Microecology of Lactobacilli and Bifidobacteria Inhabiting the Digestive Tract: Essential Knowledge for Successful Probiotic Research

Gerald W. Tannock

Department of Microbiology, University of Otago, Dunedin, New Zealand

The past decade has seen a somewhat amazingly increased interest in the use of intestinal species of lactic acid-producing bacteria in the production of milk products. Lactobacillus acidophilus and Bifidobacterium species are now common components of yogurts that are retailed throughout the world. These acidophilus-bifidus (AB) products are the most widely consumed probiotics intended for humans. Probiotics, by definition, are "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (1). The marketing of probiotic products based on milk uses this definition because statements relating to the benefits to the consumer are generally restricted to "helps maintain a healthy balance of beneficial bacteria," "stabilizes the intestinal microflora and modulates its function," "promotes the positive balance of the intestinal flora," or similar claims. Where more specific health claims have been made (e.g., "can actively reduce cholesterol levels"), they have been readily challenged by regulatory authorities. The marketing of AB products is not, it would appear, overtly based on specific scientific facts but more on the basis of a theoretical concept. The definition of a probiotic and the statements made by dairy food manufacturers beg the following questions: what is the nature of the improved microbial balance to which one aspires? How is it to be defined and detected? How would one recognize the benefits of achieving the required balance?

The development and marketing of probiotics would be improved if these questions could be answered. But there is a barrier to achieving the derivation of scientifically specific AB products. This impediment is identified in the definition of a probiotic, where it is implicit that consumption of living microbial cells affects the normal microflora of the digestive tract in ways that promote the well-being of the consumer. A weakness in the development of probiotic products is that knowledge of the normal microflora is incomplete, so convincing evidence that probiotic products have any impact on the normal microflora of the consumer is impossible to obtain. A thorough understanding of the normal microflora of the human digestive tract is essential for the development of probiotics that are scientifically valid.
The normal microflora of the human digestive tract is largely confined to the colon, which harbors a complex collection of microbes, mostly bacteria. The numerically predominant species of bacteria are obligate anaerobes and are represented by both Gram-positive and Gram-negative genera. Possibly 400 bacterial species can inhabit the colon, but for any one individual 30 to 40 species account for 99% of the microflora. Numerically predominant species attain populations of about $10^{10}$ colony-forming units (CFU) per gram of intestinal contents (feces), wet weight. Thus, the colon contains at least $10^{12}$ bacterial cells (2). Knowledge of the intestinal (fecal) microflora of humans has been derived mostly from comparisons of the microbial content of fecal samples collected from subjects ingesting contrasting diets and following different lifestyles. This research has provided a large body of information regarding the complexity of the colonic community, but also an almost complete lack of agreement between research groups about the influence of diet and lifestyle on the digestive tract ecosystem (3–5). It has been concluded from these studies, however, that in the absence of major stressors the intestinal microflora is relatively stable with regard to bacterial species (6). Even so, identification of species of intestinal bacteria is fraught with difficulties and has probably contributed to the lack of agreement between results of studies on normal microflora conducted in different laboratories. Analysis of bacterial communities by species identification is rather a crude measurement, especially when an ecosystem suspected of considerable dynamism is concerned. Biological successions of biotypes of Enterococcus faecium and of strains (differentiated by plasmid profiling) of L. acidophilus and Lactobacillus fermentum have been described for mice and piglets, respectively (7,8). Fluctuations in the composition of populations of Escherichia coli, detected through the use of serotyping, in human fecal samples have also been reported (9). It is apparent that analysis of the microflora at the level of bacterial strains is necessary to appreciate fully the dynamics and complexity of the digestive tract ecosystem.

