criteria for IBS, functional dyspepsia, or functional abdominal pain received L. rhamnosus GG or placebo for 4 weeks. Those children in the L. rhamnosus GG group were more likely to have treatment success (33 vs. 5%) and reduced frequency of pain ($p = 0.02$) compared to those in the placebo group, but there were no differences in pain severity between the groups (15). In a 6-week study with 64 children with IBS, L. rhamnosus GG was not superior to placebo in the treatment of abdominal pain, but it relieved abdominal distention (14).

In a Korean study, ingestion of Lactobacillus acidophilus-SDC 2012, 2013 for 4 weeks reduced score for abdominal pain and discomfort compared to the baseline and led to better treatment success than placebo (16). On the other hand, some studies have failed to show any beneficial effect of Lactobacillus compared with the controls (12, 18–20), and it has also been reported that some Lactobacillus species may even have unfavorable effects in patients with IBS. Ligaarden et al. (21) investigated the effects of L. plantarum MF1298 in 16 subjects with IBS in a placebo-controlled double-blind crossover trial. Treatment periods lasted for 3 weeks and wash-out period for 4 weeks. Thirteen patients (81%) preferred placebo to probiotic treatment. In the probiotic period, IBS sum score was 6.44 compared to 5.35 in the placebo period ($p = 0.01$).

These results suggest that lactobacilli may be a valuable alternative in the treatment of IBS, but the effect is most probably strain specific.

**Table 2.** Randomized controlled trials of probiotic mixtures on IBS

| Probiotic mixture | Number of subjects | Duration | Result | Reference |
|------------------|--------------------|----------|--------|-----------|
| L. rhamnosus GG combination\(^a\) | 103 | 6 months | IBS score ↓ | 25 |
| VSL\(^b\)/30 | 86 | 5 months | IBS score ↓ | 26 |
| 25 | 8 weeks | Bloating ↓ | 27 |
| 48 | 4 weeks | Flatulence, colonic transit ↓ | 28 |
| 59 children | 6 weeks | IBS symptoms ↓ | 29 |
| L. plantarum LP 01 + L. brevis BR 03 or L. plantarum LP 01 + L. acidophilus LA 02 | 70 | 4 weeks | IBS symptoms, pain ↓ | 30 |
| L. acidophilus CUL60 + L. acidophilus CUL21 + L. lactis CUL34 + B. bifidum CUL20 | 52 | 8 weeks | IBS symptoms, pain ↓ | 31 |
| B. bifidum BGN4 + L. lactis AD011 + L. acidophilus AD031 + L. casei IBS041 | 70 | 8 weeks | Abdominal pain ↓ | 32 |
| B. longum LA 101 + L. acidophilus LA 102 + L. lactis LA 103 + S. thermophilus LA | 100 | 4 weeks | ↔ | 33 |

\(^a\)LGG\(^b\) combination = L. rhamnosus GG, L. rhamnosus LC705, P. freudenreichii spp. shermanii JS, and B. breve Bb99 or B. animalis spp. lactis Bb12.

**Saccharomyces**

According to two published trials, *Saccharomyces boulardii* seems not to be effective in the treatment of IBS symptoms in adults. Choi et al. (22) evaluated the effects of *S. boulardii* on quality of life and symptoms in patients (n = 67) with diarrhea-predominant IBS or mixed-type IBS. After the 4-week study period, *S. boulardii* improved quality of life better than placebo but had no effect on the symptoms of IBS. Kabir et al. (23) reported that treatment with *S. boulardii* for 30 days in diarrhea-predominant IBS patients did not result in any improvement in symptom score or personal and professional life.

**Streptococcus**

In a Danish study, a comparison of a culture of *Streptococcus faecium* and placebo was carried out in 54 patients with IBS. After the 4-week treatment period, 81% of the active and 41% of the placebo-treated patients had improved according to the physicians’ overall assessment ($p = 0.002$) (24).

**Clinical trials on multispecies probiotic mixture**

Because of the diverse nature of IBS, it has been suggested that probiotic combinations could be more effective than single strains in this particular disease. Several multispecies probiotics have shown beneficial effects in the treatment of IBS symptoms in clinical controlled trials (Table 2). Two studies with *L. rhamnosus* GG in a combination with *L. rhamnosus* LC705, *Propionibacterium freudenreichii* spp. *shermanii* JS and *Bifidobacterium breve* Bb99 or *B. animalis* spp. *lactis* Bb12 found significant reduction in IBS symptoms (25, 26). In the first study (25), 103 patients with IBS took part in the 6-month, randomized, double-blind,
Placebo-controlled study. At the end of the study, median reduction in the symptom score was 42% in the probiotic group and 6% in the placebo group. In the second study, 86 patients with IBS received L. rhamnosus GG in the combination or placebo for 5 months, and the IBS score decreased 37% in the probiotic group and 9% in the placebo group (26).

Three studies have investigated the effects of VSL#3 (a mixture of B. longum, B. infantis, B. breve, L. acidophilus, L. casei, L. bulgaricus, L. plantarum, and S. salivarius subsp. thermophilus) in patients with IBS (27–29). In the first randomized study, 25 patients with diarrhea-predominant IBS received VSL#3 powder or placebo twice daily for 8 weeks. VSL#3 reduced abdominal bloating but had no effect on gastrointestinal or colonic transit (27). In the second study, 48 patients with IBS were randomized in a parallel group, double-blind design to placebo or VSL#3 for 8 weeks. VSL#3 reduced flatulence and retarded colonic transit without altering bowel function (28). Also, in children affected by IBS, VSL#3 has been more effective than placebo in ameliorating symptoms and improving the quality of life during the treatment period of 6 weeks (29).

An Italian study compared the effects of mixtures containing L. plantarum LP 01 and B. breve BR 03 or L. plantarum LP 01 and L. acidophilus LA 02, and placebo in 70 adults with IBS. Pain score decreased significantly in the probiotic groups compared to the placebo (45 and 49 vs. 29.5%) after 28 days. Similarly, the severity of IBS symptoms decreased significantly after the use of probiotics (56 and 55.6 vs. 14.4%) (30).

Williams et al. (31) investigated the effects of a probiotic preparation comprising L. acidophilus CUL60 and CUL21, B. lactis CUL34, and B. bifidum CUL20 in 52 IBS patients. Over the 8-week intervention period, a significantly greater improvement in the Symptom Severity Score of IBS and in scores for quality of life, days with pain, and satisfaction with bowel habit was observed in the probiotic group than in the placebo group. In a Korean study, treatment with B. bifidum BGN4, B. lactis AD011, L. acidophilus AD031, and L. casei IBS041 for 8 weeks reduced abdominal pain significantly compared to placebo in adults with IBS (32). On the contrary, a probiotic combination with B. longum LA 101, L. acidophilus LA 102, L. lactis LA 103, and S. thermophilus LA 104 for 4 weeks was not significantly superior to the placebo in relieving symptoms of IBS (33).

### Meta-analyses and reviews

Several reviews (e.g. 4, 5, 34–36) and two meta-analyses (37, 38) have been published to demonstrate the data regarding the use of probiotics in the treatment of IBS. McFarland and Dublin (37) connected the results of 20 randomized, placebo-controlled, blinded trials of probiotics for the treatment of IBS. In the meta-analysis, probiotic use was associated with improvement in global IBS symptoms (RR\textsubscript{pooled} = 0.77) and less abdominal pain (RR\textsubscript{pooled} = 0.78). Too few studies reported data on other IBS symptoms to allow estimation of a pooled relative risk. It was suggested that probiotics may be beneficial in the treatment of IBS, but more prolonged studies are needed.

Hoveyda et al. (38) included 14 English, randomized, placebo-controlled studies to their meta-analysis. The length of the studies varied from 4 to 26 weeks, and different participants and doses of probiotics or probiotic mixtures were used. Combined data suggested a modest improvement in IBS symptoms after the treatment with probiotics. Seven studies reported a statistically significant improvement on abdominal pain (OR = 2.88), five studies reported a statistically significant improvement on flatulence (OR = 2.31), and four studies reported a statistically significant improvement on the symptoms of bloating (OR = 1.75). However, when using continuous outcomes reported on non-standard scales, the improvements in these symptoms were not statistically significant. It was concluded that probiotics may have a role in alleviating some symptoms of IBS, but the optimal type and dose of probiotics and especially the subgroups of patients who are likely to benefit the most remain to be clarified.

Even if meta-analysis in the area of probiotics do not make sense as the active compounds (i.e. the probiotic) are different and their mechanism may differ, one can conclude that they show much promise for the use of probiotics in the treatment of IBS.

Recently, Clarke (4) concluded in his comprehensive review that progress in the field requires adequately powered long-term multicenter trials and the embracing of bench to bedside approaches. According to his conclusion on a evaluation of 42 trials examining the efficacy of lactobacilli in IBS, there is much promise for the use of lactic acid bacteria in the treatment if IBS. Of the 42 trials, 34 reported beneficial effects in at least one of the end points or symptoms examined even if tremendous variation in both the magnitude of effect and the choice of outcomes existed.

Moayyedi et al. (34) published an interesting systematic analysis on the randomized controlled trials testing various probiotics in patients with IBS. They show not only that many trials are of good quality and that positive results were obtained with some strains but also that the studies are heterogenous and that there may be a publication bias.

Brenner et al. (35) conclude in their systematic review that most RCTs about the utility of probiotics in IBS have not used an appropriate study designing and do not adequately report adverse effects. Future studies should
follow Rome III criteria recommendations for appropriate design of an RTC.

Possible mechanisms
Numerous studies have investigated the possible mechanisms behind the beneficial effect of probiotics on IBS symptoms. Several mechanisms, such as altering intestinal luminal environment, maintenance of mucosal barrier function, and modulation of immune system, may explain the reduction of IBS symptoms after the treatment with probiotics [for review, see Lee and Bak (5)].

Alteration of the gut microbiota is related to the pathogenesis of IBS [for review, see Lee and Bak (5)]. No single abnormality in the microbiota of IBS patients has been shown, but several studies have reported alterations in the bacterial composition and stability in IBS (39–42).

Clinical studies have shown that probiotics can alter the gut microbiota with improvements of symptoms of IBS (11, 26, 43). It has also been reported that ingestion of probiotic mixture containing L. rhamnosus GG, L. rhamnosus LC705, P. freudenreichii spp. shermanii JS, and B. animalis spp. lactis Bb12 affected IBS-associated fecal bacterial phylotypes (44). On the contrary, another probiotic mixture VSL#3, which have reduced the symptoms of IBS (27, 28), had no effect on gut microbiota (45). Probiotics could influence symptoms directly through balancing the microbiota and thus normalizing an aberrant gas production.

Elevated intestinal permeability has been documented in IBS patients, and there is growing evidence that disturbances of barrier function may play a role in the development of IBS. In experimental studies, probiotics have improved barrier function [for review, see Lee and Bak (5)]. In a clinical study, L. rhamnosus GG normalized the increased intestinal permeability of children with IBS (17).

