Opportunities for Improving the Health and Nutrition of the Human Infant by Probiotics

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Abstract

The newborn is first colonized by microbes at birth. The colonizing bacteria originate mainly from the mother's gut, vaginal tract and skin. The origin of the microbiota and its development depend on genetics, mode of delivery, early feeding strategies and the hygienic conditions around the child. The indigenous microbiota of an infant’s gastrointestinal tract is modulated through contact and interaction with the microbiota of the parents and the infant’s immediate environment. After delivery breastfeeding continues to enhance the original inoculum by specific lactic acid bacteria and bifidobacteria and bacteria from the mother's skin enabling the infant gut microbiota to be dominated by bifidobacteria. These bacteria set the basis for gut microbiota development and modulation along with breastfeeding and the environmental exposures such as antibiotic administration. Modifying this exposure can take place by probiotic bacteria when breastfeeding is not possible. Thus, incorporating specific probiotics selected for the development of the infant’s gut microbiota may form a beneficial possibility for future infant feeding purposes. Many current probiotics have documented strain-specific health-promoting effects, and most of the effects that have been demonstrated in infants and children. The target in infants is to modify the gut microbiota to resemble that of the healthy breastfed infant and to counteract deviations or aberrancies present in infants at risk of specific diseases. Thus, providing specific selected probiotics to the mother to balance the intestinal microbiota during pregnancy and to the infant after birth. As the disturbed succession during early infancy has been linked to the risk of developing infectious, inflammatory and allergic diseases later in life, it is still of great interest to further characterize both the composition and succession of microbiota during infancy. With new methodologies we have been able to identify more specific aberrancies in microbiota prior to or during different disease states.
Introduction

According to current thinking, the newborn is colonized by microbes at birth. The colonizing bacteria originate mainly from the mother’s gut, vaginal tract and skin, and bacteria can transfer from the gut to the infant as shown by probiotic intervention studies [1]. The origin of the microbiota and its further development depend on genetics, mode of delivery, early feeding strategies and the hygienic conditions around the child. The indigenous microbiota of an infant’s gastrointestinal tract is modulated through contact and interaction with the microbiota of the parents and the infant’s immediate environment. Bacteria are present in the environment of the infant prior to and after delivery, and breastfeeding continues to enhance the original inoculum by specific lactic acid bacteria and bifidobacteria enabling the infant’s gut microbiota to be dominated by bifidobacteria. These bacteria set the basis for gut microbiota development and modulation along with breastfeeding and the environmental exposures. Modifying the exposure can take place by probiotic bacteria when breastfeeding is not possible. Thus, incorporating specific probiotics selected for the development of the infant’s gut microbiota may form a beneficial possibility for future infant feeding developments.

Many currently used and proposed probiotic bacteria have a scientifically demonstrated effect on human health [2, 3]. Most clinically demonstrated effects have been shown in infants and children. These may take place through a modifying effect on the relatively simple gut microbiota in infants. To improve infant nutrition we have to consider the development of gut microbiota along with the means to influence the process through dietary intervention with specific probiotics. The target in infants is to assist the development and maintenance of gut microbiota to resemble that of the healthy breastfed infant and to counteract deviations or aberrancies present in infants at risk of specific diseases. This forms the basis for healthy individual gut microbiota which promotes the health and wellbeing of the infant. Thus, providing specific selected probiotics to the mother to balance the intestinal microbiota during pregnancy and to the infant after birth will have an influence on the healthy microbiota development and wellbeing.

Probiotics: The Definition

Probiotics are live microbial food supplements with demonstrated positive effects on host health [2, 3]. This definition indicates that not all lactic acid bacteria or bifidobacteria are probiotic bacteria. The safety and efficacy of each probiotic strain has to be demonstrated in carefully controlled human clinical studies [2, 3]. Scientific reports often refer to probiotic properties without specific documentation in intervention studies. However, even closely related species and strains may have significantly different health
properties and thus have to be documented individually. An example is found in *Lactobacillus rhamnosus* studies. When two closely related *L. rhamnosus* strains were assessed in the treatment of rotavirus diarrhea, one was found to be effective and the other had no clinical effect on the duration of rotavirus diarrhea [4]. In other studies, viable *L. rhamnosus* GG (ATCC 53103) was reported effective in alleviating atopic eczema in infants. The same strain in a nonviable form was ineffective and caused side effects when administered to a similar infant population [5, 6].

