Microbial–Host Interactions: Selecting the Right Probiotics and Prebiotics for Infants

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Abstract

Probiotics were originally used to influence human health through intestinal microbiota alterations. At present, probiotics and their effects on human health have been demonstrated both within different food matrices and as single or mixed culture preparations. The health-promoting properties are known to be strain-dependent. Thus, strain identification and characterization are important: only well-characterized strains identified with modern techniques are acceptable, especially if health claims are desired. Linking the strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies are important targets. Currently there are specific strains which have demonstrated beneficial in vitro properties and clinically proven health benefits. Such specific probiotics have been included in recommendations on pediatric nutrition. The model is the microbiota of the healthy breastfed infant. Molecular methods in microbiota assessment enable more specific probiotics and prebiotics to be identified for infants with aberrancies in intestinal microbiota. Probiotic products require information on the concentration and viability of the strain(s) in the product as well as data on required dosages. Continuous control of probiotic strains or strain combinations is a must as small changes in production process or growth media may significantly affect the properties of a strain or strain combination.

Introduction

A probiotic has been defined as a ‘live microorganism which when administered in adequate amounts confers a health benefit on the host’ [1]. A
prebiotic is a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota [2].

Probiotics were originally used to influence human health through intestinal microbiota alterations but now also other modes of action. At present, the probiotics and their effects on human health are studied both within food matrices and as single or mixed culture preparations [3].

A reliable probiotic product requires an exact identification of the bacterial species and strains used and concentration and viability of the strains in the product as well as data on required dosages. Continuous control of probiotic strains or strain combinations is important as small changes in the production process or growth media may significantly affect the strain or strain combination properties.

Detailed characterization of the intestinal microbiota in the target population forms the basis of probiotic use. In infants, the target is defined as the healthy breastfed infant with the microbiota programming for long-term health. Aberrancies in microbiota preceding diseases, such as atopic diseases, form the target for primary prevention using probiotics to counteract the deviations. Aberrancies in infants and children with allergic diseases may require different probiotics for secondary prevention.

Prebiotic definition has further developed from one with an impact on beneficial microbes to the current one with demonstrated human health benefits [2]. To differentiate from probiotics, prebiotics are substances that can be characterized as non-digestible food ingredients, for example carbohydrate components, which influence health by modulating the gut microbiota. Prebiotics can be fibers, but not all fibers are prebiotic. The definition also covers non-carbohydrate components such as protein. The challenge for prebiotics is to define the target aberrancy in microbiota and to select the prebiotic that will not deteriorate an existing microbiota deviation, as no new microorganisms are incorporated.

**Healthy Microbiota Development in Infants**

Intestinal colonization prior to and following birth represents the earliest contact with microbes and the original inoculum derived from the mother. The first microbes and the succession of microbiota provide important stimuli for maturation of the intestinal immune system as well as intestinal development and the acquisition of oral tolerance [4, 5]. They contribute to the maintenance of intestinal homeostasis and mucosal barrier function. Deviations or delays in this dynamic interaction may lead to non-optimal microbiota development and predispose to disease later [6]. Thus, early establishment of a healthy gut microbiota, hallmark of that of a healthy breastfed infant, provides the key step towards long-term wellbeing. Breastfed infants have less allergies, diarrhea and respiratory and gastrointestinal infections than
formula-fed infants due to the specific composition and properties of breast milk. The major difference in the microbiota of breastfed and formula-fed infants may lie in numbers, species composition and metabolic activity of bifidobacteria, explained by the presence of bifidogenic factors in breast milk [7]. In addition, breast milk also contains microbiota-modulating bacteria including bifidobacteria [8]. The origin of these microorganisms has been debated but an endogenous origin has been suggested [9]. The mammary gland may be colonized by bacteria, which arise from the maternal gut, skin or the interaction of the breastfed infants gut bacteria [9]. Such phenomena have been reported already during late pregnancy [9]. Dendritic cells penetrate the gut epithelium to directly take up bacteria from the lumen allowing live bacteria to reach other locations through the blood stream within the dendritic cell or otherwise in a dormant state. Moreover, an increase in maternal intestinal translocation appears to occur after birth. Theses mechanisms may explain the presence of maternal gut bacteria in breast milk.

