Identification of Probiotics and Prebiotics with Antiallergenic Properties

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Introduction

Probiotics have been defined as bacterial preparations which impart clinically verified beneficial health effects on the host when consumed orally. Prebiotics are nonabsorbable carbohydrates which act by promoting beneficial members of intestinal microbiota in a manner that provides demonstrated health benefits to humans [1]. Most probiotics are currently either lactic acid bacteria or bifidobacteria, but new species and genera are being assessed for probiotic use. Common prebiotics are based on fructo-oligosaccharides from plant sources or lactose-based galacto-oligosaccharides resembling those found in breast milk [1].

Current knowledge of probiotics and prebiotics shows that mechanisms of probiotic action are multi-facetted and each probiotic or prebiotic may have specific functions affecting the host. The criteria for effective probiotics were defined based on the general properties of probiotics. It may be necessary to redefine these criteria and acquire new standards to allow the development of probiotics for specific functions and targets [2]. The same is required for prebiotics as most of them were developed as substrates for intestinal bifidobacteria in general without understanding their role in microbiota–host interactions. The focus on here is to characterize probiotics and prebiotics in

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1 A Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota (NAMI) Research Group report.
general and define the steps needed to develop probiotics and potentially also prebiotics with antiallergenic efficacy.

**Intestinal Microbiota: Basis of Probiotic and Prebiotic Development**

The generation of immunophysiological regulation in the gut depends on the establishment of indigenous microbiota. The microbiota of a newborn develops rapidly after birth and it is initially strongly dependent on the mother’s microbiota, mode of birth and birth environment, and subsequently influenced by feeding practices and the environment of the child. Most microbiota succession studies have been based on culture method studies. Recent molecular studies have indicated that the microbiota in infants develops rapidly during the first week and remains unstable for the first year of life. We know that lactic acid bacteria account for <1% of the total microbiota in infants but bifidobacteria can range from 60 to 90% of the total fecal microbiota in breast-fed infants [3, 4]. The composition of bifidobacteria microbiota in infants was first clarified by Benno and Mitsuoka [5]. Usually bifidobacteria appear after birth and within a week they have been reported to be the dominant bacterial group with *Bifidobacterium breve* and *Bifidobacterium bifidum* as the most common species present in healthy infants. Comparing breast-fed and formula-fed infants the greatest differences appear to be in lactic acid bacteria colonization and species of bifidobacteria present. In breast-fed infants *Lactobacillus gasseri* and *B. breve* are the most common species present in culture method studies [5]. Similar results have been reported with more detailed information on the distribution of bifidobacterial species using 16S rRNA primers and modern taxonomy [6]. *B. breve*, *Bifidobacterium infantis* and *Bifidobacterium longum* are frequently found in fecal samples from infants. Using molecular methods, the most common lactobacilli in breast-fed and formula-fed infant feces were *Lactobacillus acidophilus* (*sensu lacto*) group organisms [3, 7].

The development of intestinal microbiota has to be characterized in a manner defining the composition that assists the infant to remain healthy. Specific aberrancies in intestinal microbiota may predispose the infant to allergic disease. Such aberrancies include decreased numbers of bifidobacteria and an atypical composition of bifidobacterial microbiota [8]. Also aberrancies in the clostridium content and composition have been reported to be important [8–11]. Compositional differences among clostridia and their relation to bifidobacterial composition and concentration need to be assessed carefully as they may predispose the infant to allergic diseases. Similar predisposing factors may also exist in terms of microbiota and risk for rotavirus diarrhea [12]. Thorough knowledge of intestinal microbiota composition offers a basis for future probiotic development and the search of antiallergenic strains.
Optimal Characteristics for Probiotics

Tolerance to Upper Gastrointestinal Environment

The effects of gastrointestinal (GI) conditions, such as pH, bile, and digestive enzymes on the survival [13] and adhesion properties [14] of probiotic bacteria have been documented. Various bacteria show different levels of tolerance to the GI conditions. For example, the adhesion of *Lactobacillus rhamnosus* GG on mucus was reduced to 1/10, while the adhesion of *Lactobacillus johnsonii* La1 was also reduced after pretreatment with amylase, pepsin, bile and pancreatin [14]. Such properties have to be clarified for all candidate probiotics.