Probiotics often act by modifying the process of intestinal microbiota development or by affecting the composition and activity of developed microbiota [3]. In addition to microbiota effects, probiotics can also act by direct contact with the mucosal cells facilitating cross-talk between the host and microbes [7]. Current probiotics have several demonstrated beneficial effects on infant health. These include the maintenance of healthy intestinal microbiota development and counteracting deviations observed in gut inflammatory diseases or preceding them [8, 9]. The best studied effects have been demonstrated in the treatment and prevention of rotavirus diarrhea [10–12]. Other studies have been reported on the prevention of antibiotic-associated diarrhea with specific *Lactobacillus* GG [13, 14]. Probiotics have been demonstrated to reduce the risk of necrotizing enterocolitis in preterm neonates and, meta-analyses suggest that this may be the case in infants with less than 33 weeks gestation. Many of the described effects have been verified in meta-analysis studies, but only the most recent ones consider probiotics on a strain-specific basis [15–18].

**How Do Probiotics Differ?**

Each probiotic strain is a unique bacterium with its own strain-specific properties as already demonstrated when the strain differences were discussed. The unique properties are today best described by assessing the strain properties and some example strains. Genomic information has revealed a lot of new data on the probiotic properties. As an example, *Lactobacillus acidophilus* NCFM is a very acid tolerant strain which, upon entering the stomach, activates its adhesion ability by contact with stomach and bile secretions and adheres to the small intestinal mucosa [19, 20]. *Bifidobacterium longum*, on the other hand, is adapted to the lower part of the intestinal system to utilize breast milk oligosaccharides or intestinal mucus secretion as nutrient [21]. This makes *B. longum* an excellent bacterium in the intestinal tract of a breastfed infant. The strain also has special adhesive systems which may include microfimbriae to facilitate its adhesion to intestinal mucosa and probably also immune effects through this contact [21].

It has been suggested that combining specific probiotics may be useful in counteracting complicated microbiota deviations. However, combining
probiotics has also yielded significantly different clinical effects. An example again is the efficacy of *L. rhamnosus* strain GG (ATCC 53103) which has alleviated symptoms of atopic disease in infants [10]. On the contrary, when the same probiotic was administered in combination with *Bifidobacterium lactis*, *Propionibacterium freudenreichii* and *L. rhamnosus* LC 705, no clinical effect on symptoms of atopic eczema were observed [22]. In a prevention study, where the combination was administered to mothers prior to delivery and infants after birth, an effect on the prevention of atopic eczema was observed [22].

As all microbes have shuttle differences which may influence clinical outcomes, it is especially important to define the probiotic strains used as well as possible.

**Intestinal Microbiota**

*Gut Microbiota in Neonates*

The first colonizing bacteria in the infant gut originate from the mother's gut, vaginal tract and skin. Prior to birth the fetus is considered microbiologically sterile. However, this has been challenged recently by the demonstration of exposure of the fetus to bacteria in the amniotic fluid and through the placenta [23]. Thus it appears that the newborn is exposed to bacteria already prior to birth and the source of the original inoculum may vary. How the microbiota originates depends on genetics, mode of delivery, early feeding strategies, and the hygienic conditions around the child. Recently, it has been suggested that stress and dietary habits during later pregnancy and prior to birth may have a significant impact on the microbiota at the time of delivery thus influencing the quality and quantity of first colonizers of the newborn [24, 25].

*Breast Milk*

Human milk is generally considered the optimal nutrition for neonates as it contains the required nutrients and some protective compounds needed by the neonate. The diet of the mother has an effect on breast milk composition including the fatty acid composition and this in turn influences the adhesive properties of specific bacterial species and strains of bacteria. Many of the components such as oligosaccharides and fatty acids influence early microbial colonization of the infant gut. There is abundant evidence that breast milk also transmits bacteria to the infant gut [26, 27]. Many pathogens have been shown to arise in breast milk from the mother's skin and more bacteria from the infant's gastrointestinal tract and mouth [26]. However, breast milk also contains a natural bacterial inoculum which has an impact on the developing infant's microbiota [27, 28]. Perez et al. [28] have shown that some bacterial signatures in breast milk are common in the infant's feces and mother's
blood samples. In mouse studies translocation from the gut to mammary gland via mesenteric lymph nodes has been described [29, 30].