In the healthy breastfed infant, lactic acid bacteria may account for less than 1% of the total microbiota, whilst bifidobacteria can make up 60–90% of the total fecal microbiota [10, 11]. The predominance of bifidobacteria may have an important impact on later health [6]. Furthermore, allergic and healthy infants harbor different bifidobacteria [12], indicating a need for a better characterization of the healthy breastfed infant’s microbiota using methods assessing the total microbiota profile and the modulatory effects of breast milk oligosaccharides and bifidobacteria. Promotion of the bifidobacterial microbiota has been taken as a target for nutritional intervention in infants [18]. The most common approach includes supplementation of prebiotic substances to infant formulae. However, current prebiotic supplements differ significantly from the complex and dynamic mixture of oligosaccharides naturally present in human breast milk. Probiotic lactobacilli and bifidobacteria have also been used, but the strains usually found in present preparations differ from those found in breastfed infant feces. Mimicking breast milk oligosaccharides and bacteria requires careful selection, microbiological and immunological characterization, preclinical and clinical assessment of the components alone or in combination and, finally, stringent safety assessment. This will lead to selection of improved probiotic bacteria and prebiotic components for allergy prevention and health maintenance, the healthy breastfed infant microbiota being a model and goal.

**Intestinal Microbiota Deviations**

Combining these data with the first molecular and culture-based studies has suggested that significant deviations in early intestinal microbiota may precede the development of atopic diseases [6]. Less diverse microbiota composition has been associated with the development or presence of atopic
diseases [6]. Kalliomäki et al. [6] reported a reduced ratio of bifidobacteria to *Clostridium histolyticum* group bacteria at the age of 3 weeks in infants who later became atopic. There is supporting evidence that allergic 3-month-old infants have higher counts of clostridia, but the species distribution needs to be verified. Also, the total composition of *Bifidobacterium* species differs in infants later developing allergy [6].

Deviations or aberrancies in the composition and activity of bifidobacterial community between healthy and allergic children or children later developing atopic disease have been reported [13, 14]. Infants developing allergies have a more adult-type of *Bifidobacterium* species, such as *B. adolescentis*, already early in infancy [unpublished data; 6]. These are potentially derived from the mother indicating that the mother–infant transfer of microbiota is influenced by probiotics [8] and further promoted by breast milk [15].

The recent development of high-density microarrays has enabled the comprehensive analysis of microbiota composition in a more universal and culture-independent way [16]. Therefore, the previous reports of infants in whom atopy was or was not developing during follow-up have to be re-assessed. Fecal microbiota composition at the age of 6 and 18 months using a

<table>
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high-throughput phylogenetic microarray allowing the simultaneous detection of virtually all bacterial phylogenotypes present in the intestine has lead to an overview of the total microbiota. These studies have confirmed the earlier findings [6] and further emphasize that bifidobacteria are key players in healthy gut microbiota development with *Bifidobacterium longum* as the most important species in the infant population. It is also clear that specific clostridia clusters need to be further characterized and potentially other minor components of microbiota including *Akkermansia* may be associated with the development of atopic eczema [unpublished data]. Further studies are ongoing to assess the microbiota differences at the total profile level, and specific subgroups between allergic and non-allergic infants form the basis for selecting the right probiotics and prebiotics for prevention studies.

**Search for New Probiotic Strains for Allergy Prevention in Infants**

Clinical and experimental studies demonstrate the importance of the gut microbiota in guiding the inflammatory responses in the infant. The gut microbiota of the healthy breastfed infant constitutes a good source of probiotic strains but several factors influence the outcome (fig. 1). In addition, orally consumed probiotics pass along the entire gastrointestinal tract and therefore, by selecting new candidate probiotics from members of the normal microbiota, the strains are likely to have the prerequisite survival and specificity. Probiotic bacteria should also counteract the inflammatory process by stabilizing the gut microbial environment and the intestine’s permeability barrier, particularly via intestinal IgA and/or mucin production. Probiotic effects may also be mediated by control of the balance between pro- and anti-inflammatory cytokines [13].