The normal microflora of the digestive tract, in total, has marked influences on the animal host (2). These influences have been demonstrated by comparing the characteristics of animals harboring a normal microflora (conventional) with those of animals without a normal microflora (germ-free). These comparisons have shown that the biochemistry, physiology, and immunology of animals is modified by the presence of the normal microflora (Table 1). While the impact of the normal microflora as a whole on the host has been documented through comparisons of germ-free and conventional animals, the influence of specific microbial components of the normal microflora has not, until recently, been demonstrated. The derivation of mice that do not harbor lactobacilli as part of their normal microflora has been a major advance in our understanding of the importance of lactic acid-producing bacteria in the digestive tract ecosystem (10). Derived from conventional mice by a combination of antibiotic treatment and gnotobiological methods, the lactobacillus-free animals have been maintained and bred in isolators for several years. Comparisons of the characteristics of lactobacillus-free mice with counterparts that had been intentionally colonized with Lactobacillus strains, but in which an otherwise identical microflora was resident, have shown that lactobacilli inhabiting the gastrointestinal
### Table 1. Comparison of selected properties of germ-free and conventional animals

<table>
<thead>
<tr>
<th>Host characteristics</th>
<th>Conventional</th>
<th>Germ-free</th>
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<tbody>
<tr>
<td>Bile acid metabolism</td>
<td>Deconjugation, dehydrogenation, and dehydroxylation</td>
<td>Absence of deconjugation, dehydrogenation, and dehydroxylation</td>
</tr>
<tr>
<td>Bilirubin metabolism</td>
<td>Deconjugation and reduction</td>
<td>Little deconjugation</td>
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<tr>
<td>Cholesterol</td>
<td>Reduction to coprostanol</td>
<td>Absence of coprostanol</td>
</tr>
<tr>
<td>(\beta)-Aspartylglycine</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Intestinal gases</td>
<td>Hydrogen, methane, and carbon dioxide</td>
<td>Absence of hydrogen and methane</td>
</tr>
<tr>
<td>Short-chain fatty acids</td>
<td>Large amounts of several acids</td>
<td>Small amounts of a few acids</td>
</tr>
<tr>
<td>Tryptic activity</td>
<td>Little activity</td>
<td>High activity</td>
</tr>
<tr>
<td>Urease</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>(\beta)-Glucuronidase (pH 6.5)</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Organ weights: heart, lung, liver</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Cardiac output and oxygen utilization</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Mucin content of intestinal mucus</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Extent of degradation of mucins</td>
<td>More</td>
<td>Less</td>
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<tr>
<td>Cecal size (rodents)</td>
<td>Smaller</td>
<td>Larger</td>
</tr>
<tr>
<td>Enzyme activities associated with duodenal enterocytes</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Intestinal wall</td>
<td>Thicker</td>
<td>Thinner</td>
</tr>
<tr>
<td>Intestinal mucosal surface area</td>
<td>Greater</td>
<td>Smaller</td>
</tr>
<tr>
<td>Rate of enterocyte replacement</td>
<td>Faster</td>
<td>Slower</td>
</tr>
<tr>
<td>Peristaltic movement of contents through small bowel</td>
<td>Faster</td>
<td>Slower</td>
</tr>
<tr>
<td>Body temperature</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Serum cholesterol concentration</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Larger</td>
<td>Smaller</td>
</tr>
<tr>
<td>(\gamma)-Globulin fraction in blood</td>
<td>More</td>
<td>Less</td>
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Tract have an important influence on intestinal biochemistry (Table 2). This is due either to modification of the enzymic activities of other members of the normal microflora of the large bowel (azoreductase, \(\beta\)-glucuronidase) or to the association of this metabolic feature with the lactobacilli themselves (bile salt hydrolase). The predictive value of these murine observations with regard to the human colon remains to be tested, but the measurement of the impact of the consumption of probiotics on the normal microflora should surely include these analyses.

