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Gut Flora in Health and Disease: Potential Role of Probiotics

Report of the workshop on probiotics 22-24 April, 2004, Amsterdam

**M. Heselmans¹, G. Reid², L.M.A. Akkermans³,
H. Savelkoul⁴, H. Timmerman³ and F.M. Rombouts^{5*}**

¹Science writer, Wageningen, The Netherlands

²Lawson Health and Research Institute, and University of Western Ontario, London Ontario, Canada

³Department of Surgery, University Medical Center, Utrecht, The Netherlands

⁴Dept. of Cell Biology and Immunology, Wageningen University, Wageningen, The Netherlands

⁵Dept. of Agrotechnology and Food Sciences, Wageningen University, Wageningen, The Netherlands.

Abstract

In a young evolving science, there are always more questions than answers. That is also the situation in the emerging field of Probiotics, and this was made very clear at the International Probiotics Workshop in Amsterdam. In the report of this workshop, we present a selection of the most urgent questions in the field of probiotics. In addition, we propose a few strategies for the future of probiotics research. During the workshop, 120 experts - from disciplines including Human Nutrition, Gastroenterology, Nutritional Therapy, Cell Biology, Microbiology and Immunology - discussed new views on microbe-host interactions and the role of probiotics in prevention and alleviation of gastro-intestinal, atopic and auto-immune diseases. There is a general consensus among the experts that administering defined strains can help in preventing and curing gut flora related diseases: the first clinical trials show a promising role for probiotics. But the system is very complex, and most underlying mechanisms are still unclear. Rapid progress in this field will depend largely on the collaboration between fundamental researchers from different disciplines and medical specialists. Besides, more clinical studies are required to convince authorities and the public of the value of microbial therapies.

Do we need another term for Probiotics?

Many people including those who buy products containing probiotics in supermarkets and health shops do not seem to have problems with the term probiotics. Probiotics

are seen as 'good' bacteria that improve your health. As the FAO/WHO defined more precisely: 'Probiotics are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host'. In fact the term probiotics is relatively well defined by the International Scientific Association for Probiotics and Prebiotics (www.isapp.net). But the term is misused by a number of companies that bring products on the market of dubious quality and with vague unproved claims. This situation prompted some of the speakers to suggest abandoning the term Probiotics. "Shouldn't we leave the term Probiotics?" one of them asked. He was supported by others who suggested it is difficult to prove a general health claim such as 'this strain improves your gutflora, which will make you feel better'. How can a company validate such a broad claim? Contrary to probiotics with such a general health claim, the claims for probiotics curing particular diseases can be proved with clinical trials. So, for use as a registered therapy to cure particular gutflora-related diseases, some delegates proposed the term 'bacterial therapy' or 'microbial therapy' or 'bacterial immunomodulators'.

However, not everybody agreed with choosing a new term for probiotics. "Whether or not the producers want to sell it as a food or a drug, all probiotics must, by definition be proven to confer a health benefit that is measurable and shown by clinical trials", said Dr. Gregor Reid from the University of Western Ontario in Canada. So rather than change the name, he stated, producers need to change, so that consumers and healthcare providers are clear what is truly a probiotic and what is not. Also the FAO/WHO preferred to retain 'probiotic' with the hope that in time its proper definition will be adhered to.

So there was no agreement about a special term for medical use of probiotics. However, one can argue that it is possible to use both new terms such as 'bacterial' or 'microbial therapy' and the term 'probiotic', depending on the context.

How can fundamental 'mechanism-studies' be integrated with clinical 'disease-studies'?

During the symposium, several specialists demonstrated direct and indirect effects of particular bacterial strains on immune cells, the mucosal barrier and gut-motility. Others showed for example the effects of stress on gut permeability or on the regulation of the mucosal barrier function by neuro-immune factors. Almost all experiments in this scientific field of mechanisms are done in vitro and in animal models – mostly rats and mice. In addition to these fundamental studies, medical specialists demonstrated the effects of defined strains on gut-flora-related diseases in patients. Dr. Dominique Brassard from Rhodia summarised the results of the first clinical trials with bacterial therapies. In Pubmed, he had counted 97 randomised controlled trials with patients. Most studies,

For the programme of this workshop and abstracts of lectures, readers are referred to:

<http://www.probiotics-amsterdam.org>

*For correspondence. Email frans.rombouts@wur.nl.