The genus *Bifidobacterium* constitutes an attractive supplement for infants due to both its predominance in the gut microbiota of the healthy breastfed infant and its excellent safety records. Lactobacilli and bifidobacteria colonize mainly the small and large intestine, respectively. Bacteria isolated from human milk are attractive as probiotics for neonates since they fulfill some of the criteria generally recommended such as human origin, a history of safe prolonged intake by infants, and adaptation to mucosal and dairy ecosystems. Among the bacteria isolated from human milk, species of *Lactobacillus* and *Bifidobacterium* are considered among the potential probiotic bacteria and, actually, some strains are included in probiotic products. The use of culture-independent molecular techniques has further revealed the occurrence of lactobacilli and other lactic acid bacterium species, Gram-positive bacteria including *Bifidobacterium* and certain Gram-negative bacteria in human milk of healthy women [8, 9, 17]. Comparison of the microbiotic composition in the mother, mother’s milk and the infant can verify the presence of specific species and strains.
An effective probiotic should reside at desirable target sites sufficiently long and at sufficient concentrations to elicit probiotic effects. Probiotic strains have to overcome different gastrointestinal conditions, such as pH in the stomach, bile and digestive enzymes in the intestine, in order to survive and reach their site of action. These may be important for probiotic action. Different probiotics differ in their levels of tolerance to gastrointestinal conditions. In addition, these gastrointestinal conditions may also modify the properties of probiotics. For instance, the effects of gastrointestinal conditions (pH, bile, digestive enzymes) and the effects of acid and bile resistance acquisition on the adhesion of probiotic bacteria have been documented. The presence of difference substances such as calcium ions or exopolysaccharides produced by probiotic bacteria can modify the bacterial adhesion to intestinal surfaces.

Other main selection criteria for probiotics have included competitive exclusion of pathogens. Probiotics compete directly or hinder the adhesion of pathogens on stereo-specific receptors on the intestinal surface. This may also influence the development of intestinal microbiota in infants. The outcome of microbiota development and competitive exclusion depends on the specificity of the bacteria and bacterial adhesins for the receptors and the relative concentration of the competing bacteria. Adhesion on the intestinal surface lengthens the retention time of a probiotic, and it is particularly important for the small intestine due to the short transit time. The effective

**Fig. 1.** Why probiotic effects differ?
dosage of a probiotic is determined by the relative affinity for the receptor sites. *Lactobacillus rhamnosus* GG, which has been reported to have good adhesion and colonization abilities, was found effective in shortening rotavirus diarrhea in infants. On the contrary, *Lactobacillus bulgaricus*, which could not adhere to and colonize intestine, had no effect on infant diarrhea. Adhesion to the intestinal mucosa has been related to certain beneficial effects of probiotic lactobacilli. Similarly, a highly adhesive strain *Bifidobacterium lactis* Bb12 has also been found to be effective. The ability of probiotics to adhere to intestinal mucus is likely to reflect the persistence of a probiotic in intestinal contents, but it may not necessarily be related to their capacity to successfully adhere to intestinal tissue. Adhesion may also be different depending on the target population. Thus, adhesion of the strains to intestinal mucus or tissue from the specific target population should be assessed for strain selection. Moreover, adhesion and even temporary multiplication of probiotic bacteria at the target sites would result in enhanced concentrations of probiotics at the optimal places of action, achieving the desirable responses even at a lower dosage [33]. However, it is not clear whether all probiotics can grow in the intestinal environment. No commercial probiotics have been reported to be able to establish permanently in the human intestine [20]. However, long-term colonization studies are required to further understand the microbiota effects following early probiotic administration. It has been shown that some probiotics attach to intestinal mucosa and can be recovered in biopsies much longer than in feces. Thus, adherence studies need to complement fecal recovery assessment, preferably in biopsies. Focusing on adhesion and immune modulation, preferential binding of probiotics on the specific antigen-processing cells (macrophages, dendritic cells) may be even more important than adhesion to epithelial cells.