Adhesion

Adhesion on the intestinal surface lengthens the retention time of a probiotic, and it is particularly important for the small intestine. The resident time of intestinal material in the small intestine is relatively short. *L. rhamnosus* GG, which has been reported to adhere and also colonize also the small intestine, was found to be effective in shortening rotavirus diarrhea in infants [15, 16]. *Lactobacillus bulgaricus*, which could not adhere to and colonize the intestine, had no effect on infant diarrhea. Similarly, a highly adhesive strain *Bifidobacterium lactis* Bb12 has been proved to be effective in preventing and treating acute diarrhea in infants [17]. Recent information on the genome of *B. longum* indicates that the strain has specific gene sequences that assist in the adherence to intestinal mucosa especially in the colon [18]. The genetic coding may predispose some strains and species to inhabit specific target sites in the intestinal tract of infants and these properties should be carefully characterized for each strain.

The ability of probiotics to adhere to intestinal mucous glycoprotein is likely to reflect the persistence of a probiotic to intestinal contents, but it may not necessarily be related to their capacity to successfully adhere to intestinal tissue [19]. A probiotic bacterium that binds strongly on mucin glycoprotein would compete with pathogens for adhesion on the mucous surface, but may have a high turnover rate on the mucosal surface, due to their continuous dislodgement from the intestinal surface together with mucus that they bind to. Conversely, a probiotic bacterium that penetrates the mucous layer may adhere to the epithelial surface [19, 20].

Specificity to Target Sites

Orally consumed probiotics pass along the entire GI tract and, therefore, selecting new candidate probiotics from members of the normal microbiota, the candidate strains are likely to have prerequisite survival and specificity depending on isolation location. An effective probiotic should reside sufficiently long at desirable target sites and at sufficient concentrations to elicit probiotic effects. 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 [19, 21, 22].

**Growth and Metabolic Activity**

Without adhesion to the intestinal mucosa, the concentration of probiotic bacteria would be diluted to an insignificant level following a meal or drink. It is not clear whether all probiotics could grow in an intestinal environment. No commercial probiotics have been reported to be able to establish permanently in the human intestine, which suggests that even if there is cell division the specific growth rates are not fast enough to replenish detached probiotic cells on the intestinal surface [1].

Growth in the intestinal tract increases population size and the metabolic products of probiotics, and their ability to alter GI bacterial activities. We have yet to see evidence demonstrating the growth of probiotics in the GI tract. Some probiotics attach to the intestinal mucosa and can be recovered in biopsies much longer than reported by Alander et al. [23] and Zoetendal et al. [24]. Thus, adherence studies need to complement fecal recovery assessment, preferably in biopsies. The importance of viability is underscored in reports in which immune-enhancing effects during probiotic treatment of rotavirus diarrhea were only observed with viable probiotics [25]. Another aspect of viability concerns metabolite production. Acid and peroxide production by bacteria are linked to growth, but secondary metabolites are non-growth linked and produced when cells are not multiplying. These metabolites may play an important role in locally modulating GI microbiota.

**Prebiotic Characteristics**

**Bifidogenicity**

The basis for prebiotics is the selective stimulation of bifidobacteria observed in the assessment of fecal or intestinal contents [1]. This is the major characteristic for each candidate prebiotic. Rather than the current requirement for enhanced bifidobacterial concentrations in feces, one key factor for antiallergenic properties may be the influence on the bifidobacterial composition in the intestinal tract. We have reported that different bifidobacterial species and strains may be involved when antiallergenic properties are considered. Infants that later develop allergies or are allergic are more often colonized by adult type of bifidobacteria, *Bifidobacterium adolescentis*, whilst children that remain healthy have a different bifidobacterial composition [10]. Thus, future prebiotics for antiallergenic properties may need to be formulated to promote specific species and strains of bifidobacteria, not just the *Bifidobacterium* ssp in general.
Microbiota Effects of Prebiotics