Transfer of Breast Milk Microbiota
Expressed human milk contains commensal bacteria which are specific to the environment and the mother. Breastfeeding exposes the milk duct to skin bacteria and also intestinal bacteria from the neonate. In normal breast milk the bacterial content depends on contamination with the skin, skin bacteria in general, breastfeeding (feedback from the infant) and contamination with gut bacteria. Additionally there are the bacteria originating from the mother’s gut. These bacteria and their combinations accelerate the adjustment of the infant’s gut microbiota to adapt to the environment of the mother and child. They are likely to have a significant impact on the succession and establishment of the type of microbiota (fig. 1).

In recent studies it was demonstrated that breast milk contains around 3,000 bacteria/ml and an average of almost $10^3$ bifidobacteria/ml [26, 27]. The milk from an individual mother may contain from 1 to 5 different bifidobacterial species. Transfer of the maternal bacteria via breast milk may be a natural means of reinforcing the colonization of the gastrointestinal tract of the neonate. Thus, breast milk forms a unique personalized source of bacteria typical of the particular environment in which the mother and infant reside.

Impact of Breast Milk Microbes on the Health of the Child
The microbial provocation originally obtained from the mother changes over time. Yet it appears that large numbers of bifidobacteria and lactic acid bacteria form an essential supplementary bacterial inoculum to the breastfed infant. Generally, a competitive exclusion effect between breast milk microbes and their impact on gut microbiota is a factor that may shape the succession of microbiota during breastfeeding and weaning. By reinforcing the original inoculum from the mother and by providing breast milk oligosaccharides, the mother acts as a constant source of both probiotic bacteria and prebiotics to the newborn. This supplementation is likely to modify and shape the normal gut microbiota. As several studies have suggested a protective impact of breastfeeding against different intestinal tract-associated diseases, the colonization effects may provide one explaining mechanisms for the observed effects [30, 31] and suggest a basis for the development of healthy gut microbiota.

Succession of Microbial Communities
The step-wise process of indigenous microbiota establishment begins with facultative anaerobes such as the enterobacteria, coliforms, lactobacilli and
streptococci first colonizing the intestine. These are rapidly succeeded by bifidobacteria and lactic acid bacteria.

The development of infant gut microbiota is usually characterized by the following steps: early colonization at birth with facultative anaerobes depending on the mode of delivery with rapid succession by anaerobic genera such as \textit{Bifidobacterium}, \textit{Bacteroides}, \textit{Clostridium} and \textit{Eubacterium}. Molecular methods indicate that lactic acid-producing bacteria may account for less than 1% of the total microbiota while bifidobacteria can range from 60 up to 90% of the total fecal microbiota in breastfed infants. In formula-fed infants the microbiota is more complex, but depends on the composition of formula. The new techniques indicate that the greatest difference in the microbiota of breastfed and formula-fed infant lies both in the bifidobacterial numbers and species composition within the intestinal microbiota, while the lactic acid bacteria composition appears to be rather similar. \textit{Bifidobacterium breve}, \textit{Bifidobacterium infantis} and \textit{B. longum} are frequently found in fecal samples of breastfed infants, whereas the most common lactobacilli in both breastfed and formula-fed infant feces constitute \textit{L. acidophilus} group microorganisms such as \textit{L. acidophilus}, \textit{L. gasseri} and \textit{L. johnsonii}. In general, the differences between the breastfed and formula-fed infants have decreased along with the improved composition of infant formulas [32].
**Probiotic Effects on Immune Responses**

Specific probiotic bacteria are involved and may assist in the development and maintenance of the gut immune homeostasis by either influencing microbiota composition and metabolic activity or by directly modulating immune responses through gut mucosal signaling pathways. These mechanisms strengthen the epithelial barrier function and may inhibit pathogen adhesion to mucosal surfaces and consequent growth and invasion. Probiotics can interact with the mucosal immune system via the same pathways as commensal bacteria, specifically via interaction with epithelial cells and dendritic cells. Through this interaction there is an influence on both innate and adaptive immune responses. Probiotic effects on immune responses appear to be immune-regulating rather than immune-activating. In vitro and in vivo studies in mice and humans have shown that probiotics may predominantly modulate dendritic cell and T regulatory cell activity rather than T-helper responses per se. For example, *Lactobacillus casei* Shirota strain was shown to increase the Th1 response in an animal model of diabetes but to inhibit Th1 responses in an animal model of allergic disease, suggesting an effect at the T-regulatory cell level [8–10]. As with probiotics in general, each effect is unique to a defined strain and no extrapolation can be made on the properties of closely related strains.