Dysregulation of immunity can contribute to the pathogenesis of several diseases including IBS. Probiotics have been shown to have various effects on the immune system, and thus, they may be beneficial in the treatment of IBS [for review, see Lee and Bak (5)]. O’Mahony et al. (7) noticed that patients with IBS have abnormal ratio of IL-10/IL-12, which is an indicator of a proinflammatory state. This ratio was normalized after 8 weeks treatment with B. infantis 35624. These results suggest that probiotics may have an immune-modulating role in the treatment of IBS.

An inflammatory component seems also to be one possible factor in IBS, especially in so called postinfectious IBS (46). Also, animal models clearly suggest that inflammation could contribute to the symptoms of IBS, since there seems to be a causal relationship between mucosal inflammation and altered sensory-motor function.

It has also been suggested that the role of visceral sensitivity and the dysfunction of brain-gut axis are important factors in the development of IBS symptoms (47, 48). L. acidophilus NCFM was able to increase the visceral pain threshold in rats and to upregulate μ-opioid and cannabinoid receptors in colonic epithelial cells and in the colonic epithelium of rodents (49).

As listed above, numerous mechanisms are possible in the action of probiotics in IBS. A probiotic contains thousands of genes, which may potentially influence the clinical effects as was pointed out by Marteau (50). New discoveries will hopefully have an impact on the understanding on intestinal motility and visceral sensitivity and thus the mode of action of probiotics in the treatment of intestinal disorders.

Conclusions
There already exists rationale behind the use of probiotics in the treatment of IBS although solid strain-specific efficacy and emerging data identifying potential mechanism is needed. Careful trial design, strain selection and blinding, adequate sample size and trial length, optimum dosage, safety, and long-term tolerability must be considered when designing and evaluating clinical studies.

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Gut microbiota and infant distress – the association between compositional development of the gut microbiota and fussing and crying in early infancy

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Keywords: infant crying; fussing; gut microbiota; Bifidobacterium; Lactobacillus; diary

Excessive crying in an otherwise healthy child coincides with several environmental alterations and maturational processes: changes in the sleep and feeding patterns, immunological, endocrinological, and neurological maturation, thermoregulation, compositional development of the gut microbiota, and improvement of the immunological defences, including the gut barrier functions. An intimate interrelationship between diet, the immune system, and microbiome has been recognized when explaining susceptibility to disease later in life, subsequent to the demonstration that the establishment of the gut microbiota provides an initial and massive source of microbial stimuli for the maturation of the gut-associated lymphoid tissue, particularly for the IgA plasma cells, conferring the first line of host immunological defence. Notwithstanding the extensive and multidisciplinary scientific interest centred on infant nutrition and gut microbiota, research so far has been unable to conclusively ascertain the determinants underlying infant crying, a common problem manifesting itself at the peak of these maturational processes; the duration of crying increases after birth, reaching a maximum during the second and the third month of life (1). Infantile colic is a specific condition characterized by paroxysmal, excessive, and inconsolable crying, with a duration exceeding 3 hours a day for 3 days or more a week (2).

Introduction

The attempts to control excessive crying, above crying related to acute infectious diseases, have focussed on varied dietary regimens of the affected child. Indeed, significant lessening in crying has been achieved by reducing the allergens: a subgroup of infants with excessive early fussing and colic-type cry manifests atopic diseases, or a heightened risk thereof, and food allergy (3–5). A potential role of specific probiotics is also suggested, as aberrant composition of gut microbiota has been reported in colicky infants during the time of colic (6–8).

Aim

The aim of the present study was to establish whether there is an association between the compositional development of the gut microbiota and amount of fussing and crying in early infancy. We focussed on the entire spectrum of crying as opposed to the most often assessed colic cry (9).

Methods

Behavioural patterns of 88 infants during the 7th and 12th week of life were recorded by parental diaries. During the first 6 months of life, infants’ gut microbiota was analysed firstly by quantitative polymerase chain reaction (qPCR) and fluorescent in situ hybridization (FISH) assays and secondly by PCR-denaturing gradient gel electrophoresis (PCR-DGGE). The study was approved by the Ethics Committee of the South-Western Finland Hospital District (9).

Results and discussion

The median (range) duration of total distress of the infants was 106 (0–478) min a day during the seventh week and 58 (0–448) min a day during the 12th week. At the age of 3 months, the proportion of Bifidobacterium counts to total bacterial count was inversely associated with the amount of crying and fussing during the first 3 months of life (p =0.03). In contrast, Bifidobacterium breve behaved contrary to this general Bifidobacterium pattern: the amount of Bifidobacterium breve was...
found to be associated with the amount of total distress \((p = 0.02)\). Furthermore, the prevalence of \textit{Lactobacillus} spp. at the age of 3 weeks was inversely associated with total infant distress during the seventh week of life \((p = 0.02)\). At the age of 6 months, after the peak period of crying, the total number of \textit{Bifidobacterium} \((p = 0.03)\) and \textit{Lactobacillus} \((p = 0.008)\) were inversely associated with the total distress experienced during the first 3 months (Figs. 1a and b). We have reported that members of the \textit{Bifidobacterium} and \textit{Lactobacillus} genus in intestinal microbiota appear to protect against crying and fussing and here we report that the specific \textit{Bifidobacterium} species are associated with infant crying and distress. Identification of specific \textit{Bifidobacterium} and \textit{Lactobacillus} strains that would have optimal protective properties would benefit at-risk infants and result in new probiotic applications.

**Conclusion**

Our findings link the composition of the gut microbiota to fussing and crying during early infancy in demonstrating species-specific effects of the gut microbiota on infant distress. A more profound understanding of the complex nature of infant crying is needed, as it is likely that there are distinct etiological factors and pathogenic mechanisms underlying the condition; detailed elucidation of these may facilitate the development of novel preventive and therapeutic options against this common problem.

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Isolation of bifidobacteria for blood group secretor status targeted personalised nutrition

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Background: Currently, there is a constant need to find microbial products for maintaining or even improving host microbiota balance that could be targeted to a selected consumer group. Blood group secretor status, determining the ABO status, could be used to stratify the consumer group.

Objective: We have applied a validated upper intestinal tract model (TIM-1) and culturing methods to screen potential probiotic bacteria from faeces of blood secretor and non-secretor individuals.

Design: Faecal samples from healthy volunteers were pooled to age- and sex-matched secretor and non-secretor pools. Faecal pools were run through separate TIM-1 simulations, and bacteria were cultivated from samples taken at different stages of simulations for characterisation.

Results: Microbes in secretor pool survived the transit through TIM-1 system better than microbes of non-secretor pool, especially bifidobacteria and anaerobes were highly affected. The differences in numbers of bifidobacteria and lactobacilli isolates after plate cultivations and further the number of distinct RAPD genotypes was clearly lower in non-secretor pool than in secretor pool.

Conclusions: In the present study, we showed that microbiota of secretor and non-secretor individuals tolerate gastrointestinal conditions differently and that a combination of gastrointestinal simulations and cultivation methods proved to be a promising tool for isolating potentially probiotic bacteria.

Keywords: gastrointestinal simulation; probiotic screening; Bifidobacterium; intestinal; ABO blood group; secretor; non-secretor

The human intestinal tract is colonised with several hundred bacterial species, whose total number can exceed trillions of microbial cells in the colon. The microbiota has an important role in human health. It contributes to the maturation of the gut tissue, host nutrition, pathogen resistance, epithelial cell proliferation, host energy metabolism, and immune response (1, 2). That host genes might play a role in determining the composition of the gut microbiota has been supported by twin studies (3) and a few pioneer studies on specific genes, e.g. fucosyltransferase-2 (FUT2) (4). Some pathogenic intestinal microbes, e.g. Helicobacter pylori and several other species of bacteria and viruses, have shown to use ABO blood group antigens as adhesion receptors (5). Some microbes, e.g. bifidobacteria and Bacteroides thetaiotaomicron, are also able to utilise blood group antigens or glycans found in ABO and Lewis antigens (6, 7).

The ABO blood group antigens are not present in the mucus of all individuals. These individuals, said to have the ‘non-secretor’ blood group, do not have the functional FUT2 gene needed in the synthesis of secreted blood group antigens (8). Hence, they do not have ABO antigens in their secretions and mucosa, while those with blood group ‘secretor’ have the antigens. In most populations, the frequency of non-secretor individuals is substantially lower than that of secretor status; about 15–26% of Scandinavians are non-secretors (9). The secretor/non-secretor status can be regarded as a normal blood group system and the phenotype can be determined using standard blood banking protocols (8). The genotype that is causing the non-secretor
(NSS) phenotype in European populations has been identified as a major mutation in the FUT2 gene (10). The non-secretor phenotype has been demonstrated to be genetically associated with Crohn’s disease (11), resistance to Norovirus infection (12), experimental vaginal candidiasis (13), increased risk for asthma (14), urinary tract infections (15), and animal haemorrhagic disease virus (16).

The beneficial effects of certain microbial species/strains on maintaining or even improving of gut balance and the growing evidence of their health effects on intestinal inflammatory diseases have caused a great interest in modulation of gut microbiota, and recently also on modulation of oral, vaginal or skin microbiota. Gut microbiota can be modulated by taking probiotics belonging mainly to Bifidobacterium and Lactobacillus genera. Many probiotic supplements and products currently on the market are ineffective in promoting the desired health effects among most individuals. Thus, there is a continuous need for microbial and/or probiotic products that are able to mediate the health effects of the microbes more efficiently. An important criterion for the selection of probiotic bacteria to be used in food products or formulations in relation to health promotion is the survival and activity of these microorganisms in the gastrointestinal (GI) tract of the consumer. In order to investigate the survival of probiotic bacteria in the stomach and small intestine in humans, intubation methods are available. However, these in vitro methods are laborious, expensive, and meet ethical constraints. Therefore, validated in vitro methods should be performed, at least for the first selection of strains. In our study, we investigated the difference in the GI survival of intestinal microbiota obtained from blood group secretor and non-secretor individuals by applying the TIM-1 system (TNO intestinal model-1) (17) followed by selective culturing as an approach for microbiota characterisation. This approach enables the isolation of secretor/non-secretor-specific potentially probiotic bacteria.

### Present investigation

#### Sample collection

Faecal and blood samples were collected from a group of healthy volunteers; blood samples were processed directly, and faecal samples were processed under anaerobic atmosphere. Of the blood samples, the presence or absence of the mutation responsible for non-secretor genotype was determined by using sequencing or a qPCR method as described in Refs. (10, 18) at Haartman Institute Sequencing core facility and at the Technology Centre, Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki. Based on this SNP-data, a secretor- and a non-secretor-pool were formed amongst the volunteers by selecting age- and sex-matched pairs from the respective groups, resulting in a total of 11 in both groups. Frozen faecal samples were pooled based on the grouping to form a secretor (S) and a non-secretor (N) faecal pool.

#### Isolation of new strains by TIM-1 model

The TIM-1 (TNO in vitro GI model-1) of the stomach and small intestine (17) simulates to a high degree the successive dynamic processes in the stomach and the small intestine allowing sampling from several sites of the simulated GI tract in time. The model offers the possibility to simulate very closely the pH curves and the concentrations of enzymes in the stomach and small intestine, the concentrations of bile salts in the different parts of the gut, and the kinetics of passage of food through the stomach and intestine. It has been validated for the survival of probiotic microorganisms (17) and shows such a high in vitro–in vivo correlation that the system can be used for predicting survival of bacteria during transit through the GI tract.