Most studies on the prebiotic effects on intestinal microbiota have concentrated on bifidobacteria and little is know about the effects of many prebiotics on other intestinal microbiota compositions. One of the oldest prebiotics is lactulose, which has been extensively studied and used in adults and infants. It has the required bifidogenic effects reported and lactulose resembling lactooligosaccharides are also found in breast milk. Lactulose also has a long history of safe use in intestinal microbiota management in adults and children [26, 27]. The potential antiallergenic properties of lactulose have not been assessed. This requires work on intestinal microbiota composition and long-term effects in human subjects. Fructo-oligosaccharides have mainly been assessed in adults, but recently also applications in infant formula have been introduced. The long-term microbiota effects of early fructo-oligosaccharide administration in infancy are not yet known.

Future prebiotic assessment needs to focus on intestinal microbiota as a whole and the search for prebiotics or prebiotic mixes with antiallergenic properties. Assessment of beneficial as well as potentially harmful components are needed in a qualitative and quantitative manner. The same targets apply as indicated for probiotics.

Characterizing Antiallergenic Probiotic and Prebiotics

It is clear that new selection criteria are needed in addition to the traditional ones if antiallergenic efficacy is to be obtained. One proposed scheme that requires a relatively long time and interaction by multidisciplinary research groups is presented in figure 1. It is based on characterizing the normal microbiota for infants who remain healthy for several years. Healthy infants are compared to infants who later develop allergic diseases and the microbiota aberrancies need to be monitored. At the same time, bifidobacteria and lactobacilli in healthy infants are characterized and assessed for their influence on normalizing microbiota aberrancies. Within the proposed scheme it will take several years to identify new probiotic candidates for future clinical trials. Such strains should be carefully characterized prior to application in human studies (table 1). Investigations needed for this assessment are presented in table 2.

Probiotics, Prebiotics and Intestinal Microbiota and Immunity

One of the main selection criteria for probiotics has been competitive exclusion of pathogens. Probiotics compete directly or hinder the adhesion of pathogens on stereo-specific receptors on the GI surface [28]. They can also
Fig. 1. Proposed approach for isolating and characterizing new probiotic strains with antiallergenic properties. NAMI = University of Turku combined research program on Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota.

influence the development of intestinal microbiota in infants. The outcome of the competition would depend on the specificity of the bacteria adhesins for the receptors and the relative concentration of the two competing bacteria. The effective dosage of a probiotic is thus determined by the relative affinity for the receptor sites.

Probiotic bacteria have been shown to modulate the intestinal and systemic immune responses [2, 29–31]. Activation of immunological cells and tissues likely requires close contact of probiotics with the immune cells and tissue on the intestinal surface [32]. Interestingly, both lactobacilli and bifidobacteria, which mainly colonize the small and large intestine, respectively, given as probiotic supplements, were able to modify immunological reactions related to allergic inflammation, but lactobacilli were ineffective in protection against cow’s milk allergy [2, 32–34]. In this respect, preferential binding of probiotics to the specific antigen-processing cells (macrophages, dendritic and epithelial cells) [35, 36] may be even more important than the location of adhesion. We have also shown that the cytokine stimulation profiles of different bifidobacterium strains vary and strains isolated from healthy infants mainly stimulate noninflammatory cytokines. Probiotic properties also vary in
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**Table 1.** Properties of probiotics to be assessed during the development of new strains and new probiotic functional foods