**Immunomodulatory Potential of Probiotics: A Model for Preclinical and Clinical Testing**

Many of the probiotic effects are mediated via immune regulation, in particular via control of the balance of pro- and anti-inflammatory cytokines, as well as by improving the intestine’s immunologic barrier, particularly intestinal IgA responses. Data are accumulating to suggest that even closely related probiotics possess different adhesion and other in vitro properties, this possibly explaining differences in the immunomodulatory performance [13]. The cytokine patterns induced by bifidobacteria or lactobacilli have been shown to be strain-specific, and different *Bifidobacterium* strains may induce distinct and even opposing responses [13]. Probiotic bacteria have been demonstrated to induce regulatory cytokine production and T-regulatory cells’ in vitro and in vivo induction of IL-10 and TGF-β production, and modification of Th1 and Th2 cytokine production has also been reported. Specific probiotic bacteria
have been shown to counteract inflammatory processes also by stabilizing the gut microbial environment and the intestine’s permeability barrier, and by enhancing the degradation of enteral antigens, thereby reducing the antigenic load [for review see, 24].

Recent in vitro and clinical studies indicate that differences in the immunomodulatory effects exist between candidate probiotic bacteria [19, 21]. Moreover, distinct regulatory effects have been detected in healthy subjects and patients with inflammatory diseases. On this basis, important prerequisites for successful selection of probiotics requires rigorous preclinical characterization of the specific strains on the one hand, and profound understanding of the pathogenetic mechanisms of the target disorder on the other. Only then can probiotics/prebiotics be used as innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction, and downregulate hypersensitivity reactions, which comprise their principal effects thus far.

To take an example, the benefit of probiotic intervention in children with atopic eczema and cow’s milk allergy indeed lies in cessation of the vicious circle of inflammation, clinically manifested as control of disease activity, promotion of normal growth, and a shortening of the duration of symptoms [21]. In accordance with such a concept, the beneficial effects of probiotic-supplemented extensively hydrolyzed formula on eczema severity were only observed in children with proven cow’s milk allergy on challenge and not in those with negative cow’s milk challenges. The latter group of patients thus manifested no specific treatment target for orally administered probiotics or extensively hydrolyzed formulas [22, 23]. On the other hand, Lactobacillus GG and Lactobacillus GG and B. lactis together prevented atopic dermatitis and atopic sensitization, respectively [24], while the non-probiotic Lactobacillus acidophilus had no preventive effect [25].

Other Desirable Properties of Probiotics

Other desirable properties include their impact on pathogens, inhibition, competitive exclusion, displacement and aggregation to prevent the action of pathogens. It has been reported that these can easily be characterized in vitro, but the challenge lies in defining combinations of probiotics that beneficially influence microbiota development, the metabolic activity of microbiota and other host–microbe interactions required for healthy development.

Selection of New Species of Probiotics and Interaction with Pathogens

The most commonly used probiotics are natural intestinal strains of Lactobacillus and Bifidobacterium species. Other intestinal microbes may also play a beneficial role in human health. The complexity of the gut microbiota provides a very promising source of new probiotics. D-Lactate production may have deleterious effects in individuals with gut resections or intestinal inflammation, but lactate-utilizing bacteria may contribute to reduce D-lactate levels within the large intestine.
Bacteria found in breast milk, such as staphylococci and streptococci, can be useful to reduce the acquisition of undesired pathogens by the newborn. It has been shown that viridans streptococci inhibit oral colonization by methicillin-resistant *Staphylococcus aureus* in infants. Interestingly, the presence of viridans streptococci is a feature characteristic of the healthy infant gut in contrast to that of atopic infants [26]. *Escherichia coli* can be isolated from breast milk and the species is also among the first colonizers of the infant gut. Although this species harbors 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 nonspecific natural immunity in both full-term and premature infants.

**Combining Specific Probiotics**

Specific combinations of probiotics may be selected for complex microbiota deviations or aberrancies. Under such circumstances combinations of probiotics may together have an impact on specific microbiota deviations or as single strains in different parts of the gastrointestinal tract [34]. The impact of strain combinations can be antagonistic, compatible or synergistic and such effects should be characterized when constructing strain combinations for specific targets [34].

Strain combinations can be tested in vitro and reasonably reliable results can be achieved. In some cases probiotic combinations show clear benefits as described in combinations counteracting the adhesion of *Enterobacter sakazakii* [27] or in adhering toxins and heavy metals, and thereby decontaminating the gastrointestinal tract.