Brassard remarked, show encouraging results for the prevention or treatment of the following diseases with the following strains:

- Protection against atopic disease in infants by administration of probiotics during pregnancy and breast-feeding or to children (*Lactobacillus rhamnosus* GG)(LGG)
- Prevention of infection after surgery or transplantation (*Lactobacillus plantarum* 299)
- Prevention of acute diarrhoea (LGG, *L. reuteri* SD2112)
- Reduction of abdominal bloating and pain in patients with irritable bowel syndrome (*E.coli* Nissle 1917)
- Prevention of pouchitis onset and treatment maintenance of patients with ulcerative colitis (VSL#3)
- Prevention of recurrent urogenital infections (*Lactobacillus* GR-1 and RC-14)

At the conference, some speakers confirmed these encouraging results and gave details on some proven effects in these conditions. However, one of the problems is how to integrate fundamental in vitro and animal model studies dealing with the mechanisms into clinical trials dealing with the effects of bacterial strains on patients. If you know that avirulent *Salmonella* in an in-vitro monolayer of epithelial cells inhibits IL-8 induction by growth factor TNF-alpha, what does that say about the inhibition of IL-8 in a real gut system where many human cells and bacteria interact with each other? The connection between fundamental insights and medical claims was an issue in several debates. For example, Dr. Einar Husebye from the Ullevål University Hospital showed that *Clostridium*, *Escherichia coli* and a mix of lactobacilli and bifidobacteria have different effects on the gut motility in germ-free mice.

But how can you apply this fundamental knowledge to patients with diarrhoea? As Husebye admitted: "It's a very long way to go from basic scientific evidence in a germ free situation, to a normal situation where you add one strain to 1300 other species." And there are more pitfalls in applying the results of mechanism-studies to the situation in patients. Diarrhoea is a multifactorial disease, not only influenced by a disturbed gut motility but also by other possible factors including toxins and a disturbed adsorption of water and secretion. Upon which factors does the administered strain act in patients with diarrhoea? You can make the same arguments for the effects of bacteria in patients with for example colon cancer. As Joseph Rafter of the Karolinska Institute of Stockholm has shown, there are many mechanisms involved in colon cancer that can be influenced by gut-bacteria. Some *Lactobacillus* strains bind mutagens/carcinogens in vitro, some prevent uptake and distribution of mutagens from the gut in rats, and others break down nitrosamines in rats. What are the administered strains doing in the patients?

But even though integration is difficult, mechanistic studies are important, and must somehow be part of clinical investigations in the future.

Should we start by studying the diseases or by studying the functions and mechanisms?

The gap between mechanistic studies and clinical trials leads to another question: should we start by studying the effects of particular strains on particular patients with particular diseases, or by studying the functions of the bacteria in the gut? According to Dr. Huub Savelkoul from the Wageningen University in the Netherlands it is better to start by studying a few diseases, and looking for particular strains that provide benefits to the recipient in a defined way. When the first therapies succeed, he expected, the public and authorities will be eager to invest more in the mechanisms behind the effects.

However, one problem that needs to be overcome is that not all diseases themselves are easy to define. For example, clinical trials and mechanistic studies show a promising role for *Escherichia coli* strain Nissle 1917 in the alleviation of Inflammatory Bowel Disease (IBD). But IBD can take on several forms as Dr. Wolfgang Kruis from the Evangelisches Krankenhaus Kalk explained. The Irritable Bowel Syndrome (IBS) is even an "enigma," Dr. Louis Akkermans from the University of Utrecht told the audience. "We know little of its pathogenesis. Psychologists, physiologists and dieticians all have different views."

Nevertheless, *E. coli* strain Nissle 1917 seems to diminish the symptoms of IBS, as the first randomised placebo controlled studies have shown for three subgroups of patients (total amount: 117). The Nissle strain had an effect in patients with post infectious IBS, with IBS started after antibiotic therapy, and in patients with alternating stool habits. But the question still remains, how do they act in the host? There are speculations, but nothing is proven, and where do you start when the pathogenicity is not clear?