In this study, the TIM-1 model was used with faecal samples to facilitate the isolation of microbes with good survival under GI tract conditions. The screening of the target bacteria was performed by adding the faecal slurries to TIM-1 and plate culturing the ileum effluent to grow the surviving bacteria. We collected isolates

### Table 1. Growth media and conditions for bacteria cultivation

| Agar medium | For which bacteria | Incubation temp. (°C) | Incubation aerobic/anaerobic | Incubation time (h) |
|-------------|--------------------|------------------------|----------------------------|---------------------|
| Beerens     | Bifidobacteria     | 37                     | Anaerobic                  | 72                  |
| RB          | Bifidobacteria     | 37                     | Anaerobic                  | 72                  |
| Rogosa      | Lactobacilli       | 37                     | 48 h aerobic/24 h anaerobic | 72                  |
| LAMVAB      | Lactobacilli       | 37                     | Anaerobic                  | 72                  |
| RCBA        | Anaerobes          | 37                     | Anaerobic                  | 72                  |
| BBE         | Bacteroides        | 37                     | Anaerobic                  | 72                  |

RB (raffinose-bifidobacterium agar), LAMVAB (Lactobacillus anaerobic MRS with vancomycin and bromocresol), RCBA (reinforced Clostridial agar with China blue and horse blood) and BBE (bacteroides bile esculin agar).
from bifidobacteria, lactobacilli, bacteroides, and general anaerobes and focused on characterising isolated bifidobacteria strains to deeper detail.

The frozen faecal samples were taken from the freezer and directly transferred into the anaerobic cabinet, where they were mixed together with artificial saliva and water. The non-secretor pool had in total 12.1 g and the secretor pool in total 9.8 g faecal material. A mixture of 95 ml artificial saliva and 200 ml water was prepared, and in this mixture, the total amount of the faecal samples of one pool was mixed. A 1 ml sample was taken from each mixture for plating. The bacterial slurries were then introduced into the gastric compartment containing 5 ml gastric residue.

The experiments in the TIM-1 model were performed under the average physiological conditions as found in the healthy human GI tract (19). The gastric emptying, intestinal residence time, and gastric and duodenal pH-curve mimicked the situation as found in humans in a fasted state. The concentrations of electrolytes, enzymes, bile, bile salts, and pancreatic juice were adjusted at the average concentrations as described for healthy humans. During the TIM-1 run, ileum effluent was sampled. The simulated ‘ileocaecal valve’ delivered the intestinal contents into a sampling bottle with 250 ml of diluent (cystein tryptone salt solution, containing 1.0 g tryptone, 8.50 g NaCl, and 0.30 g L-cystein hydrochloride per litre) at 10 ($\pm$2)°C. In this way, the sample was diluted to minimise the effect of bile and pH after the passage through the TIM-1 system on the remaining surviving bacteria. Every 60 min, the collected volume was measured and sampled. A 15-ml aliquot of the ileal effluents (Ie) of time points Ie-1 (60–120 min), Ie-2 (120–240 min), and Ie-3 (240–300 min) was collected for direct plating. Thus, in each TIM-1 experiments, four samples (intake, Ie-1, Ie-2, and Ie-3) were plated. The growth media and conditions used for cultivation of each species are presented in Table 1. Representative colonies were collected from culture plates for further analysis.

**Characterisation of isolates and strain properties**

Isolates were collected from each of the targeted culture plate types and stored in skimmed milk for further characterisation. Bacteroides and anaerobes were not analysed further in this study (Table 3). The bifidobacteria and lactobacilli isolates were first analysed using random amplification of polymorphic DNA (RAPD) to rapidly and roughly screen the different strains amongst the isolates. DGGE-analysis was performed with the RAPD-identified bifidobacteria and lactobacilli strains as the primary species identification method (Table 3). At this point, we focused solely on bifidobacteria and continued their identification by 16S sequencing of bacterial DNA. Not all 16S sequence measurements

| Non-secretor | Secretor |
|--------------|---------|
| Sample       | Beeren  | LAMVAB | RCBA | BBE |
| Intake       | 4.1E+08 | 2.9E+08 | 2.9E+08 | 2.9E+08 |
| Ie-1         | 3.5E+08 | 1.5E+08 | 8.4E+08 | 5.5E+08 |
| Ie-2         | 1.7E+07 | 1.6E+07 | 7.4E+07 | 2.1E+07 |
| Ie-3         | 2.4E+07 | 2.4E+07 | 4.6E+07 | 1.4E+07 |
| Total Survival | 5.5E+08 | 5.5E+08 | 7.4E+08 | 5.5E+08 |

| % Survival |
|------------|
| 0.1        | 2.0 |
| 0.1        | 1.6 |
| 0.0        | 0.0 |
| 2.5        | 2.5 |
| 0.0        | 0.0 |
| 1.0        | 1.0 |

Table 2. Survival of intestinal bacteria (number of cfu’s) in the TIM-1 samples in non-secretor pool and secretor pool.
were successful, and ID based on DGGE-phenotyping was used in these cases (Table 3). The DGGE-analysis was performed as described previously in Ref. (4). Briefly, the DNA from bacterial cultures was collected in log-phase and DNA was extracted by using the FASTDNA® SPIN KIT FOR SOIL (Qbiogene, USA). The partial 16S rRNA gene was PCR amplified with *Bifidobacterium* specific primers, run in DGGE gel with denaturing gradient from, stained with SYBR Safe, and documented with an UV-table and AlphaImager HP (Kodak, USA) imaging system. RAPD was performed as described in Ref. (20). The isolates were lysed followed by PCR amplification with a random primer set OPA-2 (bifidobacteria) or OPA-3 (lactobacilli). The PCR products were separated by gel electrophoresis and the fingerprints of the isolates from the same individual were compared visually. Substrate utilisation and/or enzyme activation were determined with two techniques: OD-measurement with Bioscreen (Growth Curves Ltd., Finland) and colour reaction with Rosco Diatabs (Rosco Diagnostica A/S, Denmark). Prior to both measurements, the bifidobacteria strains were grown for 48 h under anaerobic conditions in +37°C first on RCM-agar-plates and subsequently in RCM-broth. In Bioscreen measurements after precultivation, a 5% inoculum of each strain was cultivated in duplicate in 0.5% fucose-, lacto-N-biose-, or glucose-solutions mixed in low carbohydrate general edible medium (21) under anaerobic conditions at +37°C with slow shaking for 48 h. Starting- and end-point OD-measurements were performed with Bioscreen. The average growth of 0.5% glucose was deducted from 0.5% fucose and lacto-N-biose results. In Rosco Diatabs, the manufacturer's instructions were followed for both substrate utilisation and enzyme activation disks. The precultivated bacteria were washed with PBS and diluted to OD 1.0 in PBS. 100 μl of each dilution was pipetted to 96-well plate, and respective Rosco Diatabs disks were placed to the wells. The plate was cultivated in +37°C under anaerobic condition for 24 h, and the results were determined by visual inspection of the colour reaction. Rosco Diatabs Oxgall disks were used to test the bile acid tolerance of the screened bifidobacteria strains. Tolerance for bile acid was tested using Rosco Diatabs Oxgall as instructed by the manufacturer by plating PBS-diluted bifidobacteria colonies on RCM-agar together with Oxgall disks. The tolerance was determined by measuring the inhibition zones surrounding the disks. Zones with maximum diameter of 9 mm were determined as resistant (R) and rest as sensitive (S).

**Results**
A difference in the survival of faecal bacteria (especially bifidobacteria and total anaerobes) in the TIM-1 model was observed between the non-secretor and secretor sample pools (Table 2), although the numbers of bacteria in the intakes were nearly identical. From the non-secretor pool, fewer bacteria survived the transit through the TIM-1 system than from the secretor pool, although the lactobacilli count (using Rogosa-agar) was the only count higher in the non-secretor pool. The highest differences in survival, up to more than 10 times higher cfu's, were detected in secretor pool bifidobacteria and anaerobes. Also, the visual inspection of the culture plates suggested a difference in the diversity and microbiota composition between the two sample pools. The difference in the number of obtainable bifidobacteria and lactobacillus isolates and the number of distinct genotypes (determined by RAPD) was clearly lower in the non-secretor pool than in secretor pool (Table 3), further supporting the difference in the intestinal survival properties of the microbiota of secretor and non-secretor individuals.

In growth experiments with specific substrates (lacto-N-biose and fucose), eight of the 36 bifidobacterial strains utilised fucose and six strains used lacto-N-biose slightly better than glucose (Table 4). The S-pool users belong to both *B. adolescentis* and *B. catenulatum* species and the N-pool to *B. adolescentis* species. In the Rosco enzyme activity and substrate utilisation tests, *B. animalis* species were detected to be the best users of the studied mono/disaccharides, and several *B. longum* species had the lowest utilisation capabilities of the studied mono/disaccharides, and several *B. longum* species lacked the β-galactosidase activity. All in all, N-pool strains were detected to be more enzymatically active compared to S-pool strains. α-Fucosidase activity was not found in any of tested strains. Five *B. animalis*, three *B. adolescentis*, two *B. catenulatum*, and one *B. longum* strains were found to tolerate

| Group          | No. of RAPD types (no. of isolates) | Species                  |   |
|----------------|-------------------------------------|--------------------------|---|
|                | Secretor  | Non-secretor                  | Secretor | Non-secretor |
| Bifidobacteria | 31 (276)  | 11 (196)                      | 6        | 5            |
| Lactobacilli   | 21 (126)  | 8 (65)                        | 9        | 6            |