Two approaches to investigating the impact of a probiotic product on the normal microflora, and hence on the human host, are therefore evident. The first is to monitor the biochemistry of the intestinal contents; the second is to monitor the composition
of the bacterial populations inhabiting the intestinal tract. Since it has been shown that the bacterial content of the feces is representative of the colon, all analyses can be carried out on fecal samples (15). Analysis of the composition of bacterial populations, as discussed above, requires differentiation between bacterial strains. This can be accomplished by genetic fingerprinting of bacterial isolates by methods now well established for epidemiological use in medical microbiology: ribotyping and pulsed field gel electrophoresis of genomic DNA digests.

Ribotyping is a method by which restriction fragment length polymorphism of DNA can be detected in bacterial strains of the same species or different species of a particular genus. The method uses ribosomal RNA (rRNA) sequences as the basis for a broad-spectrum probe for strain differentiation. DNA extracted from bacterial isolates is digested with appropriate restriction endonucleases, the resulting fragments of DNA are separated in an agarose electrophoretic gel, transferred to a hybridization membrane, and probed with radiolabeled DNA encoding one or more (16S, 23S) rRNAs. Because bacteria have multiple copies of the rRNA operons in their chromosome, several fragments containing rRNA sequences are observed after hybridization with the labeled probe. The pattern produced by hybridization is referred to as the ribotype of the particular isolate (16). Fragment length polymorphism, revealed by comparison of ribotypes, permits differentiation between bacterial strains (Fig. 1).

Bacterial strains can also be differentiated by digesting their DNA using restriction endonucleases that cut rarely and thus produce a relatively small number of large DNA fragments (17). The large fragments can be separated in an agarose gel by means of pulsed field electrophoresis, which generates a pattern characteristic of the bacterial strain (Fig. 2). Pulsed field gel electrophoresis separates large DNA fragments by exposing them to alternating electrical fields. The alternating field forces the fragments to change orientation rather than to migrate immediately after the field is changed from one direction to another. The rate of reorientation is size-dependent, so larger fragments change direction more slowly than small ones. The DNA fragments are therefore separated by the retardation of net movement rather than by sieving.

Before studies of the impact of the consumption of a probiotic on the normal microflora can be embarked on, it has been necessary to establish a base of knowl-
FIG. 1. Autoradiographs showing examples of ribotypes produced by hybridization of Bifidobacterium infantis ATCC 15697 16S rDNA to DNA from Bifidobacterium strains. Row 1: DNA digested by PvuII. Row 2: DNA digested by NarI. Row 3: DNA digested by BamHI. Lanes (all three rows): 1: B. infantis ATCC 15697; 2: B. angulatum ATCC 27535; 3–9: Isolates of bifidobacteria from feces. The ribotypes in each of the lanes (all three rows) have been aligned by comparison with a standard preparation (B. infantis ATCC 15697 DNA digested by PvuII), which was included in each agarose electrophoretic gel. The top of each row (all nine lanes) corresponds to the same position in the gel. From McCartney AL, Tannock GW (16), with permission.
edge concerning the stability and complexity of the microbial community inhabiting the healthy human colon. Because probiotics are composed of specific strains of lactic acid-producing bacteria, the communities inhabiting human colons needed to be analyzed in terms of the collection of bacterial strains that were present. Recent studies of the *Lactobacillus* and *Bifidobacterium* populations detected in human fecal samples have provided a fascinating glimpse of the complexity, dynamism, and specificity of these collections of lactic acid-producing bacteria (17). In one study conducted in my laboratory by Anne McCartney and Wang Wenzhi, the fecal microfloras of two humans were monitored with respect to these bacterial populations over a 12-month period. In this study, *Bifidobacterium* and *Lactobacillus* populations were enumerated on plates of appropriate selective media. Following enumeration of bacterial colonies, 10 lactobacillus colonies and 10 bifidobacterial colonies were randomly picked, subcultured to ensure purity, and genetically fingerprinted. Because the colonies were picked from plates inoculated with a dilution of fecal homogenate that gave 30 to 300 discrete colonies per plate, only the numerically dominant strains of the total populations were analyzed.