In the case of a syndrome, it is wise to start by studying the effects of particular strains on different subgroups of patients. Kruis has done this also for IBD. He has evaluated clinical trials for three subgroups of patients: patients with Crohn's disease and ulcerative colitis (efficacy as yet not positively proven), ulcerative colitis (proven efficacy in maintaining remission) and pouchitis (proven effects in primary prevention and secondary alleviation). But even if you have distinguished different effects on different types of patients, you are still a long way from understanding the mechanisms behind the effects.

Both Dr. Gerald Tannock from the University of Otago (New Zealand) and Dr. Tore Midtvedt from the Karolinska Institute in Stockholm proposed to start by studying the functions of bacterial strains and defining the precise interaction between bacteria and human cells. "Do not think disease," Midtvedt said. "Focus on genotypic and phenotypic description of your strain and go for the function." A disadvantage of focusing on functions and precise, molecular and cellular mechanisms is the huge complexity of the interaction between immune cells, hormones, bacterial compounds, bacterial species etc. But the advantages are broader understanding of the whole gut system and broader applicability of the knowledge gained. Furthermore, with advanced

technologies such as micro-arrays, the shotgun method for sequencing all the bacterial DNA in the gut, and nano- and microscopic technologies that allow us to follow molecules directly in the cells, it has become easier to define interactions between (bacterial and human) genes, proteins and metabolites. “We actually are going to see how cells react with a bug,” stated Reid.

During the conference, speakers and attendees put forward proposals for closing the gap between fundamental and clinical studies. The most important conclusion was that rapid progress and major breakthroughs in the field of probiotics will depend largely on the collaboration between fundamental researchers from different disciplines (such as molecular biology, bacterial ecology, psychology, nutrition and immunology) and medical specialists. Accessible databases with all the studies on different levels, from mechanistic studies up to clinical trials, can help consolidate such a network. A database can be made for each particular gut-related disease that can be cured or treated with probiotics.

How can probiotics be used to modulate the immune system?

An example of promising fundamental research that requires close collaboration with clinical research concerns the role of the gut flora in the host defence system.

The lectures of Dr. Ailsa Hart, Dr. Ger Rijkers, Dr. Lionel Bueno and Dr. Erica Isolauri demonstrated the pivotal role of dendritic cells in the balance between tolerance and active immunity to antigens from micro-organisms and food components. These dendritic cells are able to ‘sense’ the intestinal microflora. Depending on the strain they sense, the dendritic cell’s maturation signals for the gut-associated lymphoid system differ, leading to different interleukins inducing tolerance or stimulation of the immune system.

Specific strains of the gut microbiota have been demonstrated to alleviate intestinal inflammation, normalise gut mucosal permeability dysfunction and down-regulate hypersensitivity reactions. One or more of these favourable properties may be present in a variety of microbial strains of different origins.

However, because of the different effects particular strains can have on the host immune system, it is very important to know the role of a specific strain in the gut before it is used more widely. As Isolauri stated, “Specific immunomodulatory properties of probiotic strains and their safety should be precisely characterised before the development of clinical applications for extended target populations.”

How can in vitro and animal studies, or those with biomarkers and randomised human trials be integrated?

More studies are necessary to convince the public and regulatory agents of the potential effects of probiotics as therapeutic regimens. Human studies are especially necessary. “We have done enough animal studies to know that probiotics protect against diseases,” said Joseph Rafter (Karolinska Institute, Sweden). “But the

human data are weak.” So how can you integrate in vitro and animal model studies with human studies to improve the validation of claims and risk assessments?

Rafter showed how the EU-funded SynCan project – Synbiotics and cancer prevention in humans – has started on an integrative approach. A twelve-week randomised, double blind, placebo-controlled trial of a food supplement in polypectomised and colon cancer patients was combined with a carcinogenicity study in rats. The researchers gave the rats the same food supplements as the patients, containing the prebiotic inulin, enriched with oligofructose, and a strain of *Lactobacillus rhamnosus* and *Bifidobacterium lactis*. During the study they measured the same 25 biomarkers (colon mucosa biomarkers, faecal activity markers and immunological and inflammatory response markers in colon and blood). In this way, the researchers were able to compare the short-term effects in humans – twelve weeks -with the long-term effects in rats, actually until they died.