**Probiotic Benefits to Infant Nutrition**

The best documented benefits of specific probiotics have been demonstrated in the reduction in the risk of gastrointestinal diseases such as necrotizing enterocolitis, rotavirus diarrhea, antibiotic associated side effects, and the treatment and prevention of atopic diseases. Probiotic combinations, apart from *B. lactis* Bb12 and *L. rhamnosus* GG combined, have not been successful in infants. Several intervention studies, especially on atopic diseases, are currently on their way, under evaluation and being published as indicated in a recent review [33].

The practical benefits of specific probiotics and specific probiotic combinations in infant nutrition may lie in the early microbiota modification.

First, modifying the microbiota of the pregnant mother is important. This approach may provide benefits to the microbiota and the wellbeing of the mother during pregnancy by influencing both the composition of intestinal microbiota and the consequent metabolic activity. Specific bifidobacteria and lactic acid bacteria have been demonstrated to be transferred from the mother to the infant during delivery and during breastfeeding [34]. Thus, the balance of mother’s intestinal tract microbiota and vaginal microbiota may influence the outcome in the infant. Microbiota may also predispose infants to later health problems as has been reported for atopic diseases [35] and recently for obesity development [36, 37].
Second, specific probiotic bacteria may be important for providing microbial stimulus to the intestinal microbiota during early infancy to assist in establishing a healthy gut microbiota and the barrier against harmful microbes and detrimental dietary components. Similarly, as the infant receives lactic acid bacteria and bifidobacteria via breast milk during breastfeeding, these are likely to deliver some of the beneficial effects associated with breastfeeding, such as protection against diarrheal diseases, atopic diseases and even obesity. Thus, it may be important to correct potential deviations in infant microbiota and to offer formula-fed infants bacterial stimuli in a form of safe probiotic lactic acid bacteria and bifidobacteria. It could be that the probiotic bacteria-supplemented formulae may better mimic the effects provided by breastfeeding. These aspects have been discussed by the North American and European expert group reports [38, 39].

Conclusions

The most important focus point in probiotic research for infant nutrition is to recognize the individual properties of specific probiotics. Each strain is different and the properties of each strain and the combinations of each strain are unique. Therefore, the scientific documentation behind probiotics always focuses on specific probiotic strains or specific probiotic combinations.

The current scientific contributions to infant health clearly include shortening of the duration of rotavirus diarrhea, reduction in risk of rotavirus diarrhea, reduction in the risk of antibiotic-associated side effects, alleviation of atopic eczema symptoms, and prevention of atopic eczema, and balancing of the deviated infant microbiota.

The healthy human microbiota is metabolically active and acts as a defense mechanisms for our body. Deviations in its composition are related to multiple disease states within the intestine but also beyond the gastrointestinal tract. Components of the human intestinal microbiota or organisms entering the intestine may have both harmful or beneficial effects on human health and clearly the genomic approach on the human infant side and the probiotic side will assist in formulating new approaches to benefit infant health.

The available information focuses mostly on the crucial role of infant microbiota and the first colonization steps to later health. Especially bifidobacteria play a key role in this process. The mother–infant contact has an important impact on initial development. The mother provides the first inoculum at birth, promotes the bifidogenic environment through prebiotic galactooligosaccharides in breast milk and introduces environmental bacteria through her skin and other contact with the infant, thus providing the means to promote guidance to the development of individually optimized microbiota under the existing environmental conditions for each infant.
The future target is to further clarify both the sequelae and the succession of microbial communities especially during and after weaning and during the first years of life. Another target is to understand the use of specific probiotics and prebiotics to influence microbiota development and maintenance as well as dietary management of the reported health-related microbiota deviations.