The total numbers of bifidobacteria were stable throughout the study period in both subjects, but numbers of lactobacilli were less constant (Fig. 3). Analysis of the bifidobacterial populations using ribotyping or pulsed field gel electrophoresis of
FIG. 3. Total populations of Bifidobacteria, Lactobacilli, Bacteroides, and Enterobacteriaceae strains in the feces of two human subjects: (A) subject 1; (B) subject 2. Symbols: □, Bifidobacteria; ○, Lactobacilli; ●, Bacteroides; ◊, Enterobacteriaceae. From McCartney AL et al. (17), with permission.
genomic DNA to differentiate between the bacterial strains showed major differences between the subjects. Subject 1 harbored five strains of bifidobacteria throughout the 12-month period, and one strain was numerically predominant (Fig. 4). In contrast, subject 2 harbored a more complex bifidobacterial population (five or six strains per sample), and the composition fluctuated throughout the 12 months (Fig. 4). One Lactobacillus strain was numerically predominant throughout the study in both subjects. Strains of bifidobacteria and lactobacilli common to both subjects were not detected.

In a further study conducted in my laboratory by Katsunori Kimura, the Lactobacillus and Bifidobacterium populations detected in fecal samples collected from 10 healthy humans were analyzed (unpublished observations). Two fecal samples, with an interval of about 3 months, were collected from each subject. The Lactobacillus
and Bifidobacterium isolates were fingerprinted by ribotyping and pulsed field gel electrophoresis of genomic DNA digests, as in the previous study. All 10 subjects harbored a similar number of bifidobacteria in both fecal samples, the total populations of these bacteria being between $10^9$ and $10^{10}$ per gram (wet weight) of sample. Each subject had a characteristic collection of bifidobacterial strains detectable in the two fecal samples. Indeed, they could be identified on the basis of their bifidobacterial strains (perhaps providing the basis for a new forensic identification test)! Five of the subjects harbored a relatively simple collection of bifidobacterial strains (less than five strains in any fecal sample), whereas the remaining subjects had a more complex bifidobacterial microflora (five or more strains). This observation fitted well with the results of the 12-month-long study described above.

In contrast to the stability in the size of the bifidobacterial population, numbers of lactobacilli differed markedly between human subjects. Lactobacilli could not be detected in the fecal samples of one subject, and some subjects had relatively small populations of lactobacilli ($10^4$ to $10^5$ per gram of feces), while others had relatively large populations ($10^6$ to $10^9$). The populations sometimes varied between the two fecal samples collected from the subjects. As in the case of the bifidobacteria, characteristic strains of lactobacilli were detected in each subject. Most subjects had a simple collection of strains (one or two), as was observed in the 12-month-long study.

The species identity of representative strains of Bifidobacterium and Lactobacillus detected throughout the 12-month study was determined using classical methods (Gram-stain morphology, carbohydrate fermentations). The strains detected in the samples collected from subject 1 belonged to Bifidobacterium angulatum or B. longum, while B. infantis, B. adolescentis, B. catenulatum, B. angulatum, and B. bifidum were detected in subject 2. Subject 1 harbored L. acidophilus, and subject 2 Lactobacillus crispatus. The adequacy of current identification methods for bifidobacteria and lactobacilli (commercial kits) is debatable, however, and in our opinion they are not reliable.

The results of these two studies provide much interesting information for discussion. First, the observations have implications for the interpretation of past and future studies of the microecology of the human intestinal tract. The studies support the view that the fecal microflora of adult humans shows considerable stability in composition when considered in terms of the total populations of bifidobacteria. The populations were maintained at constant size in all individuals whether over a 3- or a 12-month period. For a given individual, the bifidobacterial microflora can be remarkably stable, as in the case of subject 1 in the 12-month study, with one strain predominating throughout the study. In other subjects (e.g., subject 2 in the 12-month study), a complex collection in which no one strain predominates can be detected. The complex collection may be dynamic in that specific strains seem to appear, disappear, and sometimes reappear (subject 2, Fig. 4). It would be interesting to investigate the colonization resistance of these two subjects in relation to the consumption of a probiotic food. Perhaps implantation of a new bifidobacterial strain in the intestinal tract of subject 1 would be impossible because of the stability of
the bifidobacterial microflora? Would implantation be more readily achieved in the case of subject 2, in whom the composition of the bifidobacterial population appeared to fluctuate? Or would the converse be true?