This kind of combined rat-patient study can be useful for validating or discounting biomarkers. The current biomarkers lack a qualitative check, complained Rafter. “It’s nice to see that probiotics diminish the DNA-damage, but there is no proof that less DNA-damage diminishes the risk of a tumour. You can make the same argument for all the biomarkers.” The lack of validated biomarkers is a problem for regulatory agents too. “Biomarkers are important,” said Dr. Peter Borriello from the HPA Colindale in London.

However, some experts at the symposium were not so interested in focusing on biomarkers. They preferred to focus on epidemiology studies in which hundreds or even thousands of patients are followed for long periods of time, even ten or twenty years. These enable you to see if there really is a relation between probiotic use and delayed development of a disease. “It’s not so expensive,” someone from the audience said. “You only need to ask patients who use probiotics and those who do not and see if he or she has developed a tumour.” These types of studies need close collaboration between several hospitals from different countries. As featured in Nature, 22 January 2004, some groups already are organising broad patient studies. For example Dr. Francisco Guarner, a gastroenterologist at the Vall d’Hebron Hospital in Barcelona, is helping to organise an EU-backed clinical study involving 360 patients suffering chronically from one of two types of IBD – ulcerative colitis or Crohn’s disease – at centres in Ireland, Spain, Finland and France. The patients, all in remission, receive either a strain of *Lactobacillus salivarius* UCC or a strain of *Bifidobacterium infantis* - both strains reduce gut inflammation in experimental animal models.

How should we judge clinical studies?

According to Brassart, probiotics must be subjected to the same scrutiny that is applied to other pharmacological therapies, i.e. randomised, controlled, double-blind clinical trials, especially if you want them to retain the designation “probiotic” and make specific therapeutic health claims. Clinical trials are still required to prove an

effect in the prevention of diarrhoea, or in any effective treatment of patients with IBS or IBD. Among 1395 references concerning probiotics on Pubmed database, 97 were on randomised controlled trials in humans (7%). But interpretation of these studies is still difficult due to their large variability in relevance, parameters studied and probiotic strains that are used. Dr. Brassart proposed therefore three criteria for analysing articles which claim therapeutic benefits of probiotics:

- Are the results of the study valid? This includes a consideration of the biases inherent in the study design, randomisation, follow-up and analysis, blinding, study controls and reporting.
- What are the results? Once the answer to the first question is judged to be positive, the magnitude and precision of the results can be considered. This is largely dependent on the study size.
- Will the results help in caring for patients? This question connects the results to real-life patients, considering the similarity of study subjects to other patients, and whether the study endpoint is significant for patient care. Consideration is also given to weighing the therapeutic risks and benefits. This also considers the expected result of not giving the therapy in question.

Rafter proposed criteria for good intervention studies to measure the effect of pre-and probiotics on colon cancer:

- They have to be done with three types of subjects: healthy persons, cancer patients and high risk groups.
- Their design has to include randomised, double-blind trials with control groups, over a length of time that is appropriate for the markers employed and with information on the patients' diet.

However, not everybody in the audience agreed with the need for double-blind studies with placebo-control. "We shouldn't follow too easily the recommendations prescribed for the pharmaceutical industry," a specialist stated. "Consistency is at least as important as double-blind."

Which bacteria are residents, which are transients?

Dr. Gerald Tannock made clear that it is of primary importance to be able to distinguish between resident and transient or autochthonous and allochthonous micro-organisms. Studies using *Lactobacillus ruminis* as model gut micro-organism are beginning to reveal the molecular base of autochthony. Also Dr. Tore Midtvedt elaborated on the continuous balance-imbalance that exists in our gut microflora. Lots of micro-organisms come and go, although some may stay for a while.

The transients come and go, but the residents in our gut are rather stable, as Dr. Arthur Ouwehand from the University of Turku (Finland) summarised. These residents can differ at strain-level from one subject to the other. Tannock showed that bifidobacteria strains differ in young babies of 25-35 days in Ghana, New Zealand and the United Kingdom. For example *B. infantis* was detected in the faeces of 23 Ghanaian children, while this

strain was absent in the faeces of children in New Zealand and United Kingdom. In New Zealand 16 children had *B. longum* while the figure was eight in United Kingdom and two in Ghana (Young et al. 2004. *Clinical Diagnostic & Laboratory Immunology*. Submitted for publication).