<table>
<thead>
<tr>
<th>Property</th>
<th>Target and method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity to species and target</td>
<td>Source or origin to be assessed, gut commensals as a source, target defined</td>
</tr>
<tr>
<td>Resistance to pH</td>
<td>Model systems for gastric and bile effects</td>
</tr>
<tr>
<td>Adhesion and colonization</td>
<td>Several model systems to be used for adhesion (e.g. cell cultures, mucus, intestinal segments)</td>
</tr>
<tr>
<td>Competitive exclusion</td>
<td>Colonization in human studies adhesion and competitive exclusion of pathogens in in vitro and in vivo model systems</td>
</tr>
<tr>
<td>Immune regulation</td>
<td>In vitro and human studies</td>
</tr>
<tr>
<td></td>
<td>- Cytokine profile</td>
</tr>
<tr>
<td></td>
<td>- Contact with immune cells</td>
</tr>
<tr>
<td></td>
<td>- Adhesion related to immune effects</td>
</tr>
<tr>
<td></td>
<td>- Improvement of gut barrier and permeability disorders</td>
</tr>
<tr>
<td>Function specificity</td>
<td>Adhesion</td>
</tr>
<tr>
<td></td>
<td>Immune function</td>
</tr>
<tr>
<td></td>
<td>Competitive exclusion</td>
</tr>
<tr>
<td>Safety</td>
<td>Safety clearance and post-market monitoring</td>
</tr>
<tr>
<td>Technological properties</td>
<td>Stability and activity throughout the processes</td>
</tr>
<tr>
<td>Sensory assessment</td>
<td>Acceptance of probiotic and prebiotic products</td>
</tr>
<tr>
<td>Efficacy assessment</td>
<td>Human clinical intervention studies with final product formulations, at least two independent studies to show efficacy in target populations and safety in all consumer groups</td>
</tr>
</tbody>
</table>

**Table 2.** Examples of target-specific searches for optimal probiotics

<table>
<thead>
<tr>
<th>Target for probiotic action</th>
<th>Selection criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleviation of lactose maldigestion symptoms</td>
<td>High lactase producing strongly site specific adhesion LAB</td>
</tr>
<tr>
<td>Intestinal inflammation</td>
<td>Site specific adhesion properties, anti-inflammatory cytokine expression, mucosal properties to alleviate permeability disorder and gut microbiota aberrancy</td>
</tr>
<tr>
<td>Alleviation or food allergy symptoms, reducing the risk of atopic diseases</td>
<td>Adherence to small intestine, induction of local TGF-β production, proteolytic properties</td>
</tr>
<tr>
<td>Reducing the risk of colon cancer</td>
<td>Target-specific adhesion to distal and or proximal colon, mucosal butyric acid production, competitive exclusion of inflammatory bacteria, toxin binding and promotion of nontoxigenic mucosal microbiota</td>
</tr>
</tbody>
</table>
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**Table 3.** Future challenges for the health-promoting probiotics

- Preclinical testing of probiotics: efficacy and safety
- Different and often opposite immunological effects of the specific strains of gut microbiota
- Different role of gut microbiota and the effects of probiotics and prebiotics in the healthy versus inflamed mucosa
- Identification of specific strain in prevention and management of infectious and inflammatory diseases
- Identification and mechanisms of effective dietary components
- Development of novel functional foods with specific probiotics, prebiotics and supporting dietary compounds

This respect, *Lactobacillus casei* has been reported to induce IL-12 and transforming growth factor-α from murine dendritic cells while *Lactobacillus reuteri* causes IL-10 production and downregulates the effects of *L. casei*. The gut microbiota provides crucial maturational signals for the immune system in infancy and interferes and also actively controls gut-associated immunological homeostasis later in life. Indeed, it is not because of food antigens but because of the antigens of the microbiota that fully matured gut-associated lymphoid tissue is the largest immunological organ containing greater numbers of T cells than the rest of the body combined. Duchmann et al. [37] have demonstrated that healthy individuals are tolerant to their own microbiota. Specific strains of the gut microbiota have also been shown to contribute to a T-helper cell population that maintains a disease-free state of the gut. These interactions should be taken into account when selecting the candidate probiotics (table 3).

**Conclusions**

It is clear that intestinal microbiota development is of major importance to the health of the newborn. It appears that due to their concentration and composition bifidobacteria are more important than lactic acid bacteria during early intestinal colonization. These factors form the basis for selecting probiotics from the currently available ones, suggesting bifidobacteria as the first option, and specific lactic acid bacteria that may stimulate intestinal bifidobacteria as the second option. The qualitative effects of current probiotics and prebiotics on intestinal microbiota should be understood prior to being used in infant foods.