The total population of lactobacilli of subject 2 in the 12-month study showed considerable variation between samples, and the enterobacterial population, which was also monitored, fluctuated unpredictably (see Fig. 3). The results of monitoring these populations over a 12-month period showed the importance of conducting lengthy baseline studies in experiments aimed at evaluating the effect of dietary modification, such as the consumption of a probiotic, on the microecology of the intestinal tract. Short-term experiments, which are the usual subjects of scientific reports, could give misleading information on the significance of probiotic products. If, for example, in the case of subject 2 (see Fig. 3), samples collected at weeks 9 and 14 and then at weeks 23 and 27 had constituted the unmodified-diet periods during a trial and samples collected at weeks 17 and 20 constituted the probiotic period, then a dramatic but misleading effect of dietary modification would have been recorded. Our studies highlight the requirement for fundamental knowledge of the normal microflora in order for the impact of dietary components on the intestinal ecosystem to be investigated reliably.

Because of the variation in some population sizes between individual humans, the dynamic nature of some of these populations, and the unique *Lactobacillus* and *Bifidobacterium* strains that each individual had, it is clear that in experiments entailing dietary modification, subjects must serve as their own control. In other words, comparisons in these studies must be intra-individual not inter-individual.

Which would be preferable as probiotic bacteria: bifidobacteria or lactobacilli? Would bifidobacteria be the more sensible choice because they are much more numerous in the human intestinal ecosystem than lactobacilli? Yet their population size was similar in all of the humans that we studied. Why would humans need to supplement their diet with bifidobacteria when their colon is full of them? Numbers of lactobacilli vary considerably between human subjects, so perhaps there may be more logic in supplementing the diet with these bacteria? One subject studied in my laboratory was not colonized at a detectable level by lactobacilli. Could this individual ever be colonized, or is there an innate "anti-Lactobacillus factor" operating in the intestine of some humans? Do *Lactobacillus* strains given as probiotics become part of the lactic acid-producing microflora, or are they supernumerary to the resident (indigenous) population? If they become part of the microflora, do the probiotic bacteria eliminate a proportion (perhaps all) of the resident strains?

What criteria should be used to choose probiotic strains? Since individuals have their own, unique strains, it does not seem likely that a "universal" colonizer will be found. This surely is where experimental animal studies must be a guide as to the microflora-associated characteristics that can be influenced by lactic acid-producing bacteria and that can therefore act as indicators of probiotic activity. Genetic fingerprinting of *Bifidobacterium* and *Lactobacillus* strains has provided a means of detecting the numerically predominant strains harbored by a particular individual. Study of the characteristics of these strains, particularly those that have
been shown to be long-term inhabitants of the intestinal ecosystem, may reveal the mechanisms by which bacteria colonize the human colon. Identification of specific strains will also assist in studying host–microbe relations, such as the interaction between the normal microflora and the immune system of the animal host.

The way forward is clear. The impact of the consumption of probiotic products on the intestinal ecosystem can be studied by using a combination of biochemical assays, enumeration of major groups of normal microflora components, and analysis of lactic acid-producing bacterial populations by genetic fingerprinting. As knowledge of the interaction of lactobacilli and bifidobacteria with the immune system becomes more detailed, immunological assays may well be added to this armamentarium.

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REFERENCES


**DISCUSSION**

Dr. Hanson: I suppose the individuals that you looked at were healthy and did not travel? More specifically, could they have been exposed to antibiotics used in food production?