Resident strains can also change over the years in one individual. Tannock's group followed the faecal microbiota of children during the first six years of their lives, and showed that different strains predominate over different times. Bifidobacteria dominate in the first months, but are pushed out by other species after weaning. At the age of 4 years the composition of the microbiota in an individual is quite stable, but a study of a person at the age of 60 years the composition appears to be more diverse with increased numbers of *Clostridium*. The changed composition may influence the susceptibility for probiotics.

The probiotics are believed to be transient. "They don't colonise," Ouwehand concluded. According to several specialists, adherence to human cells is therefore not important for the selection of a strain. Besides, how can you test adherence? As Ouwehand remarked, "You can see in vitro that a strain adheres to human cells, but what does that say about adherence in the gut? These kinds of questions have never been asked, never been tested." However, under some conditions, you can influence the resident-population at least for a while.

How can we predict the effects of an administered strain on the host?

There are probably more than 1300 species of bacteria in the gut. Together they play an important role in metabolic activities including acid production, antimicrobial formation and vitamin synthesis. They also play a role in providing the host with 'energy' for example by fermenting lactose and fibres. They appear to enhance resistance to colonisation of potential pathogens depending upon the inoculum size of the latter, and they influence intestinal motility, maturation of the newborn gut and development and modulation of the immune system. The impact of certain bacteria on the host can be considerable. For example, *Bacteroides thetaiotaomicron* may switch on/off 400 genes in the host, thereby considerably influencing molecular architecture of the gut surface.

However, for most species we do not know what they actually do in the gut. To complicate it further, even closely related strains can act in different ways. Tannock gave the example of ten tested bifidobacterial strains, provoking defined and decisive but different effects in the immune system. Only four stimulated to a greater or lesser degree the production of the anti-inflammatory Interleukine 10 by the T-cells. This is an important property because IL-10, preferentially produced by the regulatory T-cells, provides an increasingly appreciated target for immunotherapy. It is thus widely assumed – however not proven - that probiotics can exert their positive effects on the gut immune system by inducing regulatory T-cell populations. But it is important to realize that closely related strains react in a different way.

Another difficulty in predicting the precise impact in the gut of an administered strain concerns the different conditions in vitro and in the gut, as Dr. Michiel

Kleerebezem from Nizo Food Research in the Netherlands explained. By using an in-vivo expression technique, he was able to show that a number of genes in *Lactobacillus plantarum* WCFS-1 are expressed more than a 100 fold in the duodenum, compared with the expression level in a laboratory medium.

One way of studying the functions of the different strains is by using molecular genetic tools such as sequencing and micro-array technology. Kleerebezem showed how his group has selected the strains with the gene coding for the multidomain receptor responsible for mannose adhesion. If you know which functions you want and if you have isolated the genes responsible for these functions, it is quite easy to select for the strains with these particular genes. Besides, micro-array technology is likely to generate a lot of important information on the functions of bacterial genes in real time within the gut.

Will the strain reach the right compartment?

Selecting the strain with the right genes is not enough. The next question is: does the organism reach the gut compartment, and once there, does it function as predicted? Dr. Midtvedt has devoted a lot of attention to "compartmentalisation" in the gut, in terms of microbial survival and biological activity of organisms in different compartments. A point less well addressed was *where* in the gut various probiotics exert their specific effects. The small intestine is probably very important for immunomodulatory actions. But if this is the case, it should be realised that passage-time of non-adhesive micro-organisms does not exceed four hours. This time period may be too short for significant multiplication and functionality.

Unless delivery systems are used that protect bacterial passage to the gut, strains likely will need to be able to resist lysozyme, acid, bile and pancreatic enzymes. However, with only in vitro tests it is not possible to predict the resistance in the gut. As one of the experts explained, a bug can be resistant to a low pH, but after dinner the pH in the stomach is much lower, and two minutes later it's different again.

"So how do you optimise the delivery system," asked Reid. "Can you have a system that goes to one compartment in the small bowel and another compartment in the colon?" According to him there are technologies for this purpose such as different types of encapsulation.

Are the probiotics dead or alive, and does it matter?