**Table 3.** Bifidobacteria and lactobacilli isolates collected from the TIM-1 model experimentation sample plates.
Table 4. Substrate utilization, enzyme activity, and bile tolerance of bifidobacteria isolated from secretor and non-secretor individuals

| Species         | Lacto- | Lactose | Sucrose | Mannose | α-Glucosidase | β-Glucosidase | β-Galactosidase | α-Galactosidase | α-Fucosidase** | Bile resistance |
|-----------------|--------|---------|---------|---------|--------------|--------------|----------------|----------------|----------------|----------------|
|                 | Fucose | Glucose | n-biose |         |              |              |                |                |                |                |
| Secretor        |        |         |         |         |              |              |                |                |                |                |
| *Bifidobacterium sp.* | 0/1   | 0/1     | 1/1     | 1/1     | 1/1          | 1/1          | 1/1            | 1/1            | 1/1            | 0/1            |
| B. adolescentis | 4/8    | 2/8     | 6/8     | 8/8     | 7/8          | 3/8          | 8/8            | 8/8            | 8/8            | 8/8            |
| B. catenulatum  | 2/4    | 2/4     | 4/4     | 4/4     | 3/4          | 4/4          | 4/4            | 4/4            | 4/4            | 0/1            |
| B. longum       | 0/8    | 0/8     | 6/8     | 8/8     | 4/8          | 5/8          | 8/8            | 3/8            | 8/8            | 0/3            |
| B. animalis     | 0/4    | 0/4     | 4/4     | 4/4     | 4/4          | 3/4          | 4/4            | 4/4            | 0/1            | 4/4            |
| All             | 6/25   | 4/25    | 21/25   | 25/25   | 20/25        | 16/25        | 25/25          | 18/25          | 25/25          | 0/8            |
|                 | (24%)  | (16%)   | (84%)   | (100%)  | (80%)        | (64%)        | (100%)         | (72%)          | (100%)         | (92%)          |
| Non-secretor    |        |         |         |         |              |              |                |                |                |                |
| *Bifidobacterium sp.* | 0/1   | 0/1     | 0/1     | 0/1     | 0/1          | 1/1          | 1/1            | 1/1            | 1/1            | –              |
| B. adolescentis | 2/3    | 2/3     | 2/3     | 3/3     | 3/3          | 3/3          | 3/3            | 3/3            | 3/3            | 0/3            |
| B. catenulatum  | 0/2    | 0/2     | 2/2     | 2/2     | 2/2          | 2/2          | 2/2            | 2/2            | 2/2            | –              |
| B. longum       | 0/4    | 0/4     | 3/4     | 3/4     | 2/2          | 4/4          | 4/4            | 4/4            | 4/4            | 0/3            |
| B. animalis     | 0/1    | 0/1     | 1/1     | 1/1     | 1/1          | 1/1          | 1/1            | 1/1            | 1/1            | 0/1            |
| All             | 2/11   | 2/11    | 8/11    | 11/11   | 8/11         | 8/11         | 11/11          | 11/11          | 11/11          | 0/7            |
|                 | (18%)  | (18%)   | (73%)   | (100%)  | (73%)        | (73%)        | (100%)         | (100%)         | (100%)         | (0%)           |

* Bioscreen growth marked positive if (OD after growth with 0.5% substrate − OD after growth with 0.5% glucose) > 0.
** Due product shortage, α-fucosidase production was measured only from total of 15 strains.
− States for not measurement.
bile and 55% of the N-pool strains tolerated bile well compared to 32% of the S-pool.

Conclusions
In the present study, we show the difference in the GI survival properties of the faecal microbiota of blood group secretor and non-secretor individuals. In addition, we present a rapid approach for screening potential personalised probiotic strains from human faecal samples. We combined the traditional plate cultivation, still the most efficient way to collect live bacterial isolates, with an accurately upper GI tract conditions simulating model.

The faecal samples used in this study were collected from healthy individuals and pooled according to donor’s blood group secretor or non-secretor status. The secretor individuals express by definition ABO blood group antigens in, e.g. their GI secretions, whereas non-secretors express only Lewis a-antigen. Our group recently published a novel finding stating non-secretors and secretors have marked differences in their faecal bifidobacteria populations, non-secretors having both clearly lower overall counts, and fewer bifidobacteria species present (4). In the current study, we were able to show that the secretor status associated difference in the microbial species composition reflects also the phenotypic properties of the intestinal bacteria especially those influencing the tolerance to upper GI conditions (22).

We utilised our previous findings as a basis to screen secretor status-specific probiotic strains with a novel approach. The TIM-1 system has been widely applied to study the upper GI survival properties of single bacterial strains (17, 19). In our approach, we utilised the TIM-1 for the preselection of potentially acid- and bile-tolerant intestinal bacteria. The model offers the possibility to study the survival and behaviour of probiotic and pathogenic microorganisms in the stomach and small intestine, and the results can be used to predict the survival of probiotic bacteria in the human intestinal tract. The isolate cultivation results supported our hypothesis well, as the bacteria in the non-secretor faecal pool had significantly lower survival rate in the TIM-1 simulation compared to the secretor pool bacteria. Especially non-secretor bifidobacteria and anaerobes survived very poorly compared to respective species in secretor pool. However, only B. animalis species were isolated in the ileal effluents in both simulations, thus being only strains able to withstand the harsh conditions of the upper GI tract. Due to the excellent acid and bile tolerance, B. animalis is widely used in probiotic products in Europe.

Interestingly, the users of lacto-n-biose were found only amongst B. adolescentis (two in both S- and N-pools) and B. catenulatum (two in S-pool). As lacto-n-biose is the basic backbone of all ABO blood group glycan structures, these strains, and especially two strains tolerating bile (N-pool B. adolescentis and S-pool B. catenulatum), might be potential probiotic Bifidobacterium candidates aimed to utilise ABO blood group structures and warranting further studies.

In conclusion, we showed that the tolerance of the intestinal microbiota to upper GI conditions differs between secretor and non-secretor individuals, secretor bifidobacteria surviving in larger number, whereas non-secretor bifidobacteria had broader glycolytic activity and better bile tolerance. In addition, we compiled a selective pretreatment and culturing approach, which appears to be a promising tool for isolation of potentially probiotic bacteria.

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Defining microbiota for developing new probiotics

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The human body harbors complex communities of microbes that play a prominent role in human health. Detailed characterization of the microbiota in the target population forms the basis of probiotic use. Probiotics are defined as live bacterial preparations with clinically documented health effects in humans, and independent of their genus and species, probiotic strains are unique and their beneficial properties on human health have to be assessed in a case-by-case manner. Understanding the mechanisms by which probiotics influence microbiota would facilitate the use of probiotics for both dietary management and reduction in risk of specific diseases. The development of high throughput sequencing methods has allowed metagenomic approaches to study the human microbiome. These efforts are starting to generate an inventory of bacterial taxons and functional features bound to particular health or disease status that allow inferring aspects of the microbiome’s function. In the future, this information will allow the rational design of dietary interventions aimed to improve consumer’s health via modulation of the microbiota.

Keywords: microbiota; probiotic; diet; health

The human microbiota (HM) is a complex system of many microbial communities inhabiting a diversity of environmental niches throughout the human body. HM exhibits large variation among individuals in relation to internal and external factors, such as genetic factors, age, diet, and health, and remains in a complex equilibrium. Although the exact composition of the microbiota is not known, advances in genomic technologies have recently begun to unravel our microbial partners. It is known that about 75% of the gut microbiota is covered by already known and dominant phyla (Actinobacteria, Firmicutes, and Bacteroidetes), while a fraction of about 25% is still unknown. It is estimated that each individual houses a consortium of 1,000–1,150 prevalent bacterial species (1) whose collective genome (microbiome) contains at least 100 times as many genes as the human genome.

The development of the human gut microbiota is a complex process that has been traditionally assumed to start at birth, although recent reports indicate that microbial colonization may begin earlier as bacteria have been detected in meconium, umbilical cord, and amniotic fluid (2–5). In infants, microbiota development is a fast process that depends on the first inoculi received from the environment, and also the maternal microbiota, mode of delivery, type of feeding, including weaning food practices and the use of antimicrobials (6). The early GIT microbiota is often dominated by one or a few genera and among breast-fed infants, *Bifidobacterium*-dominated microbiotae are more frequent than among infants fed with formula, but other compositions are also common. A large shift in microbiota composition accompanies the introduction of solid foods into the diet (7–10). It is likely that the beneficial impact achieved for the infant with breastfeeding is a combination of a balanced supply of nutrients, bioactive proteins, and indigestible oligosaccharides, as well as bifidogenic bacteria in breast milk (11). Compilations of long-term studies have shown that breast-fed infants have lower risk of diabetes (12), hypercholesterolemia (13), cardiovascular disease (14), and obesity (15) in adulthood than formula-fed infants, although the causality is difficult to ascertain.

Human microbiota composition and health

In recent years, the increase in microbiota-related research has provided important advances toward establishing the identity of specific microbes and microbial groups or microbial molecules contributing to various aspects of host physiology and health. Studies on human microbiota should include microbial ecology and analysis.
of the complex metabolism of the microbial community, as well as various host–microbial interactions occurring at the interface between microbes and host intestinal epithelia. Such studies should lead to an understanding of the impact of the microbiota on human health and disease. Concurrently, host factors involved in various aspects of development and maturation targeted by the microbiota have been identified.

A balance among microbial groups present in the human gut is crucial for maintaining health. When this balance is disturbed, the host–microbe relationship can progress toward a disease state. Altered intestinal colonization by commensal microorganisms as well as high interindividual variability and reduced microbial diversity have been reported in preterm infants (16, 17), increasing the risk to develop later disease. Several gastrointestinal pathologies such as irritable bowel diseases (IBD) or syndromes (IBS), necrotizing enterocolitis (NEC), obesity, various forms of colitis, and even autism have been linked to disturbances in microbiota or alterations of the intimate cross-talk between these microbes and human cells (18). Numerous studies have also linked early gut microbiota to the development of atopic diseases, but no specific microbes have yet been identified with consistently harmful or protective roles regarding atopy (19–21). However, some reports have suggested that the gut microbiota could regulate host energy homeostasis and adiposity, as differences in microbial composition can explain an increased capacity of the obesity-associated microbiome to harvest energy from the diet (22, 23).

Other studies have examined gut microbiota composition in human obesity and type-2 diabetes and the impact of weight reduction on microbiota (24–26). All these works suggest that there could be a link between gut microbiota composition and host’s health. Further studies will confirm if the rationale for modulating the gut microbiota by means of probiotics could also derive in health improvement.

Challenges for probiotic development based on microbiota research

There is a growing interest in studying beneficial microbes from the human microbiota with specific functions, which could eventually be used as probiotics in foods or supplements to improve human health and prevent or treat diseases. According to the FAO–WHO definition of probiotics – ‘live microorganism which when administered in adequate amounts confers a health benefit on the host’ (27) and food regulations that are currently in force, those beneficial effects must be scientifically demonstrated. It is important to underline that probiotic strains are unique, limiting the extrapolation of results from one strain to another. It is well-known that different bacterial strains of the same genus and species may exert completely different effects on the host. Therefore, the specific properties and characteristics of individual strains should be well defined and the effect on health of each strain should be demonstrated in a case by case manner. Then, the selection of potential probiotic strains from appropriate sources depending on the target population constitutes a promising approach. Some clear challenges have been identified through this study.

Novel uses and applications of probiotics

In general, any disorder in which an aberrant microbiota or an unappropriate immune response may play a role are potential targets for probiotic intervention, even though they may not take place at gut level. Studies have shown that administration of probiotics to pregnant women, nursing mothers, or newborns can influence the establishment and composition of infant gut microbiota (28–30), impacting early and later in life. Probiotic bacteria have been usually used to treat and prevent some gastrointestinal disturbances such as IBD, IBS, or diarrhea, and new evidences support the use of probiotics in the prevention and treatment of a number of diseases including atopic diseases, immune disorders, obesity, and diabetes, although new extraintestinal applications are the getting interest of industry and consumers. Disturbances in microbiota have been identified in other intestinal disorders, including diverticulitis and extraintestinal conditions, such as elderly people suffering severe frailty. Further, patients with severe systemic inflammatory response syndrome showed lower levels of bifidobacteria and lactobacilli and higher levels of pathogenic microorganisms than healthy subjects. Reduced levels of bifidobacteria have also been shown in multiple sclerosis patients. In addition, the current evidence for a role of bacteria (commensals, probiotics, and pathogens) as key modulators of gut–brain communication (31) suggests the potential role of probiotics on the gut–brain axis. Although, so far, probiotics have not been tested in these settings, these studies indicate potential targets for the future development of probiotic products.