**Potential Future Approaches**

The knowledge on intestinal microbiota acquisition and intestinal microbiota development increases rapidly. This facilitates the understanding on the impact of probiotics on intestinal microbiota composition and metabolic activity. Successful probiotics have two main characteristics: the ability to colonize the infant intestinal tract, influence the microbiota, and its composition and activity effects and immunological properties. These have been the main targets for probiotics. Recent studies have also indicated that intestinal microbiota can act on the host signaling system to have an impact on host health. Such developments enable the thorough understanding of intestinal microbiota and help to identify the right probiotic strains or combinations to counteract or to normalize particular well-characterized microbiota aberrancies. These will require carefully designed intervention studies to establish the suggested effects and to apply them to practical infant nutrition guidelines and practices.

**References**

Discussion

Dr. Björkstén: Thank you for this elegant presentation and I agree basically with everything you said. However, I would like to suggest that perhaps we should have an alternative approach. One may question the evidence for strain specificity. I think that the traditional reductionist approach of one molecule or one specific bacteria may not be an optimal approach. The internal ecology is as complex as the ecology of a jungle. Going for one specific strain would of course be wonderful for the industry.

Dr. Salminen: To a certain extent I would agree and I was actually not promoting a single strain approach at all. I was rather demonstrating that especially in the infant gut microbiota, when we try to modulate it, we have to be primarily safe rather than sorry. Therefore we have to select the safest alternatives, and I am quite convinced that these alternatives won’t work for you and me. With such a complex environment, only in early childhood do we have the luxury of a simple microbiota which can be influenced in a simple way. That is exactly the reason why most of the studies showing clinical efficacy to probiotics are actually from infants.

Dr. Björkstén: I agree with this. I think at this stage it is alright to include various strains of lactobacilli in the meta-analyses, provided that the studies were done properly. It may be that differences in the outcome of various studies are more a consequence of different environments than of different strains. You quoted Dr. Prescott’s and my recent review summarizing the allergy-prevention studies [1]. Three of them were positive and one negative. Although there were different strains, the major difference between the studies may actually be that in the negative study, treatment only started after birth, while in the three positive studies treatment started in the pregnant mothers.

Dr. Salminen: That is certainly one explanation, but I still question and I have asked the producers several times to provide information on the strain that was used in the negative study, because it would be very interesting for the scientific community to understand what the properties are in a particular strain that might have influenced the outcome. So far I have not had an answer from the producer concerning the strain or the preclinical and safety studies on it.

Dr. Hernell: 25 years ago Dr. Björkstén and myself were involved in writing guidelines for milk banks in Sweden and there was always concern about the bacterial contamination of breast milk – that was before the hygiene hypothesis. When the bacterial count in the breast milk was too high, we recommended that the mothers clean their breasts and nipples and the amount of bacteria dropped very significantly. Have you checked what happens with the bifidobacteria in the milk if the mother cleans her breast?

Dr. Salminen: We of course tried to have sampling procedures as clean as possible; if the sampling procedures were not clean we would have had much higher concentrations.
For instance, there is a study from Spain that almost suggested that breast milk like yogurt has $10^7$ CFU/ml and I am sure that part of those bacteria were really from the sampling procedures and from the skin. For us having perhaps $10^2$ to $10^3$ CFU/ml, I think this is realistic and we significantly reduced contamination by doing that. The important thing that should also be discussed is that when we think about personalized nutrition, breast milk is really personal in the way that the bacteria are also adjusted to the environment of the mother. It is meant for that particular baby living in that particular environment, it is not meant for somebody living in New York City or somebody living in Japan, but that particular surrounding; that is what makes it virtually impossible to personalize the bacterial content unless the approach is to use a safe bacterium, a specific member of the healthy intestinal gut microbiota, in infants who remain healthy for years to come.

Dr. Prescott: I have more of an answer than a question. The strain that we used is also known as LAFTI-10. It was used by Clancy et al. [2] who recently published a paper on it. It is produced by DSM Foods in Australia. The company that provided it to me asked us to use a different trade name, so I hope that helps.

Dr. Salminen: That certainly helps but it also returns us to our discussion yesterday that the basic principle in all studies would be to use the International Culture Collection numbers so that we would know what type of bacteria there is.

Dr. Prescott: Absolutely, and as I previously indicated we commenced this study in the 1990s before any of the other studies had been published. It was very difficult to know which strain to choose in any of these studies, and our choice was limited by what was available at the time. There was also very little information in the area at the time, so I am sure you agree.