The in vitro tests on combinations have assessed a *L. rhamnosus* (two strains), *Propionibacterium freudenreichii* and *B. lactis* combination. This combination was then demonstrated to be effective in clinical studies on irritable bowel syndrome patients [28]. The same probiotic combination did not have an impact on the alleviation of allergy symptoms in infants, while *L. rhamnosus* GG was effective as demonstrated earlier [29]. When the combination was given along with a galacto-oligosaccharide (GOS) the resulting effect was atopic eczema prevention [30]. Thus, these reports demonstrate the significant potential of identifying microbiota aberrancies and selecting suitable probiotic combinations to counteract them also in the case of atopic diseases.

**Search for New Prebiotics for Allergy Prevention in Infants**

Traditionally, bifidogenic factors mean prebiotic substances promoting bifidobacterial growth in the gut. As this still holds true, it should be considered
that bifidobacteria from breast milk and formula supplemented with bifido-
bacteria can also be ‘bifidogenic’. This is also the case for other components 
of breast milk, including anti-inflammatory factors and cytokines. Thus, the 
term bifidogenic needs to be redefined to provide a new basis for probiotic 
and prebiotic identification and characterization also using suitable matrices 
for their administration. However, bifidogenic factors, in addition to microbes, 
can also include other substances which differ between breast milk and for-
mula milk.

The difference in the microbiota of breastfed and formula-fed infants lies 
in numbers and species composition of bifidobacteria. Breast milk bifidogenic 
factors include carbohydrates and peptides [35]. Human milk typically con-
tains a very complex mixture of oligosaccharides at a concentration of about 
7–12 g/l, constituting an important fraction of human milk [36]. Promoting 
the Bifidobacterium biota is often considered a treatment target and very 
commonly the approach to this end includes the supplementation of prebi-
otic carbohydrates to infant formulas. Oligosaccharides from human milk 
have been found to inhibit the adhesion of enteropathogens, to prevent diar-
rhea [37], and to stimulate cytokine production and activation of cord blood 
T cells [38]. Unfortunately, oligosaccharides in the milk of domestic animals 
are less complex, structurally different, and present at much lower concen-
trations that those in human milk [36]. This indicates that, although it would 
be almost impossible to find sources containing oligosaccharides identical to 
those found in human milk, it is very important to select or develop oligosac-
charide mixtures resembling those found in human milk.

Supplementation of infant formula with a mixture of GOS and fructo-oligo-
saccharide (FOS) stimulate bifidobacterial growth in both preterm and full-
term infants. This increase in fecal bifidobacteria by prebiotic consumption 
was found to correlate with a reduction in the carriage of potentially patho-
genic microorganisms. In addition, formula supplementation with prebiotics 
(FOS/GOS mix) has been reported to increase fecal concentrations of secre-
tory immunoglobulin and, moreover, in a randomized, double-blind, placebo-
controlled study administration of prebiotic oligosaccharides during the first 
months of life reduced the number of infectious episodes. Interestingly, the 
same mix (GOS/FOS) induces a microbiota composition that resembles that 
found in breastfed infants.

Although the number of studies focusing on the use of prebiotics to pre-
vent allergy is very limited, in a randomized, double-blind, placebo-controlled 
trial with infants at risk of developing atopy, the consumption of formula 
supplemented with a prebiotic mix (GOS/FOS) was found to be protective. 
The results showed a reduction in the development of atopic dermatitis at 6 
months of age. The follow-up of these infants indicates that this protective 
effect is still present at 2 years of age [30]. In another study, Arslanoglu et al: 
[31] administered a probiotic mix and GOS to a very large number of infants 
during the first 6 months of life and found a significant reduction in atopic
eczema. Whether this effect was due to the probiotic mix, the prebiotic or to their combination requires further clarification.

**Conclusion**

The selection of probiotics and prebiotics for infants at risk of allergies or already suffering allergic diseases poses different challenges: identification of the gut microbiota development resulting in a healthy outcome, and modifying an already deviated microbiota. Several new approaches are available with the model of a healthy breastfed infant as the target. This sets requirements for probiotics and prebiotics, and requires careful analysis of healthy and aberrant microbiota associated with these conditions. In the case of breast milk, it is likely that both specific probiotics or probiotic combinations and specific prebiotics are needed. Current developments in probiotic and prebiotic research have made it possible to personalize this approach.