Study of other gut microbiota components as probiotic

The complexity of the gut microbiota provides a very promising source of new probiotic organisms, and in many research works, gut immunologists prefer to use the terms ‘commensal bacteria’, instead. Enterocytes and dendritic cells in the gut mucosa can discriminate pathogenic from commensal bacteria, through specialized receptors and signal transduction pathways crucial for maintaining intestinal immune homeostasis and mechanisms of innate defense (32, 33). These cascades of molecular signals are nowadays only partially defined and constitute the basis of the demonstrated
immunomodulatory effect of these bacteria. In this regard, other bacteria than those commercially used as probiotics have been scarcely studied. Different microorganisms are used as human probiotics, the most commonly used probiotics are intestinal strains of Lactobacillus, Bifidobacterium, and Enterococcus species, for which technology has been developed for their industrial production. However, other intestinal microbes may also have a beneficial role in human health. Escherichia coli is among the first colonizers of the infant gut, and although this species harbor pathogenic strains, the E. coli probiotic strain Nissle 1917 has been found to reduce the number and incidence of infections, to stimulate specific humoral and cellular responses, and to induce the non-specific natural immunity in infants (34, 35). In addition to commercially used Saccharomyces boulardii, which has been reviewed extensively, a number of spore-forming bacilli have been claimed to show probiotic effects (36, 37). In fact, Bacillus subtilis strains have also been used commercially as their spores offer great manipulation and packaging advantages over other bacterial species (38). Other Bacillus strains have been studied such as Bacillus cereus var. toyoi (39) and Bacillus clausii (40). Furthermore, other gram positive bacteria have also been solidly claimed to have great probiotic potential. Different studies attribute immunomodulatory properties to strains of Propionibacterium freudenreichii used individually (41) or in combination with lactic acid bacteria and bifidobacteria in intervention trials (42). Further, humans lack the enzymes needed to metabolize oxalate, a toxic compound causing hyperoxaluria and calcium oxalate urolithiasis. This compound in humans can be eliminated through excretion in urine, forming insoluble calcium oxalate and elimination in feces, or oxalate degradation by microbiota. Oxalobacter formigenes and Lactobacillus and Bifidobacterium species are the best studied in this regard, with oxalate degradation in the lactic acid bacteria being both species- and strain-specific (43). Recently, a Bacteroides strain, closely related to Bacteroides dorei, able to reduce cholesterol was isolated from the gut microbiota of a subject with a high ability to reduce cholesterol to coprostanol (44).

Potential of probiotics to modulate microbiota
One of the major challenges found, from early research on microbiota composition, is to define the composition and complete functionality of the normal microbiota in healthy individuals. Recent studies have shown differences in the microbiota composition of healthy subjects from different locations (45–47). Could probiotic intervention strategies be developed targeted to restore this normal population profiles in cases of aberrant microbiota associated to diseases? Different research publications until present indicate that administration of Lactobacillus and Bifidobacterium probiotics did not affect the overall populations proportions in the gut microbiota; however, a significant health effect was observed, and in all cases, strains administered dominated the respective groups in the gut (48–51). A deeper understanding on the microbiome-modulating abilities of specific probiotic strains is needed and, so far, the metagenomic data available from human intervention studies with probiotics and their impact on the microbiome are limited.

Conclusions
Knowledge of intestinal microbiota development, nutrition, immunity, and specific diseases should be carefully combined with information of the genome of potential probiotic strains to find new probiotics with disease risk-modifying properties.

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Probiotics and prebiotics: health claim substantiation

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‘Probiotics’ and ‘prebiotics’ by definition should have health benefits. Health claims on microorganisms proposed as probiotics and probiotic stimulating agents (prebiotics) suggest that there is a relationship between the specific food and maintaining good health or that the food can reduce the risk of a disease. The Health Claim Regulation in European Union aims at a level consumer protection. Thereby, health claim assessment focuses on defining the probiotics and prebiotics, assessing the health relationship and evaluating studies with emphasis on controlled human intervention studies. The challenges include the focus of claims for healthy populations while most intervention studies with probiotics and prebiotics have been conducted in patients or subjects at risk of specific diseases. Another challenge is the risk reduction claim, which requires demonstrated changes in biomarkers that are generally accepted as indicators of disease risk. Existing assessment opinions from EFSA illustrate the need for further research for probiotics and prebiotics in the future.

Keywords: claims; regulation; health benefits; probiotics

The Regulation on Health and Nutrition Claims aims to ensure that consumers are not misled by false, ambiguous, or misleading claims. With the current legislation, consumers should be able to rely on clear and accurate information on food labels, enabling them to be properly informed on the food they choose. The background information and the categories of health claims and the review of scientific data on existing claims in different EU member states (Article 13.1 claims) have been reported and discussed earlier with probiotics and prebiotics as examples (1). Concerning especially gut health and probiotics and prebiotics, the European Food Safety Authority (EFSA), based on experiences gained with the evaluation of health claims, has published in 2011 a guidance document on scientific requirements for health claims related to gut and immune function to facilitate submission of application for the authorization of health claims (2). This guide addresses the beneficial effects and outcome measures that are acceptable for substantiation of claims in these areas (3).

The definition of probiotics has evolved over the years and the definition that is used most commonly is based on work of ILSI Europe and the WHO (4–11). The WHO expert group definition of probiotics states that probiotics are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (7). The prebiotic definition according to FAO states that ‘A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota’ (7). The requirements for scientific substantiation of claims are also given in the EFSA Guidance document (3).

In the case of probiotics, the main health benefits appear to be associated with maintenance of healthy microbiota or improving the resilience of microbiota, for instance, by reducing the numbers or colonization of pathogenic bacteria or viruses and by maintaining and improving the intestinal integrity and barrier function. Specific probiotics may also be associated with other health benefits including desirable modulation of lactose intolerance, bowel function and gastrointestinal comfort, diarrhea prevention and symptom alleviation, and upregulation or downregulation of immune response. A probiotic claim or a prebiotic claim is any claim, which states, suggests, or implies that a probiotic food or a prebiotic food has particular characteristics relating to its origin, nutritional properties, and health (6–8).

The evidence required for a probiotic, a prebiotic, or a combination of a probiotic and a prebiotic for a health claims can be summarized as follows:
Health Claim Substantiation

(1) Characterization of the strain or each of the strains in a probiotic mix or combination or the prebiotic components;
(2) Identification of the health relationship that is considered as a beneficial physiological effect to the target population (i.e. the general population or a defined part of it);
(3) Demonstration of health effects in a normal healthy target population.

The demonstration of the health claims has to be established in human intervention studies, and the background needed is described in Fig. 1 (3-12).

A specific challenge for microorganisms and probiotics and prebiotics has been the definition of the health relationship. Often, the claimed health relationships have been too general to assess, or they were considered by EFSA to not be beneficial. For instance, many claims attempted to document that merely increasing the proportion of lactobacilli or bifidobacteria in the gut should be considered as a beneficial health effect. EFSA has not considered a change in bifidobacteria as a generally agreed benefit, requiring further evidence associated with beneficial outcomes from human studies. So far, the evidence provided for substantiation of claims or available to the panel does not establish that increasing the numbers of any groups of microorganisms, including lactobacilli and/or bifidobacteria, is in itself a beneficial physiological effect. In fact, conceivably, the increase in bifidobacteria could even impact on potentially harmful strains; an example of this might be Bifidobacterium dentium, associated with dental caries (9).

Other examples of very general claims have been, for instance, statements such as ‘improves gut health’ or ‘boosts the immune system’, which should be more focused or identified for a specific target population.

Some claims have been judged as not eligible in accordance to the claims regulation as they pertained to treatment of pathological situations, rather than maintaining normal physiological conditions or reducing disease risk as demonstrated by the risk factors clearly indicated in the Regulation. Claims oriented at diseased people are outside the scope of the claims regulation. However, effects in specific health conditions can be accepted as evidence for effects in the general population. Some claims were constructed to reduce the risk of a disease, but the documents failed to identify risk factors. Several claims have failed because the research documents provided showed flaws in their design. Intervention studies were not always sufficiently randomized, measures for blinding subjects and/or observers were not always explained, and statistical analysis used was often either inappropriate or inadequate for a particular study design and for number of outcome measures. A flaw often encountered has been that many measures were studied, but statistical analysis failed to correct for multiple analysis. Another difficulty encountered by EFSA is that the evidence provided is insufficient to establish that the strain used in the studies is identical to the strain of the claim. There should always be sufficient information and definition of the strain used and the food constituents incorporated in the studies for substantiation of the claim.

Most probiotic studies in human subjects have been conducted in subjects who have been either ill or critically ill. A real challenge for scientists is posed by the requirement of the EU regulation where the health claims are clearly targeted for general healthy population or specific subgroups thereof, for example, elderly people, physically active subjects, or pregnant women. Such a requirement puts specific demands on human intervention studies. A review of such factors has been reported earlier (1).

Taken together, several challenges have appeared in the probiotic and prebiotic area and health claims have not been established according to the regulatory requirements. In spite of the extensive studies in the area, the focus has been outside the scope of the Regulation, as approved by the European Parliament. Thus, the research tools have to be redirected to areas that support future health claims. This can be achieved through focusing on the requirements of the regulation and through careful assessment of probiotic properties and related health outcomes. Also, the improved and more detailed guidance documents provided by EFSA will benefit all future research and applications. Learning from the experience of earlier assessment should make it possible to obtain health claims for probiotics and prebiotics in the future to provide the consumers reliable

Fig. 1. The components of health claim assessment.

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claims with new directions in helping them to make healthier choices.

Conclusions and future developments
Based on the current regulation, the documentation for substantiation of health claims for probiotics and prebiotics is challenging but not impossible. Studies have to be designed using the guidance provided by the existing regulation and the guidance documents including specific opinions published by EFSA. It is likely that new developments will be directed to achieve the levels required and acceptable specific target population and the developments will enable new products with claims to be developed.

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Nutrition economic evaluation of allergy treatment in infants and children: background for probiotic studies

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The treatment of food allergy is based on avoidance of the foods, which cause symptoms, and their replacement with nutritionally comparable foods. The cost of food allergy and elimination diets to families and society is poorly known. Our results suggest that estimation of dietary costs on the basis of dietary records was possible but challenging. In infancy, cost differences were small but vary depending on the age group with the reduction of median yearly costs around 180–240€. Thus, further studies are required for a more accurate cost estimate and an estimation of the impact of specific probiotics.