The knowledge of intestinal microbiota development, nutrition, immunity and allergic diseases should be carefully combined in the search for new probiotics and prebiotics with antiallergenic properties.
References

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Discussion

Dr. Endres: You mentioned that lactobacilli are able to enhance the growth of bifidobacteria. As we all have a similar definition of prebiotics in mind, we could say that lactobacilli have a prebiotic effect on bifidobacteria, but I think this is not the right conclusion because it is another mechanism. I think that the prebiotics create the right environment and are in a certain way food for probiotic bacteria, aren’t they?

Dr. Salminen: I quite agree with you. I was trying to point out that the probiotic effect can be similar to the prebiotic effect, but of course by definition you are absolutely right, it could not go that way. But we have been lucky in a way because many of the current lactobacillus strains used actually may have that effect.

Dr. Walker: This was an excellent overview because this is an important area of investigation that needs to be carefully analyzed. My concern is that when you put in a procaryotic against the eucaryotic, the procaryotic usually wins the battle because it can adapt so much more easily to the environment than can a whole organism, so a problem develops. One of the concerns is that prebiotics modulate the environment. As a result of their modulation you get increased bifidobacteria but, because you have other bacteria, they can adapt and the prebiotic effect is a short-term rather than a long-term effect. The other concern that I would like to have you comment on is: there was a really excellent article in Science [1] where they genetically engineered lactobacillus to produce IL-10 that could be delivered directly to the mucosal surface.
and showed in an experimental model of inflammatory bowel disease that they could
downregulate the inflammation. This is an area that we really need to be looking at
in the future because we might be able to use probiotics not only themselves, but
genetically engineer them to deliver medications/factors directly to the gut.

Dr. Salminen: I really appreciate the last comment, it is a very important area.
I left it out because we are actually discussing infant nutrition and at the moment the
genetic engineering question is such that it is very controversial even in adults. But
I quite agree, I know the study presented in Science and it certainly actually offers
some mechanisms that we should consider. From the discussions that we have in
Europe today, I would say that it certainly has great potential in the pharmaceutical
field, but perhaps we are a bit off in infant foods. We are trying to understand the
genome and perhaps, for infant food purposes, select strains or species that are natu-
ral and naturally adapted to the infant gut, such as B. longum, and the more we know
about the genome, the more choices and the more possibilities we will have. But I take
your other point also as a very valuable addition to the discussion. Of course we know
the genome in the bifidobacteria but we need to know a little bit more about what the
bacteria do to our intestinal cells and their gene expression. So we are only starting
to know the first part of a cross-talk, what kinds of signals there are perhaps in the
bifidobacteria. However, we know very little about how those signals affect our intes-
tinal cells. So in those terms I think that you are absolutely right, we need to know
more and we need to understand them, but still I think that perhaps we would use the
genetically modified organisms more in the pharmaceutical area.

Dr. Walker: My other concern is that we are being artificial when we introduce the
single organism into a gut to do a major job like prevent allergy or produce inflamma-
tion. This is a strong case for the use of cocktails of bacteria that can work in concert
to produce a potentially stronger effect than a single organism, particularly if you
chose the wrong organism.

Dr. Salminen: I agree with that comment also, but before we go into the cocktails
we have to know the safety properties of each single strain and component in the
cocktail, and I think we should understand the steps that the infant has in acquiring
its microbiota a little bit more. Because if we start very early it may be one or two
species of bifidobacteria that form 90% of the microbiota but how they then influence
the next step is not known.

Dr. Guesry: To prolong the question of Dr. Endres on the potential prebiotic role
of a certain type of probiotic bacteria, I think we should enlarge the discussion to
the point of what is the role of dead probiotic bacteria compared to living probiotic
bacteria, because we have some indication that there are sometimes synergies
between dead and living bacteria. Do you have a comment on that?