Infant formulas have become more similar to human milk through supplementation with different protein fractions, probiotic bacteria and oligosaccharides. Further developments to obtain more ‘humanized’ formula milk continue, resulting in specific probiotics that mimic breast milk bacteria, counteract intestinal microbiota deviations associated with allergic diseases, and provide protection against the specific pathogens and viruses associated with them. In the follow-on formulas, new oligosaccharide compositions and specific probiotic bacteria, more closely resembling the components of human milk, will improve microbiota development and prevent microbiota deviations, and maintain healthy microbiota in formula-fed infants.

**References**

Salminen/Collado/Isolauri/Gueimonde


Selecting Probiotics and Prebiotics


Discussion

Dr. Björkstén: I have two questions that I would like you to clarify. You mentioned that children developing atopy have more diverse microbiota. I would say that they have a less diverse flora, and that is based on studies that have been published in Sweden by Adlerberth et al. [1] and also our data from Estonia and Sweden.

Dr. Salminen: I think we are challenging the scientific community because we do the diversity index by doing a total microbiota profile, which is completely different from what has been done before. It is also completely different because there is an increasing number of unculturable microbes that have never been analyzed before, and to me this means that something special is happening during the acquisition of microbiota. You need a certain amount diversity, but perhaps not too much in the early phase, more in the later phase.

Dr. Björkstén: My second question relates to what you said about pre- and probiotics and the state-of-art. Of course I agree with what you said, but I would like to know whether it was limited to children because you did not mention *Saccharomyces boulardii* which keeps cropping up?

Dr. Salminen: The target of my talk was infant probiotics, so that I think defines the phase because there are lots of others that are important and effective if we look at adults, and that we should consider. But there are no data on the infant population yet, and I also think a lot of people would ask about some safety questions.

Dr. Szajewska: I am very much interested in bacteria in breast milk and that some of them are potentially probiotic bacteria. Could you please comment on the source of these bacteria?

Dr. Salminen: We know that some bacteria are obviously from the skin; some of them are derived from the mouth of the infant when there is a sucking movement, an exchange of bacteria, exchange of saliva, breast milk, and they are certainly thriving well in the breast milk. Once they are there, they will grow and keep well, but then there is a challenging study from the Nestlé Research Center which still needs some confirmation. There is an animal model pointing out that gut bacteria can travel to the mammary gland and can be then reutilized. We have to remember that, when dealing with galacto-oligosaccharides, the bacteria has a fantastic ability to adjust to different circumstances. There are also so-called dormant bacteria which are not alive by culture methods, but can actually travel in a dormant state in our body fluids and perhaps change the place independent of the origin, but that remains to be shown in the future.

Dr. Szajewska: You mentioned that viability is an important property of probiotics. Can you say whether it’s important that they are viable at the time of administration or
at the site? I think at the time of administration we can really prove their viability, but it might be much more difficult to show it or prove it later.

Dr. Salminen: Personally I do think that viability is not as important as we think, but on the regulatory basis I have to think about the definition that we use and the definition is viable microbial cultures.

Dr. Szajewska: We have some evidence that probiotics do not have to be viable.

Dr. Salminen: We do have a first decision from the European Food Safety Authority, not in infant foods but in animal feeds, where nonviable, rather non-culturable, bacteria in animal feed have been approved for animal feed use. This is the first time in regulatory history that proof of viability has been provided by using molecular methods on a dormant bacterium. If I talk to the WHO people, they will surely silence me if I dare consider that nonviable bacteria can be used as probiotics. But in the back of my mind, I do think that the bacterial cells can play a role and function. Our Japanese collaborators have always said that they don’t need to be alive to have an effect.

Dr. B. Koletzko: You started off by emphasizing that prebiotics should be documented as being beneficial for health. I think this is an important part of the definition if one looks at the products being sold, and for many of these products there is no scientific evidence. But you also emphasized that the definition of prebiotics is limited to non-digestible carbohydrates. From a scientific basis I wonder whether this is a wise concept? You would assume that carbohydrates serve as a source of energy metabolism of microbes, whereas clearly microbes won’t grow and won’t be able to metabolize actively without a source of nitrogen and a number of other components. A number of studies have shown that iron, phosphorus, pH, and protein are all relevant for gut microbiota. So are we missing opportunities to influence health if we limit this to carbohydrates only?