Keywords: food allergy; atopic eczema; children; nutrition; probiotic; economics

In infancy and early childhood, a balanced diet is essential to ensure age-appropriate growth and development. Dietary guidelines recommend exclusive breastfeeding until the age of 6 months and introduction of supplemental foods before the age of 6 months (1). In infants with food allergy, the introduction of supplemental foods is often delayed and the variety of foods restricted due to allergic symptoms. Mothers often feel unsuccessful when they cannot proceed according to suggested feeding guidelines (2). The organ systems most commonly involved in food allergy include the skin, gastrointestinal tract, and respiratory tract (3). Allergic reactions to food have been reported in 71% of children with severe eczema and in 51% of children who had less severe eczema initially (4). In Europe, parents perceive that 7.2% of children aged 2–3 years have food allergy (5), and diagnosed food allergy affects 5–6% of children by the age of 3 years based on double-blinded, placebo-controlled food challenge and good clinical history (6). The treatment of food allergy is based on avoidance of the foods that have been identified as allergens and their substitution with nutritionally comparable foods. The cost of food allergy and elimination of diets to families and society is poorly known. Knowledge about diet and treatment costs is needed for a basis of economic evaluations. In regard to pre- and probiotics in the treatment or prevention of eczema and food allergy, the costs of probiotic preparation should be compared against costs caused by a disease.

When two or more treatment alternatives are compared, but in which the costs and consequences of each are not examined, the terms are either efficacy and effectiveness evaluations or cost analyses. These are only partial evaluations; however, full economic evaluation includes usually both. Analyses, in which costs are related to a single, common effect that may differ in magnitude between the alternative programmes are referred to as cost-effectiveness analyses. The results may be stated as cost per unit of effect. In cost-utility analyses, utility refers to the preferences of individuals or society. The generic outcome as expressed by quality-adjusted life year (QALY) is arrived at in each case by adjusting the length of time affected through the health outcome by the utility value on a scale of 0–1 of the resulting level of health status (7).

Previous studies have evaluated the financial cost of childhood eczema, including dietary costs in Australian families. The additional annual dietary cost was 0 AUD in mild, 81 AUD (~51€ in 1997) in moderate, and 360 AUD (~227€ in 1997) in severe eczema (8). Data were collected by a questionnaire covering 12 months, but no details were given about these costs. An Italian study (9) focused also on eczema, where families were assessed by questionnaire about the use of food products with information about the name of the product, the cost,
and quantity used in 1 month. The annual cost of these food products were 117 and 452€ of family costs in moderate and severe eczema, respectively. Thirty percent of all children used food products, mainly formulas for cow’s milk allergic children, with an increase of 684€ to standard diet (9). The three most often reported cost components to families of children with eczema include hospitalization, home environmental changes, medical consultations, time off work, over the counter medicines, medications, and moisturizers (8–11).

Preference weights that are needed for a basis of cost-effectiveness analysis have been traced for different severity of eczema. Mean preference scores for mild, mild to moderate, moderate, moderate to severe, and severe eczema were 91, 84, 73, 61, and 49, respectively, in a scale of 100 (=1) (perfect health) to 0 (death) (12). In another study, the valuation survey of parents of children with eczema and general population estimated preference weights for each of 16 different health states value ranging from 0.36 to 0.84 on a continuum of 0 (death) to 1 (13). Studies related to food allergy and cow’s milk allergy have been mainly decision analyses evaluations from different perspectives like health care in from the perspective of healthcare insurers (14, 15), publicly funded healthcare systems (16, 17), and parents of infants (14). In these analyses, major costs to public health care have accrued from clinician and GP visits (44–50% of costs) and nutrition preparations (38–87% of costs). Little is thus known about dietary costs.

This study was designed to evaluate challenges related to estimation of daily dietary costs and factors contributing to these costs in infants with and without food allergy at the ages of 6, 12, and 24 months and challenges related to evaluation of food-related costs longitudinally.

**Subjects and methods**
Children (N = 80, 60.3% boys) with (n = 23) and without (n = 57) food allergy were evaluated at the ages of 6, 12, and 24 months. Nutrient intake and diet-related costs were calculated from 3-day diet records. Food prices were obtained from local supermarkets and prices of vitamin and mineral supplements and infant formula from the University Pharmacy. Growth, length of breastfeeding, and age at introduction of solid foods were ascertained. Data on reimbursements for hydrolyzed formulas used by infants with cow’s milk allergy were obtained from the Social Insurance Institution of Finland. Correlations between average daily costs and other factors were analyzed, and paired t-tests were used to analyse differences in costs. Prices of year 2006 were used.

**Results**
There were differences in energy yielding nutrient intakes between the infants with and without food allergy. Protein intake was lower (p = 0.001) and fat intake was (p = 0.04) higher in infants with food allergy at the ages of 12 and 24 months (18). The daily dietary costs for families with infants with food allergy were 1.64€ (SD 1.62, n = 21), 3.18€ (SD 1.5, n = 23), and 2.91€ (SD 0.82, n = 20) and without food allergy 1.21€ (SD 0.94, n = 55), 2.69€ (SD 0.7, n = 53), and 2.89€ (SD 0.61, n = 55) at the ages of 6, 12, and 24 months, respectively. However, the costs were not significantly higher than in infants without food allergy (12 months, p = 0.146; 24 months, p = 0.915). The infants with food allergy who used hydrolyzed formula (n = 12) had a higher daily dietary cost than those using (n = 11) soy-, oat-, or rice-based alternatives or breastfed at 12 months (3.91€ vs. 2.41€, p = 0.015). Society’s mean contribution (Social Insurance Institution) to the cost of using hydrolyzed formula was 8.67€ (SD 7.78) and 4.86€ (SD 5.15) per child at the ages of 12 and 24 months, respectively, which means that the cost to families would be much higher without a reimbursement. Daily use of probiotic preparation would add daily dietary cost by 0.5–0.7€ (2012 price) per day. Longer breastfeeding was related to lower dietary costs in infants with and without food allergy at 12 months (r = −0.58, p = 0.004; r = −0.37, p = 0.006).

**Discussion**
Nutrient intakes have been reported in studies earlier, but food-related costs have been seldom evaluated. In infancy, this is challenging when the age of introduction of infant formula or complementary feeding varies. Management of infant’s food allergy by elimination diet only modestly increased the family’s daily dietary costs. Provided that specific probiotics or prebiotics are effective to alleviating the symptoms like eczema, previous case scenarios and measured preference weights may serve a basis for calculation of QALY. This, however, means that the severity of eczema is measured at more frequent intervals with reliable and validated measures of severity and own specific measure for those having gastrointestinal symptoms, for example visual analogue scale, to identify the differences resulting from probiotic or prebiotic use. Thus, from the economic perspective, the reduction of median yearly costs should be 180–240€ (2012 value), which means a shift from severe to moderate or moderate to mild eczema. When the costs of probiotic or prebiotic use are estimated, the total reduction in costs can be evaluated either against this common measure or the number of prevented cases.

**Conclusion**
The estimation of dietary costs on the basis of dietary records was possible but challenging. In infancy, cost differences were quite small. The diet is put into practice in different ways even in infants with cow’s milk allergy. Introduction of solid foods and breastfeeding were related to lower dietary costs, but otherwise mothers...
choices when and how to start complementary feeding seems to be more relevant. In those infants with more severe allergy, hydrolyzed formulas are the main reason for increased costs. Daily average costs can be used to estimate yearly costs because more accurate data are difficult to obtain. It would require longitudinal dietary records over months, which are usually impossible to obtain.

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Nutrition economics: towards comprehensive understanding of the benefits of nutrition

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There has been an increase in the knowledge and interest on nutrition, and functional foods have gained popularity over the last few decades, and the trend is increasing. Probiotics and prebiotics are among the most studied functional foods. Nutrition economics has been defined as the discipline dedicated to researching and characterising health and economic outcomes in nutrition for the benefit of society. The concept and its application to probiotics and prebiotics will be discussed in terms of health and economic benefits and their evaluation. Health economics and concrete applications showing how to maximise long-term nutritional benefits will contribute to motivate consumers in making food choices based on a rational understanding of their own interest. We present a model that shows that nutrition economics can be used as an analytical tool for product and service network development.

Keywords: Nutrition; economics; public health; effectiveness; product development; service networks

The interaction of foods, nutrition, and public health is increasingly recognised as a key element of health and wellbeing and also a factor in quality of life. Scientific evidence is gradually unraveling the link between food and health maintenance as well as the potential of food to prevent or delay disease development.

In this context, functional foods have gained popularity over the last few decades, and the trend is increasing. Functional foods, which are found in virtually all food categories, can be described as foods that provide more than simple nutrition and that supply additional physiological benefit to the consumer (1). A substantial international market exists for functional foods both in terms of market value and variety of premium products.

Currently, in the field of health care, the economic assessment has rapidly developed, emerging from the increasing pressure on health care budgets and the growing interest in cost-effective and evidence-based health care. This has resulted in an increasing number of cost-effectiveness studies being completed – in fact a particular research discipline, called health economics, has evolved – and a growing role of health-economic arguments in policy making.

However, the fields of functional foods and health economics have only recently been interconnected (2), and reliable data on cost-effectiveness or cost benefits of nutritional interventions for health management are often lacking. The medical profession, in cooperation with the pharmaceutical industry and the hospital community, has introduced science-based economic evaluation methods for the assessment of health management programs. In this area, also standardised treatment protocols, in particular in the hospital setting, have been introduced and assessed. This has allowed to establish the health-economic principles of cost-benefit and cost-effectiveness assessments. Until now, however, no specific approach has been established for estimating the potential of food in improving the allocation of the available health care resources, in spite of a clear need by both policymakers and health professionals, as well as consumers. A recent report by the World Bank states that cost-effectiveness of functional foods in reducing disease burden and lost productivity is an important research
gap (3), even though ‘the popularity of functional foods is increasing and the effect on the food industry is evident’.

The objective of this work is to propose a schema that sketches how nutrition economics can be used to emphasise the relevance of nutritional benefits, to provide a framework for nutrition guidance and a tool for designing new foods in a perspective of personalised nutrition advice as a future procedure for individuals, companies, and societal structures. Examples will be taken from the field of probiotics and prebiotics as these are among the most studied foods for health benefits (11). The WHO expert group definition of probiotics states that probiotics are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (4). The prebiotic definition according to FAO states that: ‘A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota’ (5).

Although the functional properties and healthiness of probiotics and prebiotics will increase the nutritional quality and health benefits of a food product, consumers are different in their food choices motives (6). The critical sensory properties contributing to liking of product may be very different for consumers considering health and ethical concern as important motives, compared to consumers considering convenience, mood, and familiarity as important. Liking again is a crucial factor in enjoyability on food markets. This type of knowledge increases the challenges for development of functional food and also nutritional education.

This paper presents a novel framework of economic analysis where more traditional health economics methods are linked to product development and service network creation. The framework introduces the idea of a positive feedback system between economic analysis of nutrition and product and service network development. That is, business development can benefit from economic analysis of nutrition, and simultaneously service network is a crucial element for wider realisation of system level benefits of nutrition.

Complexity of food choices

Food choice is often a very individual decision. Food products contain many kind of sensory and non-sensory factors that may have an influence on the individual choice. Sensory factors include experiences perceived with both chemical and physical senses. Nutritional information, health claims, together with price, origin, and brand may play an important role to motivate consumers in making their choices. In addition, the consumer-related personal factors such as genetic variation, age, gender, tradition and values, attitudes, and demographic and socioeconomic status have a complex effect on the degree of pleasantness and acceptability and moreover to food perception. We live effectively in our personal sensory worlds. Based on the economic science definition of selecting foods, individual consumers may choose food items based on rational understanding of maximising long-term benefits for themselves.