The considerations about compartmentalisation lead to the question, for which biological activities the administered bacteria have to be alive, and for which activities are dead cells also adequate. There are examples of dead bacteria having an effect, but there are also examples of dead bacteria having no effect, less effect or even an adverse effect. Probably the effect will depend on the way the bacteria have been killed and on the way they act upon the host. In certain cases DNA per se or a protein from a dead bacterium may be enough to fulfil a function but this would not constitute a probiotic – simply a pharmaceutical type product.

Sadly, too many companies sell products claiming to be probiotics when in fact their contents are either all

dead at time of use, or with insufficient viability to retain the claim of being probiotic. The viability of bacteria is not generally tested at the time the product is used by the consumer.

What is the optimal dose?

"Everybody tries to avoid the question of doses," a member of the audience remarked during one of the debates. Probiotics in different doses, varying from a few millions to a few billions per portion, are available on the market. But little is known about the optimal dose of probiotics in most applications. And to what extent does the dosage matter anyway? The administered dose is not likely the same as the effective dose in the gut. As Dr. Ger Rijkers from the University of Utrecht remarked, "An administered probiotic will multiply 100 fold or 10 fold in the gut, depending on the person. So there can be huge differences between the administered dose and the real effective dose in the gut." It is difficult to test the dose effect on health and well-being in general, but with more specific claims, research groups can do dose-response studies. Dr. Peter French from VRI BioMedical Ltd in Australia stated that his company has done dose-response studies. They measured the effect on the immune system at five different doses. According to French, industry should start considering this factor. "The sceptical consumers expect us to do this kind of studies. That they are too expensive is no argument."

Should we use single strain or multistrain probiotics?

There are reasons to believe that mixtures of strains belonging to one or more species would perform better than single strains. Multistrain probiotics could in principle be targeted against different risk factors. Binding to different receptors can be an advantage too, as Rijkers pointed out. For example, polymorphism exists in Tol-Like Receptors (TLRs) expressed on dendrite cells (DCs). Insofar as probiotic strains bind to TLRs to exert their immunological effects, it would be obvious that mixtures of strains eliciting these immunological effects could be more effective by binding to different polymorphs of TLRs.

Dr. Nada Rayes from the Charité Universitätsmedizin in Berlin described a first clinical trial that has compared the effect of multiple strains with that of one strain. This was done in a double-blind, randomised placebo-controlled trial with 2*33 liver transplant recipients and 2*30 patients after pylorus preserving. A preparation of different probiotic strains significantly reduced the bacterial infection rate following liver transplantation and markedly after pylorus preserving compared to treatment with only prebiotics and compared to treatment with one probiotic strain. In addition, the treatment was well tolerated, and without serious side effects.

However, in general regulatory agents still insist on each component of any drug being tested separately, and for a company with 8 probiotic strains, testing each strain combination would be financially prohibitive.

How can we guarantee the safety of probiotics, also in the long term?

Before a microbial therapy is put on the market, regulatory agents want to be sure that it is safe. Borriello gave an overview of the possible risks of probiotics. He stated that probiotics should not cause harm to the consumer, should not transfer (antibiotic) resistance determinants and should not facilitate transfer of virulence factors. In the last 30 years, 180 cases of lactobacillaemia have been reported and 69 cases of infective endocarditis in which lactobacilli were involved. "They do exist," concluded Borriello, "But they are very rare." Besides, the cases with common bacteria had nothing to do with oral intake, but with lack of hygiene in the hospital. In surgical site infections lactobacilli are absolutely unimportant. Host risk factors are extremes of age, an immunocompromised state and underlying disease.

However, Borriello said, any probiotic is a potential pathogen, so it is important to improve the current safety tests. Indirect safety tests such as those proposed in the 1970s (absence of enzymes such as azoreductase, nitroreductase, β -glucuronidase and lack of formation of biogenic amines and deconjugated bile acids) are not widely supported. That is also the case for several in vitro tests. "What does an in vitro study with a monoculture say about the effect in the gut?" Boriello asked. Other types of tests such as formation of capsules around bacterial cells and the ability to translocate across the gut wall into the blood stream may be more relevant. He gave his own examples of safety tests, including a 30-day rat feeding study, a challenge test with immunodeficient mice, a rabbit infective endocarditis model and human volunteer studies. The best kind of studies, according to Boriello, are those in which you measure everything you can in patients and in volunteers, and also conduct interviews. You have to do these studies with many people and over a long term.