Dr. Savilahti: You started by remembering our mothers as the source of gastrointestinal microflora, and then you said that some lactobacilli come from vegetable sources. How large a proportion of gastrointestinal flora originates from nonhuman sources, from the soil, from drinking water, from the feces of other animals? It is known that children who grow up on farms have fewer allergies, which is thought to be because they inhale lipopolysaccharides but they actually also eat feces from the cattle, and we know that if there are a lot of cats and dogs, allergy is reduced. Do these other sources permanently change our microbiota?

Dr. Salminen: I don’t think it is important whether they permanently change; what happens in early childhood is. For instance we haven’t seen the probiotics that are used today to permanently colonize anybody, but it is important how the success of the microbiota is facilitated during early life, and certainly the success is different if you live in a different environment. Several speakers have pointed out that there are differences between countries and certainly if we live in the US, Europe, Australia, Japan, most of our food technologists have always been taught to kill all the bacteria in our food supply. We have worked together with Indonesian researchers, and there is a totally different environment. There are the natural lactic acid bacteria, bifidobacteria from all environmental sources, from the food supply, from water, from the hygienic conditions, the exposure is totally different. So basically we are trying to reverse what food technology has done to us, trying to introduce some of the friendlier bacteria back, the ones that we have always wiped out during the last 50–60 years.

Dr. Savilahti: Are these bacteria human specific or are they specific for other species?

Dr. Salminen: Bacteria are very difficult. I know that the early definition of probiotics actually said that we should have bacteria of human origin, but what is of human origin? Does it mean that any bacteria we have eaten and stays for a while in our intestinal content, or something that is only adhering to our mucosa that we never see in the fecal samples? I think it is easier to define what you have, especially in infants; you
have a healthy gut microbiota defined in such a way that those children remain healthy for years to come, and you look at the composition and the types of safe bacteria there that you can take. We have all sorts of passersby that cause transient diarrhea in adults and children; we have viruses that cause transient diarrhea, change the metabolic profile and activity of our intestinal microbiota, so it is quite difficult to say whether something is really of human origin or whether it is specific to humans. We then also have to understand the adaptability of bacteria. There was a wonderful article on \textit{Bacteroides fragilis} in \textit{Science} 2 years ago \cite{3} and, just by its position in the gastrointestinal tract, a single strain can take at least 12 different phenotypes that act totally differently, and it depends on the stimuli provided by the intestinal mucosa, by the interaction with the intestinal mucosa, and the same bacteria which we analyze in the feces is one form but it could be 12 different forms in the upper intestinal tract. So bacteria are very adaptable, and whether they are really specific or not to one mammalian species I wonder. We did a study with Dr. Arthur Ouwehand in our laboratory where we compared the species specificity, looking at opossums from Australia, crocodiles, cats, cows, humans, and actually there was no real species specificity \cite{4}. Adhering bacteria adhered; if you want to displace a pathogen you can do it in an opossum or a crocodile as well as you do it in a human. So the idea is to select the ones that are really members of what is healthy and normal to your species.

\textbf{Dr. Isolauri:} I want to summarize what we mean by identifying the strain and identifying the population. Our studies \cite{5,6} are frequently compared with that of Brouwer et al. \cite{7}. In our studies \cite{5,6} at weaning breastfed infants were given extensively hydrolyzed formula with or without probiotics, then after that treatment period they were challenged and then only those who had cow’s milk allergy fulfilled the study criteria. The opposite was done in the Netherlands study \cite{7}, in which formula-fed infants of the same age were given extensively hydrolyzed formula, and then an open challenge was made with cow’s milk which was negative. So even though the patient population was infants, some were allergic, some were not, some had gut involvement, and others didn’t, but then they had different microbiota to begin with. So it is also very important to chose the food matrix and the target population.

\textbf{Dr. Klassen-Wigger:} Thank you for your presentation that nicely summarized the current evidence on the efficacy of probiotic strains that can be of interest for an infant population. However, some of the strains that you were referring to are D-lactate producers and as such current regulation does not allow their use in infant nutrition. Do you think that the scientific community is trying to pave the way for changing this legislation or at least trying to get a bit more evidence about this?