Understanding the individual indicators is the key point leading us to next generation and personalised product development. More information is clearly needed for the decision making and wider efforts should be placed by the food manufacturers to develop healthy and tasty choices to promote good balanced and sustainable nutrition. In a similar manner, food service providers and nutrition professionals should propose services, which will successfully result in cost savings at varying levels – for the individual, for health care, and for the whole society. These factors should be thoroughly assessed not only to take into account the expenses of societal programmes needed to induce a change in behaviour but also especially in terms of the rising costs of so-called lifestyle diseases and stress symptoms disturbing well-being, which are increasing in prevalence. These include among others diabetes, osteoporosis, obesity, and cancer as well as the still high level of cardiovascular diseases. The balance for dietary prevention and treatment costs against health care costs and use of pharmaceutical preparations needs to be more clearly identified and cost benefits evaluated for each step in risk reduction, prevention, and treatment.

Economic aspects of nutritional benefits

Nutrition economics has been defined as the discipline dedicated to researching and characterising health and economic outcomes in nutrition for the benefit of society. Within the setting of a production-driven context, cost-effect calculations will often take other aspects into consideration, such as environmental issues and the use of natural resources. Hence, the correction mechanisms of possible positive or negative externalities add a complementary dimension, which will be discussed below based on the following three items:

(1) Maintaining health by appropriate nutrition (Better health equity for different socioeconomic classes, improved productivity, and reduced health care costs);
(2) Alleviating symptoms and reducing risk of disease by nutrition (Reduction of absenteeism from work, optimisation of resource utilisation by reduced hospital stays, and more focus on those patients who need attention);
(3) Improving wellbeing and quality of life by nutrition (increased mobility and access to services, enhanced service utilisation).

All the above-mentioned themes may at times also apply to probiotic and prebiotics. The first theme is actually the simplest one and requires only the application of
nutrition recommendations. In the food patterns of the general population, this would concern the contribution of probiotics and prebiotics to a healthy condition through their interaction with the gut microbiota. Most investigators conclude that results are promising; nevertheless, more research on the causality between products and effects as well as on the extent of the impact is needed to provide concrete estimations about the efficiency.

The second effect of nutrition might often be comparable to the one of health care, decision of a treatment can be based on cost savings (or other measures of pharmaeconomic). A good example here of can be found in interventions trials that study the impact of probiotics on diarrhoea and in particular the case of acute paediatric diarrhoea (AAD) (7) and antibiotic-associated diarrhoea (8) The incidence of AAD varies with the class of antibiotic used and with the characteristics of patients treated: AAD has been observed in a wide variety of patient populations including orthopaedic, obstetric/gynaecologic, intensive-care-unit patients, as well as ambulatory patients in the outpatient setting. One of the mechanisms by which antibiotics cause diarrhoea is by alteration of the commensal gut microflora. In such cases, a medical decision or recommendation to use probiotics aims at minimising the deleterious impact of antibiotics on the gastrointestinal flora. Currently, several initiatives are looking more closely into the health-economic consequences of these strategies and their potential to reduce health care expenditures.

In the case of prevention, the second approach becomes more complex, since the cost-effectiveness of preventive care is often difficult to measure, but on the other hand, assessment of symptom alleviation can be easier. An interesting possibility in this context is the use of modellisation techniques, as illustrated by a pilot analysis that assessed the cost-effectiveness of the use of prebiotics for the primary prevention of atopic dermatitis in an at-risk population (10). Atopic dermatitis is the most common inflammatory skin disease in children, affecting 10–15% of children in the developed world and, increasingly, those in the developing world. This study showed that the favourable health benefit associated with the use of prebiotics results in positive short- and long-term health economic outcomes.

The main differences between both are the health status of the user and realisation of the assumed benefit of the nutrition. In the case of an existing symptom, the relief can be observed more or less rapidly, while in the second case, a healthy person reduces his/her risk of disease somewhere in the future. This is presented in Fig. 1.

These themes are closely linked to the traditional health economics and methods of health economics are more or less straightforwardly applicable [for requirements of viable analysis, see (9)]. The third theme is the most challenging since general wellbeing is not very simply measurable, i.e. individual utility functions are neither very simple nor well-identified. Respectively, the effects are not very clear, and typically, there are plenty of interactions between different factors having an effect on individual wellbeing. Since effects are also often under dispute, it is justified to say that consumers having individual preference do make very much decisions under uncertainty. The consumer then knows the effect of the diet only after having tried it. If the response time is short, we can talk about experience goods in economic terms, but in the case of very long-term risk reduction type of goal, it is possible that customer never knows for sure if the diet worked. In the latter case, the product or service is called credence good. As the manufacturer producing the food has more information on the health functionality of the product than the consumer can readily verify, this can lead to asymmetric information. As a result, the ability of the functional food industry to credibly communicate will play a critical role in the marketplace success and subsequent health benefits in the population. Positive government regulations regarding heath claims and food labelling, such as those that allow food containing plant sterols to claim CHD prevention, would obviously go a long way towards ameliorating information asymmetry concerns by giving consumers as well as health care providers an impartial source of information on the credence aspects of functional foods.

The level of uncertainty and limited ability to identify the effects leads to the approach of this paper. That is, we see the market as a discovery process and in this endeavour, there should be different kind of actors, enabling not only supply and demand to exist but also to make them meet. Fig. 2 illustrates the system consisting of the economic interplay of nutrition, product development, and service (or value) network creation. The role of the economic analysis is twofold. Firstly,
economic analysis increases the knowledge about the effectiveness of the certain product, diet, or nutrition intervention. This knowledge can be used when marketing the product for the customers (individual benefits – how consumer’s wellbeing improves by using the product), applying for public support (system benefits – how consuming the product reduces health care costs), or gathering partners for larger scale supply (partner benefits – how promoting a product helps partners to fulfil their value proposition to their customers). By service network, either a healthier diet or even a simple effective product can be made familiar to more consumers. Interaction between service provider and consumer both makes the consumer more committed to using products with positive health effects and also allows to collect information about the needed product development. Hence, the service network both boosts the demand of original products and safeguards the product evolution.

This rather abstract conceptualisation underlines the importance of nutrition economics. That is, health economics and concrete applications showing how to maximise long-term nutritional benefits will contribute to motivate consumers in making food choices based on a rational understanding of their own interest. The model also shows that nutrition economics can be used too as an analytical tool for product and service network development.

**Conclusion**

A general methodology for nutrition economics is urgently needed for the background estimation and to provide tools for individual consumers, food manufacturers, food service operators, health care systems, and nutrition and health professionals. A societal impact of dietary changes should also be assessed based on costs and savings for the community as a whole.

We present a novel framework of economic analysis where more traditional health economic methods are linked to product development and service network creation. The framework introduces concepts with a positive feedback system between economic analysis of nutrition and product and service network development. Thus, business development can benefit from economic analysis of nutrition and simultaneously service network is a crucial element for wider realisation of system level benefits of nutrition for individual consumers and the society.

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Fermented milk containing *Lactobacillus GG* alleviated DSS-induced colitis in mice and activated epidermal growth factor receptor and Akt signaling in intestinal epithelial cells

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*Lactobacillus rhamnosus* GG was assessed for its ability to alleviate DSS-induced colitis in mice and activate epidermal growth factor receptor and Akt signaling in intestinal epithelial cells. In this study mice were treated with DSS to induce colitis and they were given *Lactobacillus* GG fermented milk to assess the effect of probiotic on colitis. *Lactobacillus* GG fermented milk significantly reduced the colitis associated changes suggesting a protective effect against DSS induced colitis.

Keywords: *Lactobacillus* GG; fermented milk; colitis; EGF receptor; intestinal epithelial cell

*Lactobacillus GG* (LGG, ATCC53103) is a widely used probiotic bacterium, which was originally characterized with strong adhesion to human intestinal mucus and epithelial cells. This bacterium can temporarily colonize the human colon following oral administration, and many health promoting effects have been documented during its persistence in the human intestine. Among these health benefits is its strain-dependent characterized ability to alleviate/improve the intestinal disorders of host animals such as antagonisms against various pathogenic bacteria and viruses associated with diarrhea, and inflammatory bowel disease (IBD). Therefore, additional knowledge about the underlying mechanism and key components related to these LGG actions will greatly help to characterize this probiotic bacterium much more effectively.

Recently, LGG was found to secrete two new functional soluble proteins, p40 and p75, in an artificial chemical culture medium (1). These functional proteins expressed apparent anti-inflammatory effects and regulated the survival and growth of epithelial cells in several cell culture and animal studies (2). Therefore, the production of these functional proteins might be one of the important requests for LGG to express its characterized health promoting effects to host intestinal function. However, it is still unclear if these functional proteins could be produced by LGG in the fermented milk, which is one of the most important food lines to use LGG as probiotics. The present study was conducted to demonstrate if these functional proteins could be detected from fermented milk product containing LGG (LGG-milk), and then investigate actions of LGG-milk on cultured epithelial cells and DSS-induced colitis in mice.

**Experimental**

A total of 17 commercial fermented milks were tested for the possibility to secret p40 and p75 using Western-blotting analysis with anti-p40 and anti-p75 antibodies.

Young adult mouse colon (YAMC) cells were cultured with 1:100 to 1:2,000 diluted supernatant of LGG-milk for 2 h. Cellular lysates were prepared for Western-blotting analysis using anti-phospho EGF receptor-Tyr1068 and anti-phospho Akt antibodies to detect EGF receptor and Akt activation, respectively.

In order to evaluate the preventive effect of LGG-milk on DSS-induced colon epithelial injury and colitis, female...
C57BL/6 mice were treated with 3% DSS in drinking water for 4 days to induce colon injury and acute colitis. LGG-milk (500 μl) was gavaged to mice 6 days before and during DSS treatment. Inflammation and injury was assessed using a score system.

**Results**

Fourteen fermented milk were found to contain p40 and p75, respectively. The highest concentration of p40 and p75 was found in the LGG-milk (Fig. 1). These results suggest that relative stronger ability to produce p40 and p75 might be one of the specific respects of LGG-milk.

LGG-milk activated EGF receptor and Akt in a concentration-dependent manner in the YAMC cells as same as those observed in the previous studies (Fig. 2).

The colitis (score: $6.2 \pm 0.84$) was significantly lower following LGG-milk treatment (score: $3.4 \pm 3.14$) compared to the other (Fig. 3). The shortening of the colon induced by DSS ($6.38 \pm 0.39$ cm), as a marker for colitis, was reduced by LGG-milk treatment ($7.48 \pm 0.48$ cm) (Fig. 3). These results suggest that LGG-milk may protect mice from DSS-induced colitis.

**Conclusion**

These studies suggest that LGG can secrete functional soluble proteins, p40 and p75 in the fermented milk. These functional proteins could be considered as among the key components to determine the potent health promoting effects of LGG-milk to host animal.

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**Fig. 1.** The functional proteins p40 (A) and p75 (B) identified in LGG-milk.