For guidance in safety considerations consult the EC Regulation no. 258/97 concerning novel foods and novel food ingredients. http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=31997R0258&model=guichett

Different regions, different bacteria, different needs

Almost all clinical studies with microbial therapies are done with people from developed countries, but there may be more variety in gut flora between people from different regions of the world than between the flora of people in the same region. As mentioned earlier, Dr. Tannock showed the differences in flora between children in New Zealand, United Kingdom and Ghana. Having said that, Dr. Reid stated that there are similarities in the vaginal flora of women in different continents, and why wouldn't there be, since bacteria have inherited the earth long before it separated into continents and long before humans emerged. Thus, there are many similar organisms in all of us, but there are different organisms too.

The needs of different population groups are different too. More studies are needed to examine the role of probiotics in Third World countries, and for example to reduce the risk of diarrhoea and HIV/AIDS. Dr. Reid

showed how fermented milk products, in combination with community building, could play a role in preventing deaths and infections in countries like Tanzania, Kenya, and Nigeria. In terms of HIV, there is a strong correlation between loss of vaginal lactobacilli and increased risk of disease. Thus, by restoring the vaginal lactobacilli, it may be possible to reduce the risk of HIV in women. His group is working on a concept whereby local African families make their own probiotic yoghurt, provide it for children and eventually form a small business to make it available for others.. "This is not a Western solution for Africa," Reid emphasised, "Local groups used to eat fermented milk products for years." What does come from Western countries is the technology to study the vaginal and gut flora and to select better strains.

Probiotics cannot replace a healthier lifestyle

Much of the world's current chronic disease burden finds its origin in modern lifestyle such as nutrition, environment and stress. Probiotics can diminish the risk of chronic diseases, but everybody knows that good health is better promoted by spiritual harmony, physical exercise and good nutrition. According to Dr. Stig Bengmark, from University College London Medical School, humans are drifting too far away from the situation to which they are genetically conditioned by evolution. We used to eat four to ten times more fibre and our food used to be laden with micro-organisms, of which lactobacilli were probably very important. We have a dual digestive system: the one based on our own enzymes, occurring in the small bowel, and the one based on microbial fermentation in the large bowel. The flora in the colon produce organic acids but they also release antioxidants, growth factors, anticoagulants and produce immunomodulins. In conclusion, we need both prebiotics (fibres, flavonoids etc.) and probiotics, which were in more generous supply in the past when we had a less refined daily diet. So, as Bengmark emphasised. "*Lactobacillus plantarum* cannot replace eating plants and vegetables. My attitude is if we eat correctly, we don't need probiotics." However, others stated, until suitable probiotic enriched foods are available, supplementation of the diet is the only way to replenish the organisms we excrete each day.

Summary

So rapid progress in the field of probiotic science will depend on more interdisciplinary and international collaboration. Here we summarise the outcomes of the workshop:

- Much is to be gained by further exploration of the favourable roles that probiotics can play in maintaining health and in alleviation of chronic diseases. There is an especially promising future for applications in the clinical setting. Probiotics cannot always replace a healthier lifestyle, but they show great promise in alleviation of gut and other diseases.
- In the future, consideration should be given to whether a new term is needed for specific uses of probiotics, such as therapeutic cure of disease. For now, we need to monitor how regulatory agencies in different countries handle food, dietary and drug uses of probiotics, and

whether producers of unproved products either get off the market, provide clinical proof of their product's efficacy or call their products not probiotics.

- There is much more to be learned about the functions of the gut microbiota if we are to be able to design intelligent and effective applications of probiotics. A key step will be to develop systems that allow real-time analysis of the bacterial composition, gene expression, host responses and proteins produced within gut compartments. Such techniques may only be 5-10 years away.
- Rapid progress and major breakthroughs in this field will depend largely on the collaboration between fundamental researchers from different disciplines (such as molecular biology, bacterial ecology, microbiology, psychology, nutrition and immunology) and healthcare specialists.
- To convince authorities and the public of the value of probiotics there is a need for education and large-scale, clinical studies within hospitals and community settings in different countries. These studies will also provide insight into the safety and long term effects of administered strains.
- To alleviate 'poor-region' diseases such as HIV and diarrhoea it is important to find locally adapted solutions in collaboration with researchers in Africa, Latin America and Asia.