Dr. Salminen: I think you have touched upon a very important area both in terms
of intestinal microbiota research and in terms of probiotics. Most of the genetic
methods that are used today in assessing microbiota include all, both viable and
nonviable bacteria, and if we compare the viable bifidobacteria counts, for instance,
usually the counts in gut samples are lower than the genetic methods for the total
number of bifidobacteria. In a recent review that we wrote with Dr. Isolauri [2] we
suggested that one should also consider nonviable bacteria because even though
the bacterial cells are not presently alive in the gut, they are probably present in
the mucosa and possibly influence a local effect. So I think you are absolutely
right, but we also have to do studies on nonviable bacteria. Again I would refer to
Dr. Isolauri because she has done diarrhea studies with viable and nonviable bacte-
ria, and if I recall well I think the clinical effects were similar but the immune effects
were not the same [2]. So they may be efficacious in some aspects and we should look
at that.
Dr. Guesry: In that case it is another dogma that we have put down this morning since the living bacteria is part of the definition.

Dr. Salminen: Let me just point out that I use the commonly accepted definition of probiotics. We have proposed another one that will also take into account nonviable bacteria.

Dr. Sorensen: Thank you for a very nice review of a topic I know nothing about, which I think explains why I am going to ask you this question. Why do you exclude non-pathogenic *Escherichia coli* from your list of useful bacteria? There is plenty of evidence that infants by 1 year of age have developed antibodies and isoagglutinins. I understand there are antibodies against galactosamines on the surface of *E. coli*, so they clearly have an effect on infant immunity and a normal child may never have had diarrhea but plenty of evidence of having many *E. coli*. Why do you ignore them?

Dr. Salminen: I do not discriminate against them at all. I was only trying to point out that there is no straightforward categorization of harmful or not so harmful. I think clostridia are another example. If we lose all the clostridia in our gut, we might be in trouble and it is probably the same with the *E. coli*. Again, the early colonization of the gut is so much dependent on the environment in which we are born that we cannot say that one bacteria, one species, not even some genera, are really strictly harmful, but we should rather find a way not to kill them, not to eradicate them, but to live together with them. That comes back to the composition: how do we understand the composition? The European Union has been investing a lot of money in defining healthy European gut microbiota, but we are giving up because there is no such thing. There are individual healthy gut microbiota that between us will probably be dramatically different. I would not eradicate, but would try to coexist happily with the microbes we are born with and only start eradicating when there is serious disease.

Dr. Szajewska: We know from the recent meta-analysis [3] that there is a dose-related effect, at least in diarrheal diseases. Can you please comment on the minimum dose, to summarize data, and also do you think that we have to be worried about too much bacteria being given to an infant?

Dr. Salminen: These are two very excellent questions that all of us should consider. The first question I think the clinicians should answer. From the microbiological point of view I think the dose that is used currently for most probiotics is $10^9$ colony forming units/day/person. It is some kind of average of many studies and I would say that it comes back to the unique individual strain properties. I think most of the studies have used that dose to be sure to guarantee enough exposure. How much lower you can go, I don’t think anybody knows. We can probably go lower but how much lower. There are no dose-response studies that go to $10^6-10^5$, the lowest effects are usually seen in $10^8$, in some studies $10^8$ is not enough. But then I think it comes to the other question, viability, how important is survival, what is a strain-specific property, survival in the gastric acid conditions, survival and adherence to the gut, local effects? So I think $10^9$ is a rule of thumb, but when we learn the mechanisms in more detail we could probably use lower doses with some strains. However, if you were running a clinical study, would you risk going lower without knowing?

Dr. Saavedra: I agree completely with what you just said. But from the point of view of dose, I think a lot will also depend on what the use of the probiotic is. Because a good part of what is currently presented relates to therapeutic effects of probiotics, that is to treat a particular condition once the condition has been initiated, whether it is allergy or for treatment of diarrhea, versus the application of probiotics for what we would consider an adjuvant in terms of gut health or modifications of gut function on a chronic basis. These are completely different approaches incorporating it on a long-term and chronic basis, and of course even then it is even harder to know what the
minimal doses are going to be. Can you comment on what an overdose of probiotics is? But I think it is almost impossible.