Dr. Tannock: Yes, they were healthy individuals. No restriction was placed on their diet, except that none consumed acidophilus-bifidus products. In New Zealand, it is not legal to add antibiotics as growth promoters to animal feeds, although they may be used prophylactically or for treating infections in farm animals. One subject in the 12-month study had a respiratory tract infection and was given a standard treatment regime of amoxicillin. This had no influence that was obvious to us on the total bacterial population. However, we have analyzed the antibiotic resistance of strains of enterobacteria from that subject, and you would be quite frightened at what happens to the gut, as far as the selection of resistant strains is concerned, just on a standard dose of amoxicillin over a 7-day period. We hope to publish this before too long.

Dr. Pfeifer: I have a question concerning your ribotyping technique. Does this technique allow you to discriminate among individual strains with 100% exactness, or do you in fact identify groups of strains that have the same pattern? Do you think we really have all the tools necessary to investigate the microflora in humans? What about molecular probes?

Dr. Tannock: So far as discrimination among strains by ribotyping is concerned, we find it is best to use the two genetic fingerprinting methods, so that we get sufficient sensitivity. With ribotyping, we always use at least two different restriction endonucleases, used separately in making digests. That gives us very good discrimination between the lactobacilli and the bifidobacteria, but you can improve it still further by using the second fingerprinting method, pulsed field gel electrophoresis. You could spend your entire career evaluating typing methods, but you have to draw the line somewhere.

You mentioned DNA probes. That’s certainly an area with great potential, particularly in looking at the structure of the microbial community. The good work on the nutritional interactions among bacteria in a gastrointestinal ecosystem has been done with the rumen ecosystem. We know there that there are consortia of microbes that work together to break down certain substrates and there is cross-feeding of nutrients and so on. I would suspect that this is also happening in our colon, but at the moment I don’t think we have a mental picture of the spatial relationships of one type of organism to another. What I’m suggesting is that different kinds of bifidobacteria coaggregate or bifidobacteria and bacteroides coaggregate. We could study this kind of thing very well with in situ hybridization.

Dr. Pfeifer: So do I interpret you correctly that you cannot discriminate strains by ribotyping only?

Dr. Tannock: No—I’m confident that I could tell your strains from all the rest here. I’m perfectly confident of that. I know after examining a couple of fecal samples which ribotypes of *Lactobacillus* and *Bifidobacterium* are present in their feces; it’s like a forensic test. If I get another sample from that individual, I will know who it was.

Dr. Lentze: I’m intrigued by this. Why do you think the flora is so individual from person to person? Is it purely the environment, or are there factors in the host providing this kind of individual flora?

Dr. Tannock: Well, it is my impression from these results that individuals have their own collection of strains. Why that is, I don’t know. There could be genetic influences. There
have been studies from The Netherlands on the intestinal microflora in twins \((1)\), and it appeared that the genetic constitution influenced the flora. As I said before, there are so many variables in the way humans live, and each individual is so unique, that I don’t know how one would try and tackle this problem.

**Dr. Bohles:** When we are talking about colonization of a body’s surface, have you any idea if there is a correlation or relation between the colonization of the gut and the skin? Could there be a relation between the mechanisms of colonization?

**Dr. Tannock:** The basic concepts of colonization may be similar, but the microfloras in different parts of the body are all unique to their particular origin. Thus, in my mind they are quite separate microflora.

**Dr. Guesry:** I’m quite puzzled by the bacteriological profile of your 12 volunteers compared to what we usually have in our own volunteers in Europe, and I suppose also in the United States, not to mention less developed countries. You say they were normal volunteers, and not on any type of special diet, but were they Caucasians or Maoris? Was their diet really of the same type as our European diet? And were they lactose-tolerant or lactose-intolerant, because that could also cause a change in the intestinal flora?