Dr. Salminen: You are absolutely right here, and here I also have the scientific hat and the regulatory hat. Unfortunately it is so that in the regulatory world we have to find definitions that are more or less agreeable for everybody, and currently the FAO/WHO definition goes along that line. My view is that this will be expanding because it will be rather rate-limiting in the chemical sense to stick to that. So we have the scientific avenue and the regulatory avenue, and for a while they will go different ways and hopefully find each other in the end.

Dr. B. Koletzko: You alluded to the potential of modifying maternal flora by providing oligosaccharides. We published a paper in the American Journal of Clinical Nutrition in which a small group of women was supplemented with oligosaccharides during pregnancy. While there was clearly an effect on bifidobacteria in the maternal stools, it was very disappointing that infant microbiota were not affected at all with that intervention, and also some measures of immune response in the neonates were not affected. But my question is really along the thoughts that Dr. Szajewska just raised. The data on bifidobacteria and Lactobacillus reuteri in breast milk there were about $10^2$–$10^3$ bifidobacteria reported. I was very impressed by a recent study by Kvist et al. [3] on 466 healthy women. They very nicely characterized the bacterial counts in human milk collected under apparently clean conditions, and found $10^6$ Staphylococcus aureus, $10^6$ coagulase-negative staphylococci, $10^6$ group-B streptococci, $10^6$ Streptococcus viridans, $10^6$ Enterococcus faecalis, and so on, and obviously very few infants get infected. We think there is a mechanism, such as sIgA in human milk, that protects infants from becoming infected by these huge bacterial loads. Is there a differential effect of these potential pathogens and commensals in breast milk?

Dr. Salminen: I am sorry to say that I omitted most of the other microbiota that have been characterized in breast milk, and they vary from place to place, from
country to country, from one individual to another; I have sometimes said that looking at the data from Spain, for instance, breast milk is almost like yoghurt, looking at the concentrations of bacteria there. But when considering breast milk microbiota in terms of probiotics, I actually omitted them because at the moment, again from the regulatory hat, we cannot consider their use as probiotics because we have to have something that’s absolutely safe and does no harm to anybody who is taking them, and therefore I kept to the bifidobacteria and lactic acid bacteria and even the specific species in there. But yes, it is a combination; mostly the same bacteria you find on the skin of a breast or on the skin of a mother. But applying that to a probiotic or prebiotic concept I think is very difficult because this is the part which will always be the individual exchange between the mother and the infant and it is adjusted to their living environment and provides local protection. What I had to do here is to look for possibilities to provide overall protection or overall beneficial effects. In those terms I think I can safely exclude the other types of bacteria, especially considering what we heard from Dr. Kasper about Bacteroides fragilis, and we have similar information about S. aureus. There are different expressions and different types of the S. aureus, some might perhaps even be useful for e.g. adult probiotics, but I would not apply them for infants at this state of knowledge.

Dr. Lentze: I have a question in relation to the content of microbiota in allergic children. You stated that children with eczema have a different composition of microbiota. The question is what is the hen and what is the egg? Do you mean, for instance, breastfed infants with eczema have a different composition of microbiota or does it depend on the nutrition of these children?

Dr. Salminen: I am sorry if I was not clear. First of all I was not comparing children who have eczema or those who don’t have eczema; I was comparing the microbiota of a cohort of children some of whom later developed eczema and some who didn’t. So these were the changes before any signs of eczema or differences before any signs of eczema were shown. Dr. Björkstén has looked at the differences when you already have different allergic diseases in the Swedish and Estonian population; so we have a different starting point. We start from what is there early: does it predispose you to something; is there a way of preventing the predisposition; is there some sort of inflammation prevention effect that we could apply by modifying the microbiota? Then if you already have a disease, we have to take a different approach to try to return and maintain that healthy gut microbiota. So there are two different points where we look at the same challenge.

Dr. Björkstén: We have done both. We looked at the gut microbiota at 5 days and 1, 3 and 6 months, and then outcome allergy eczema by age 2 years, and checked with regard to breastfeeding or not breastfeeding. We have both done similar studies on that. In addition to that we have of course seen differences in established disease.

Dr. Baerlocher: You mentioned the environment as an important factor. Nowadays fathers are taking more interest in the care of their newborns. What is the role of the father as an environmental factor?

Dr. Salminen: I think the father plays a role as an environmental factor because he already played a role prior to birth. There was an exchange of bacteria even before the infant was born. So he definitely plays a role, but in the early weeks of life I think the role of the mother is still more significant.