Dr. Salminen: I am sorry, by discussing the dose I forgot to discuss your second question. As long as we have been conducting safety assessment of different specific probiotic strains, there doesn’t seem to be an achievable toxic dose. However, we know from the use of lactobacilli that one has always to remember that when you have viable living bacteria, and that is another question concerning viability, under some circumstances it is viable bacteria that can cause a problem. However, I would say that with lactobacilli the problems are extremely rare as far as we know, with bifidobacteria they are almost unreported. I would almost think that bifidobacteria are even onefold safer than lactic acid bacteria. We know for instance for the *Lactobacillus bulgaricus* or *Streptococcus thermophiles* that, if I recall correctly, no problems have ever been reported for these two common yogurt strains in the literature.

Dr. Lack: We tend to think now of certain probiotics and prebiotics as being beneficial perhaps for allergy, and antibiotics as being harmful or evil. As someone who knows very little about the field I just wanted to ask, could antibiotics have a beneficial role, could they have a prebiotic effect in the sense of allowing you to then come in with the good guys (the probiotics)?

Dr. Salminen: If you really take a bright look into the future, why would one not be able to develop an antibiotic that would perhaps very specifically decrease some components of the microbiota and enhance some other microbiota parts. I think the lactobacillus question is stimulatory in a way, but the more wide-spectrum things you use, the more important microbes and indifferent ones you are wiping out, and again from the microbiological point of view it is a similar thing to your hygiene hypothesis. Most of us working with food have always been told how to kill every single bacteria in the food before moving it to the consumer. Recent studies from the US, for instance, have shown that cheese produced under very hygienic conditions is also more prone to spoilage because there is no natural bacteria to fight against, so there is no competitive exclusion against the spoilages causing bacteria. Perhaps also in the food industry we have gone one step too far, and now people, again with the European Union funding, are trying to look at selective ways of pasteurizing or sterilizing food so that you don’t kill everything, but you kill the predominant problem causing organisms and let the indifferent ones live perhaps to better preserve the food in the long-term.

Dr. Lack: You distinguish between looking at probiotics as a group and perhaps focusing rather than on genus, on species and strains with their very specific immunological effects. Do you think different host immune responses may come into play as well? I think you show differences between atopic and nonatopic responses in terms of IL-10 production. Could we explain the differences in the colonization of the gut in relation to the host immune response as a reverse causal effect?

Dr. Salminen: It could be part of it but I would leave it to the clinicians to look at the effects. I can only assess what happens in the microbiota.

Dr. Isolauri: I think you missed a slide because it was not an immune response producing more IL-10 in atopic infants, but it was the bifidobacteria isolated from atopic infants which were producing less IL-10, while in healthy infants bifidobacteria caused the high IL-10. So it was the bifidobacteria isolated from the fecal samples of the patients [4].

Dr. Saavedra: I think the question on antibiotics was a very provocative one, but at the same time from that point of view, I think one of the criteria that we chose, in particular probiotics, is that they don’t have the ability to transfer antibiotic resistance, for example, which would make things very complicated. It is again another reason why we need to go stepwise and very slowly with these strains.
Dr. Manjra: Are there any studies with regard to the duration of therapy with probiotics?

Dr. Salminen: There are studies but again I would leave that to the clinical specialists in the audience.

Dr. Neijens: How much documentation do we have on the effect of probiotics on different cell populations like coli bacteria and rotavirus because this interaction might be very important? E. coli, for instance, produce a lot of IL-12. My second question is what do we know of the effects of antibiotics given to the child on the different strains? Should we advise not to give antibiotics or to be very restrictive, or should we follow that up? My third question is what is the documentation as far as the exchange of genetic material between different strains? What do we know about the exchange of genetic material like DNA or plasmids, etc.?