**Fig. 2.** EGFR signal transduction activation with LGG milk.

**Fig. 3.** Epithelial damage in mice receiving DSS with or without LGG-milk.
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Overview of technology developments in probiotic field

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Probiotics are ‘live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host’ (FAO/WHO, 2001). This requirement, i.e. that the probiotic bacteria must be in viable form at the time of consumption, poses a number of technical challenges from food processing perspectives. Environmental stresses encountered during food processing include acid exposure during food fermentations, extremes in temperatures encountered during drying processes, in addition to oxidative, osmotic, and food matrix stresses. Furthermore, the ingested bacteria must remain viable during gastric transit, to reach the site of action in viable form to exert the probiotic effects. This imposes further stresses, as the gastrointestinal tract is naturally designed to impede the passage of microorganisms with low pH encountered in the stomach and the detergent-like properties of bile encountered in the duodenum. A number of approaches have been investigated in order to minimise the damage caused by exposure to such stresses experienced by probiotics during food processing and gastric transit. Approaches for protection of probiotic viability during food processing and shelf life include manipulation of bacterial cell physiology, application of prelethal stress to the cultures during cell preparation, selection of appropriate drying conditions, and optimisation of reconstitution conditions after drying. Furthermore, probiotic viability losses can be minimised by selection of appropriate food carriers for their delivery to the intestine. In this respect, the composition and physical nature of the food matrix can have profound effects on the stability of live probiotics during gastric transit. Encapsulation of probiotics is another approach to positively affect viability of probiotics in some matrices. Furthermore, it is important to understand the mechanisms underlying bacterial survival in hostile environments in order to develop efficacious functional foods delivering the benefits associated with the probiotics within.

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Prebiotics and probiotics – the importance of branding

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The costs of developing a probiotic or prebiotic ingredient have always been substantial. Ingredient characterization, evaluation of technological and physiological properties, and demonstrations of safety and clinical efficacy require expensive research. The demanding regulatory requirements imposed by EFSA raise the bar even higher so that the costs of acquiring the necessary clinical evidence to support labeling of these food ingredients is approaching that of pharmaceuticals. In order to justify investment in such expensive clinical development, companies require certainty that they can gain a return on investment. Patenting can provide some protection but is not always possible to patent ingredients, and the period of protection is limited. All ingredients eventually face the prospect of commoditization once patents expire. Branding strategies offer one means of maintaining adequate product differentiation to protect market share and margins over the long term.

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Probiotics and atherosclerosis – a new challenge?

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Background
Atherosclerosis is the major cause of cardiovascular disease and stroke, which are among the top 10 leading causes of death worldwide. Pathogen-associated molecular patterns (PAMPs) can activate toll-like receptors (TLRs) and activate nuclear factor kappa B (NFκB) signaling, a central pathway in inflammation, which regulates genes that encode proinflammatory molecules essential in atherogenesis. Lipopolysaccharides (LPS), which is unique to gram negative bacteria, as well as peptidoglycan (PGN), which is found in gram positive bacteria are PAMPS and ligands of TLR4 and TLR2, respectively, both of which are essential in plaque progression in atherosclerosis. Gastrointestinal tract is suggested to be the major site for absorption and translocation of TLR2 and TLR4 stimulants. Inflammation can result in a ‘leaky gut’ that leads to higher bacterial translocation, eventually the accumulation of LPS and PGN would activate TLRs and trigger inflammation through NFκB and promote further systemic complication like atherosclerosis. Probiotics, can protect the intestinal barrier to reduce bacterial translocation and have potential systemic anti-inflammatory properties.

Objective
To evaluate whether probiotics can help reduce atherosclerotic development using in vivo study.

Design
Apolipoprotein E knockout (ApoE⁻/⁻) mice were fed on high fat diet alone, with telmisartan (Tel) (1 or 5 mg/kg/day, positive controls) or with probiotics (VSL#3/LGG) with or without Tel (1 mg/kg/day) for 12 weeks.

Results
Probiotics, Tel, or a combination of both reduced lesion size at the aortic root significantly; VSL#3 reduced serum inflammatory adhesion molecules soluble E- (sE-)selectin, soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), and plaque disrupting factor matrix metalloproteinase (MMP)-9 significantly; probiotics and Tel at 5 mg/kg/day could induce changes in gut microbiota population; the efficiency of lesion reduction seemed to correlate to the microbiota composition; probiotics seemed to reduce plasma endotoxin but did not reach statistical significance.

Conclusion
Probiotics has the potential to be used as a cheap, non-invasive, and with little side effects way to reduce atherosclerosis that brings worldwide benefits.

Keywords
ApoE⁻/⁻ mice, LGG, VSL#3, gut microbiota, cardiovascular disease

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Probiotics and dental caries

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Dental caries is one of the most common infectious diseases in the world. Dental caries can be defined as destruction of the tissues of the tooth by bacterial fermentation of dietary carbohydrates. In this respect, some bacteria are considered more caries-promoting than others, as an example Streptococcus mutans. A common feature for caries-promoting bacteria is that they are acidogenic and aciduric.

The most common bacteria used as probiotics, lactobacilli and bifidobacteria, are, in theory, caries-promoting. They are excellent acid producers, they tolerate low pH-values, and they are frequently found in caries lesions. Since probiotics can be consumed several times a day and they are even given to infants, it is essential to know that they are safe.

All studies so far indicate that probiotics have rather beneficial than adverse effects on the caries risk. The best-studied probiotics Lactobacillus rhamnosus GG (LGG), Lactobacillus reuteri, and Bifidobacterium lactis BB-12 (BB-12) colonize poorly the oral cavity of adults (1–3). A recent study shows that BB-12 administered to infants twice a day showed poor retention to the teeth and oral mucosa of the infants (4). Transient colonization reduces the possible risks for oral health.

Little is known about the effects of the above probiotics on the composition of the oral flora. Short-term consumption of LGG, L. reuteri, and BB-12 have in some studies reduced counts of S. mutans (5, 6). Also, the amount of dental plaque (biofilm on the teeth) has been reduced by some probiotics. Dental plaque is not only a caries risk factor but also associated with periodontal diseases. A recent study showed that consumption of LGG and L. reuteri did not result in an increase in the plaque acid production potential, an important virulence factor of plaque (7).

There are only a few studies in which occurrence of caries has been studied in connection with consumption of probiotics. Näse et al. (5) found that LGG milk reduced caries occurrence in 3–4-years-old children. Milk with L. rhamnosus LB21 and fluoride reduced caries occurrence in school children (8). Reversals of root caries lesions were studied with L. rhamnosus LB21 milk w/wo fluoride – in that study, not only the fluoride-containing milks but also the probiotic milk had beneficial effects on root caries (9). Recently, caries occurrence was studied in 4-years-old children who had received BB-12 twice a day during infancy. The administration of the probiotic did not increase the caries occurrence (Taipale et al., unpublished results).

Clearly, long-term clinical studies with the disease occurrence as the primary outcome measure are needed to establish beneficial versus adverse effects of probiotics on oral health. Optimally, ‘old’ probiotics with proven benefits for general health could be also be used to benefit dental health.

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Prebiotic developments

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Prebiotics have been defined recently more as a concept than a functional ingredient group: ‘The selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host’. This definition has in common with earlier definitions that modulation of microbiota composition and/or activity is its cornerstone. This has long been interpreted as an increase in faecal bifidobacteria; the so-called bifidogenic effect. However, recent regulatory evaluations have, rightfully, made clear that this is not sufficient and cannot be interpreted as a health benefit an sich. Therefore, a change of paradigm might be necessary.

While there is general agreement that the intestinal microbiota plays an important role in maintenance of health, there is insufficient understanding on the precise role the different components of the microbiota play here. Even the role of the microbiota in disease is poorly understood. A number of enteric pathogens is, of course, well known. However, many gastrointestinal disease or complaints have, as yet, an unknown aetiology. Thus, while it may be desirable to reduce levels of known pathogens, it is not clear what the target should be for, e.g. irritable bowel syndrome, inflammatory bowel disease, or even antibiotic associated diarrhoea (where known pathogens explain at most 50% of the cases).

Every individual has his or her own unique intestinal microbiota. It is reasonable to assume that, when a subject is in a healthy gastrointestinal state, the intestinal microbiota is optimal or close to optimal for that particular individual. Not only prebiotics but also probiotics could help in maintaining this composition when it is exposed to challenges; improving its resilience. Current molecular biological techniques make it possible to assess the full microbial composition of a faecal sample. We do not need to worry about the fact that we do not know the majority of our intestinal inhabitants or that we focus only on the groups we like (bifidobacteria?) or dislike (potential pathogens). Improving general microbiota resilience would be potentially a valid target for, e.g. prebiotics.

Another important point in the current (and previous) definitions of prebiotics is the health benefit on the host. Health benefits observed in association with consumption of prebiotics may coincide with changes in the faecal microbiota. A well-designed study will be able to unambiguously indicate the causality between the consumption of the prebiotic (or any other active ingredient for that matter) and the observed health benefit. However, it will be more difficult to substantiate a causal link with changes in the microbiota and an observed health benefit. Even when other confounding factors can be excluded, it will be challenging to unequivocally prove the causality due to the current limited mechanistic understanding of host-microbe interactions. Therefore, future prebiotic research should focus on clear measurable health benefits.

The mechanistic understanding of the interaction between the intestinal microbiota and the host and the implications of this for host health is a specific expertise that should study this. It is relevant not only for pre- or probiotics or functional foods at large but also has even implications for medicine and pharmacology.

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Health claims on foods: challenge for clinical research companies

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Background
The Nutrition and Health Claim Regulation 1924/2006/EC, together with EFSA guidances on the scientific requirements for different types of health claims, is setting the basis for health claim substantiation in the EU.

Aim
The aim of this presentation is to bring up the key challenges that the food industry and clinical research organizations are facing when meeting these requirements.

Results and discussion
Key issues in clinical research planning to meet the requirements set for the health claim substantiation are: (1) Selection of right outcome markers since the selection of outcome marker defines actually the formulation of the health claim to be used on food or food ingredient. (2) Selection of right target population since that determines the target consumer group for the food with a health claim. (3) Selection of dose regime and food matrices used since these largely determine the conditions set for the use of the health claim. One of the major challenges in health claim substantiation is the deviant approach to risk factors or biomarkers. From the regulation point of view, a single risk factor approach is emphasized, but from the clinical and scientific point of view the pattern of different risk markers or biomarkers could, in some cases, be a more relevant choice to reflect the final health outcome. This is especially the case in the nutrition and health area because we are often dealing with weak but multiple health effects of certain food items or ingredients. Also the lack of validated well-established biomarkers potent to be affected by diet is a challenge in health claim substantiation.

The selection of right target population is often a compromise between choosing a more potential target group to obtain efficacy (i.e. risk factors elevated vs. patient groups) and choosing a rationale to generalize the results to wider population (target consumer) group.

The selection of optimal dosing regime and matrices for a clinical study is partly dependent on previous scientific data on the dose response, if existing. But equally important is the choice of feasible doses from product formulation and food consumption point of view.

Conclusion
With careful analysis of the existing data, advance planning and clinical research strategy it is possible to build up a health claim substantiation that meets the requirements both of Nutrition and Health Claim Regulation and EFSA.

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The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

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