Dr. Baerlocher: When we want to know anything about probiotics, we refer to meta-analyses. From your title, Selecting the Right Probiotics, do you think it’s wise to use meta-analyses that comprise all probiotics which are nowadays available?

Dr. Salminen: Actually we have a meta-analysis expert here, Dr. Szajewska has even taught me some of the matters that are important for meta-analysis. My personal view is that it cannot be done, but somehow perhaps you could apply the information
on breast milk composition and breastfeeding and the health effects on the meta-
analysis, and perhaps combine these two factors together.

**Dr. Szajewska:** Yes, I would agree with the last sentence that it’s better to combine the two. It gives an idea of how to pull the data on all probiotics, if all the probiotic strains show the effect in the same direction, it gives you additional information that probably probiotics might be effective for this particular indication. However, it’s very important for every meta-analysis to include subgroup analysis based on probiotic strains. At our center, we now try to perform only such meta-analyses that evaluate one probiotic strain. If not, we pool data from different strains, and we always include subgroup analysis based on individual strains.

**Dr. Mack:** From what I have heard and my understanding is that the molecular techniques have shown that the human microbiota can become quite stable. Is there evidence that the functional properties of the human microbiota are mobile?

**Dr. Salminen:** No, it’s a different perspective if we look at what is happening in the infant and what is happening in you or me. The difference being greater in the infant because we do have a successive development of microbiota, whereas some things actually show up for a while, have an influence on the total development, disappear, and we can’t find them anymore. If you look at the temporal variation, this has already been shown by the data of Palmer et al. [4]. But somehow there is direction towards where it is going, and it’s individually fluctuating on a daily basis but going towards microbiota that are sort of predefined by the original inoculum, by the environment where the baby is born, by the feeding, by the mother, by the breastfeeding. So the fluctuation is greater there and I think we need a little bit more of these metagenomic studies to understand what it means to the infant, and it is less for you and me at our age.

**Dr. Haschke:** One of your slides indicated that higher concentrations of probiotics in food supplements seem to work better, i.e. are more effective in diarrhea prevention and treatment. If we look at the concentrations of lactobacilli and bifidobacteria in human milk, they are low, almost 3–4 logs lower. We have no clue how much we should put into an infant formula, all our knowledge comes from yoghurt and this is a very rough estimate. Maybe we are completely wrong and should be much more sensitive to what is in breast milk. Could you comment on that?

**Dr. Salminen:** This is a very good question and I am again sorry if I have not been clear. The study looking at the dose response was actually a collection of information mainly from infants suffering from diarrhea. Of course if you do have a problem, you probably need more, and there is a clear dose response. You are absolutely right in the fact that the breast milk levels are much lower, but here we are talking about the daily dose and you are talking about the number of bacteria, perhaps per milliliter. If you actually do have a significant breastfeeding volume you get higher, but not quite as high as in the diarrhea studies, but you do get higher exposure. I think you are right in the sense that all of what we understand from breastfeeding and the bacteria in breast milk is needed. In a similar way to what Dr. Prescott was saying about the detrimental things, you need a constant low exposure. That is probably what provides the best outcome; you are not shuffling with the system too harshly to do something that might be unwanted by selecting the right strains, by selecting the right prebiotics if you need them, and low-dose constant exposure. From what we know at the moment, I think that is probably the best choice.

**Dr. Isolauri:** You probably need to clarify some points raised here because there again appears to be confusion about older papers based on culture techniques and the more recently developed culture-based methods. The second point is the diet. Can you summarize how important it is to look at both the diet as well as the techniques?
**Dr. Salminen:** It is a very important thing to look at the diet and the probiotics and prebiotics. Again we are unfortunately in the situation that we cannot really compare the results that we get today to something that was achieved 5 years ago because the methodology has changed so much. What was true 5 years ago is not true anymore because we know so much more about the total microbiota. So far there are very few papers, the numbers are increasing every day, looking at the whole gut microbiota profiles, which really gives you an indication where subtle changes happen and where perhaps corrective action should be taken. But by the time we get the proceedings of this symposium in the press, I think we can add 5 or 6 new quite qualified papers to explain this a little bit further.

**References**