Dr. Salminen: I think you are presenting excellent questions but we need a regulatory authority to answer. I can try to give you a couple of points. First of all the documentation between strains varies tremendously. There are very well-documented strains, there are strains that have practically no documentation behind them. So there is very wide use of the term ‘probiotic’, and there is no good single answer to that. All the probiotics that have been used by the food and clinical nutrition industry are actually very well documented for nontransfer of antibiotic resistance, whether by plasmids or by other means, so that has been one of our selection criteria. I did not define the selection criteria that we used on safety studies. So most are lactic acid bacteria, lactobacilli or bifidobacteria, and you can be sure that there is no transfer of genetic material. However, there are still some enterococci which are known to transfer antibiotic resistance, but at least the producers of such strains claim that their particular strain does not do the transfer. Now how good or bad the documentation is, I don’t know. I think we should really address the regulatory people for this.

Dr. Szajewska: From a microbiological point of view, could you please comment on why probiotics or lactobacillus GG, for example, are effective in rotavirus gastroenteritis, but are not shown to be effective in bacterial gastroenteritis? What is your explanation?

Dr. Salminen: I think there are simple explanations that are related to the clinical studies. Looking at the microbiota, at the duration of diarrhea, they may sometimes change from viral to microbial, and you may be able to alter the outcome of the microbial phase. It has also been suggested that it could be related to the absorption of rotavirus particles. Now I don’t have any evidence on that, but it has been suggested that it may be just a physical barrier between the virus and the intestinal epithelium. But I think the clinical effects are for others to relate to the bacteria.

Dr. Endres: I would like to ask Dr. Szajewska, in your study, the ESPGAN study, it has been shown that viral diarrhea responded better to an oral rehydration solution with lactobacillus GG, whereas in the case of other kinds of diarrhea there has been a less pronounced effect, hasn’t there?

Dr. Szajewska: No, as a matter of fact in the ESPGHAN study [5] we confirmed the inefficiency of lactobacillus GG in proven bacterial infectious diarrhea. That is why I was asking my question: why do you think that lactobacillus GG was ineffective? I could not find good data in the literature.

Dr. Guesry: Yes, but your study was not prophylactic since the GG bacteria were given with oral rehydration salt or solution, it was on babies who already had diarrhea, and that is for me a main difference. In our study with Dr. Saavedra, we make it prophylactic and it works. As a treatment it is another question.

Dr. Szajewska: But my question was regarding treatment, I was not asking about prophylaxis.
Dr. Isolauri: I think that question needs to be studied especially in target groups. There are very little data to say that there is no effect or there is an effect. An additional reason is that we are not thinking about why we see an effect in some populations and no effect in another. It could be that even though we use the same strain with the same name on the label, we might finally have a different kind of product, it having been handled differently. We have looked at this in some in vitro systems and finally there might be different products.

Dr. Salminen: Perhaps a comment for the industry people, the quality control for probiotics is very important. One has to be sure that it is similar to the original strain properties because especially in the dairy industry, when butter milk, yogurt or other dairy products are continuously cultured, the properties perhaps decrease and the bile acid’s tolerance also decreases. There is absolutely no published study on the strain properties. So it is important for quality control purposes to make sure that the strain remains similar to the original.

Dr. Rijntjes: Children who are allergic have another gut flora and after birth the gut flora of the mother is important for colonization. Can you tell me if there is any literature on the colonization from the gut of the father to the child, because in modern society the father is looking after the child more and more?

Dr. Salminen: I am sure that there is some exchange of bacteria as was said earlier for the real father, and I am quite sure that some of those bacteria are transmitted to the infant, but to my knowledge there are no studies looking at that aspect. But when you have contact you exchange bacteria even if you kiss, and if you have more intensive contact then you exchange more bacteria. It is quite likely that the father has some bacteria that might be affiliated to both parents.

Dr. Rijntjes: There could also be a genetic predisposition to have the gut flora of the father, when he is the real father of course.

Dr. Al-Malik: I understand from your talk that prebiotics are used to treat gut infections and also to reduce food hypersensitivity. Has there been any attempt to use these to treat respiratory allergy or respiratory infections?

Dr. Salminen: To my knowledge there are not many studies with great success. There have been quite a few studies even on the infection or the viral side and, as we heard in the morning, they have not produced any effect. So I think it comes back to defining what is your real prebiotic and is it the right prebiotic for the target.

References