\textbf{Dr. Salminen:} I think that is a very good and complicated question. If I look at it from the point of view of the regulatory authorities, for instance the European Food Safety Authority (EFSA), safety is of primary importance, and that is why there are hurdles that actually do prevent many of the new products from becoming available on the market. If I recall correctly, this matter of lactic acid product safety was studied for one of the strains beforehand, but it still doesn’t exclude the fact that, especially in German-speaking countries, the regulation is very strict. So that is another point, harmonizing the regulations even within the countries of the European Union is happening only little by little. Going back to safety, there is also the EFSA safety assessment procedure. We currently have the qualitative presumption of safety document which is available on the EFSA website (http://www.efsa.europa.eu/EFSA/efsalocale-1178620753812_1178667590178.htm). I actually recommend all of you to have a look before you go into a clinical intervention study. If you go to the EFSA website, search for QPS, you will find a number of documents that actually list the safety studies and safety assessment of different types of probiotic products in Europe. Again in the US it is a totally different ball game of course, and as far as I understand there is no such
list on the FDA net basis at the moment. But EFSA is really focusing on that, not only in the human area but also in the animal probiotics area, and there are very strict criteria for the safety assessment, which will probably make it even harder for some of these products to find their way to the consumers, or lengthen the time at least. But on the other hand, as I said, it is better to be safe when you deal with bacteria.

Dr. Björkstén: We have actually looked at D-lactic acid production by one of the probiotic Lactobacillus strains and also looked at the evidence behind the caution for D-lactic acid-producing bacteria. The studies were merely suggestions from the 1960s rather than studies and there is no novel documentation. It just shows how the bureaucracy hangs around for ever. So I don't think that it is an issue. We have always said that the mother is the source of the bacteria and that is obviously true for some strains in vaginally delivered babies. Dr. Hernell referred to studies that were done when we were younger. Dr. Gothefors in our team showed for Escherichia coli that it was equally common that the baby got the strain from outside and subsequently colonized the mother. This was under somewhat other hygienic conditions 35 years ago in Sweden than we have today. So it may be more complicated than only blaming the mother.

Dr. Salminen: I always say that you can't blame your mother; you can only thank your mother for what you have got. She has certainly done her best and under the circumstances given what can be given. It is a process and not only the mother; what has happened before defines it. We actually have a couple of papers in which we describe this mother–infant transfer of microbiota, and it must be taken into account that it is not always possible for the mother to understand or have an impact on what she is giving her offspring [8–10].

Dr. Walker: I want to come back to what Dr. Isolauri mentioned several times, that is the matrix in the succession of bacteria. Could you comment on that?

Dr. Salminen: I think the genomic information that I showed very simple examples of is actually quite important. For instance, let's think about health foods; quite often we take these capsules that pass all these triggers which are important for at least those 3 sample model strains. It should also be carefully considered whether the bacteria in a food matrix thrive on that particular matrix or whether they are given in a dormant state. I think this is as important as the strain itself.

Dr. Koletzko: Allow me to come back to the question of D-lactic acid and the comment that Dr. Björkstén made. I fully agree that in most infants and children it is probably a nonissue, but I would be careful with concluding that it is not a safety concern. We have been measuring D-lactic acid for a number of years now and have regularly seen cases of D-lactic acidosis in children with short bowel syndrome. The symptomatology is exactly as that which veterinarians describe as the drunken cow's syndrome. It is a neurological disturbance which most people probably would not associate with D-lactic acid until they have actually measured it and seen it. It is easy to treat short bowel syndrome either with dietary changes or antibiotics. But I think it needs to be looked at before we conclude that it is a nonissue in infants.

Dr. Björkstén: May I just comment on that very briefly? I didn't say that it's a nonissue in short bowel syndrome, I said that it is a nonissue with regard to the use of probiotic bacteria.

Dr. Koletzko: I agree that it is probably a nonissue in most cases, but I think we should have some data before we draw that conclusion.

Dr. Salminen: I can only say that it has been extensively considered by several authority working groups, and I don't think there is a big change for the time being. It is still being considered and I quite agree that the early studies were not well done. We also went to those to look where the ban, so to say, originates, but there are also some worries regarding the information which makes people cautious.
**Dr. Haschke:** Just to contribute to the more recent data, there is a study by Claude Bachman, presently under review, looking at a huge cohort with regard to D-lactic acid excretion when those kinds of bacteria where added to a starter formula. I can only say that in a double-blind study there was no higher excretion as compared to controls.

**Dr. Isolauri:** Just a brief comment on the mother–infant transfer of bacteria. Even though the child’s first inoculum frequently comes from the mother, it is not passive. This means that the child does not directly get those bifidobacteria which the mother carries.

**References**