**Dr. Tannock:** Well, there was a mixture of genders and some ethnic differences. I take a simple approach. I ask, “Who is going to be consuming a probiotic?” The answer will be a great mix of people, as we have in this room now, so we’ve just got to test things out in a practical way. Over this wide range of people with different genetic characteristics, possibly with different diets, living in different kinds of houses and with different atmospheric pollutants, there are thousands of variables. We are never going to be able to say that all the differences in the microflora of an individual are caused by this minor dietary factor or that. We have to look at the whole situation and say, “These people are going to go in a supermarket or a health food shop and pull that product off the shelf. What is it going to do for the average person?”

**Dr. Guesry:** Well, in fact, the bacteriological profile of your 12 volunteers was not so variable. The bifidus bacteria count was consistently high. I think that is rather a specific finding. We don’t normally see that in Europeans except in those who consume yogurts regularly.

**Dr. Tannock:** That’s a little hard to believe.

**Dr. Heine:** Can we really discriminate between probiotics and prebiotics? I mean, the bulk of the probiotic bacteria that we ingest when eating yogurt is apparently digested in the colon, and the decay products may serve as growth factors or substrates for the indigenous microflora, so I see no difference between application of live probiotics and the fate of these bacteria when they reach the large bowel.

**Dr. Tannock:** We’ll probably hear more about prebiotics later in the workshop, but my impression of the situation is that probiotics are living microbes, while prebiotics are substrates added to the intestinal ecosystem that the indigenous bacteria utilize to promote their activity. Thus, you could of course presumably mix the two concepts. This raises an interesting ecological point as to just what the intestinal microflora is subsisting on. People often think that it’s the diet of the host. There certainly will be dietary residues that may be important, but this overlooks the fact that we are ourselves producing large amounts of protein from our secretions and from shed epithelial cells. So I believe the intestinal microflora is feeding on us.

**Dr. Black:** Can you predict whether some people will have a higher colonization resistance and some a lower colonization resistance, depending on their gut flora? If we could find some way to stabilize their gut flora so that you don’t see fluctuations over time, this might be the road toward increased resistance.
Dr. Tannock: I was trying to suggest this morning that if we want to test the idea that we really can alter the balance of microbes in the intestinal tract, then lactobacilli are a good choice, because we already know there is variation among humans.

Dr. Saavedra: One of the messages I think we can get from your presentation is that despite the colonization's being a very dynamic process from the strain point of view, there is some stability to it. There is a lot of work that would support the view that there is stability within the individual and that you can tell one individual from another from the point of view of their flora. Do you have any experience regarding changes in this stability? Is there a change with age or with time in an individual?

Dr. Tannock: Those would be fascinating studies to do, but we have not done them. Consider our subject 1, in whom one strain predominated throughout the whole 12-month period. When did that individual acquire that strain? Was it when he was a baby, was it later in life, and will another strain predominate next year? I would love to do that kind of long-term study. With regard to what happens with aging, Mitsuoka's (2) work in Japan suggests that there is a difference in the lactic acid bacterial populations as people grow older.

Dr. Fuller: Were there any in vitro biochemical characteristics that distinguished the persistent *Lactobacillus* strains in the individuals in your 12-month study?

Dr. Tannock: We have not really looked at that, but these persistent strains must have some special properties. If we could find out what they are, we would learn a lot about colonization factors.

Dr. Freter: As you know, the classical studies of the stability of intestinal flora involved *E. coli* strains, and the assumption most of the time was that new strains were newly acquired and marked a break in stability. With your lactobacilli, however, it looks as if all these strains are there, and you are simply dealing with increasing or decreasing populations. One could interpret the older *E. coli* experiment in this way as well. Would you agree?

Dr. Tannock: Yes, with the kind of analysis we do, we are still relying on picking bacterial colonies from dilution plates, so we are only sampling the most numerous strains in the intestinal tract, hitting the peaks, as it were. When a strain peaks, we can detect it; then when it becomes a subdominant population, we no longer detect it. I think that the solution to this problem is to use a quantitative polymerase chain reaction to detect those subdominant strains as well.

Dr. Rowland: In your individual who had no lactobacilli in the gut, was there any evidence that this person was in ill health or had increased susceptibility to disease, and if not, what does that tell us about the role of lactobacilli? And as a corollary, what about your mice without lactobacilli? What is their disease susceptibility?

Dr. Tannock: That human subject was a perfectly healthy young person with no greater susceptibility to infection, as far as I'm aware, than anybody else. Our *Lactobacillus*-free mice are housed in isolators like germ-free animals to maintain them in a *Lactobacillus*-free state. They have sterile food and bedding and so on. They're perfectly healthy animals, and you can't tell the difference on gross appearance between them and animals with a *Lactobacillus* microflora.

Dr. Yolken: I think it is important, before drawing any conclusions, to ask about the sensitivity of your system for detecting minority strains. Do you know, for example, in people with so-called stable strains, whether you can detect 10^6, or 10^7, or 10^8 of a different strain among the predominant 10^12?

Dr. Tannock: We have done experiments to see whether we get good representative coverage of the strains in an individual's fecal microflora, in other words, how many colonies to pick to get an adequate representation. The simple answer to your question is that the more
colonies you pick and genetically fingerprint, the more complex the microflora will appear to be.

Dr. Yolken: I would agree, and that may address the question as to why, if you already have $10^{12}$ bifidobacteria, should you feed yogurt that has bifidobacteria in it? The strains may be quite different, and you may be able to establish a level of $10^7$ or $10^8$ in someone with a predominant strain present at $10^{12}$ and not have it change the predominant flora in any way. This raises the question of the biological diversity if the bifidobacter family and the fact that there may be different biological properties among different strains.

Dr. Tannock: Yes, I think I made the point that, knowing that there is such a diversity of strains, we need to know what the impact of the consumption of a probiotic organism is on the indigenous collection.

Dr. Tontisirin: You mentioned that before a probiotic supplementation trial there is a need for long-term baseline data. How long is long?

Dr. Tannock: I would say a minimum baseline period of 6 months.

Dr. Buerlocher: We are talking about intestinal microflora. But the intestine is very long. What do we know about different parts of the intestine?

Dr. Tannock: In the case of the human, I think we can say that the proportions of the bacteria in the human feces are representative of what is happening in the colon. We know from work done at the Dunn Nutrition Unit that the metabolic activity of the microflora does change from the proximal part of the colon to the distal part (3). But bacteriologically I feel confident that what we are monitoring in the feces does give us an indication of what's happening in the human colon.

Dr. Lonnerdal: I'm not a microbiologist, and I'm still on the learning curve. It seems that major advances have been made in characterizing the fecal flora and that part of your work and that of many others demonstrates that. But we must be careful not to oversimplify. You alluded in your introductory slide to a goal of making the flora similar to some ideal. That is a little shortsighted. As a nutritionist, I can draw some parallels with infant formula. The fact that an infant formula has a similar composition to human milk is no guarantee that this will affect outcome. I think it's very important to look further. For example, I found your data on bile salt hydrolases very exciting. For me, the right sort of outcome would be to examine cholesterol metabolism, which could certainly be significantly affected, and there you are talking about major outcomes. There are certainly implications with regard to health when it comes to bacterial populations, but a lot of other things are related to health also, in particular, cholesterol metabolism.

Dr. Tannock: I'm sure we'll hear of some other factors that could be investigated in relation to the microflora.

Dr. Cezard: I still have some problem in understanding what a probiotic is? Could you define it better? Don't you think that all the bacteria we eat and that colonize our intestine are probiotics?

Dr. Tannock: The concept of a probiotic is that such a bacterial strain has some special attribute that the indigenous strains do not have.

REFERENCES

