FINAL PROGRAMME
&
ABSTRACTS OF LECTURES AND POSTERS

TNO Beneficial Microbes Conference

International conference on the health impact
and future potential of beneficial microbes

29-30 May 2008
Amsterdam, the Netherlands

Organising Committee

Mrs. Dr. Marjorie Koenen
TNO Quality of Life, the Netherlands

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WELCOME AT THE TNO BENEFICIAL MICROBES CONFERENCE!

Dear participant,

The microbiota in the gastro-intestinal tract of man and animals has been shown to be important for health and disease. For instance, a clear role has been established for the endogenous microbiota in inflammatory diseases such as ulcerative colitis and Crohn's disease. But also a role in colon cancer has been suggested. Even a role in such diverse diseases or disorders as obesitas and autism has been postulated.

Moreover, over the past decades, the benefit of probiotics has been shown in various areas, including allergy, inflammatory disease, competitive exclusion of pathogens, stool habit, and even reduction of sick-days in the case of flu or stress at work. Furthermore, probiotics and prebiotics are used in infant formula to direct the development of the endogenous microbiota. For probiotics, an interaction with the mucosal immune system seems the major mechanism by which these beneficial microbes exert their benefit to the host. Numerous hypotheses on how they might work have been postulated recently. The role of prebiotics in directing the composition and activity of the endogenous microbiota is also studied widely.

The **TNO Beneficial Microbes Conference** will highlight the most recent advances in the understanding of the mechanisms behind the health benefit of probiotics and how the endogenous microbiota influences health and disease. Novel tools will be presented and the implementation of the '–omics' technology is this research area will be highlighted. An important aspect is application of beneficial microbes, both probiotics and through the endogenous microbiota, for product development in food and feed industry.

The specific topic areas are the interplay between beneficial microbes and nutrition, the epithelium, the immune system and future developments in the field of beneficial microbes in the food and feed industry.

We aim at a networking meeting to inform you on the latest scientific developments and the industry's requirements and to create a European platform for the new initiatives for the application of beneficial microbes in the food and feed industry.

On behalf of the Organising Committee,

Marjorie Koenen

TNO Beneficial Microbes Conference

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- abstracts of lectures and posters are grouped separately;
- the lectures are grouped according to the daily program;
- the posters are grouped according in alphabetical order according to the first author.

CONFERENCE PROGRAMME

Thursday 29 May 2008

09.00 Opening of the **TNO Beneficial Microbes Conference**
Dr. C.M. (Tini) Colijn-Hooymans
TNO Board of Management, the Netherlands

09.15 Keynote lecture:
Beneficial Microbes: the first 100 years since Metchnikoff and beyond
Dr. Ger Rijkers
University Medical Center Utrecht and St. Antonius Hospital Nieuwegein, the Netherlands

Morning chair:
Prof.dr. Willem M. de Vos
Wageningen University, the Netherlands and Helsinki University, Finland

INTERPLAY BETWEEN NUTRITION AND BENEFICIAL MICROBES

10.00 *New approaches to understand microbial fermentation in the gut*
Dr. Koen Venema
TNO Quality of Life, the Netherlands

10.30 *Development of intestinal microbiota and the impact of diet and lifestyle*
Prof.dr. Willem M. de Vos
Wageningen University, the Netherlands and Helsinki University, Finland

11.00 Networking break: coffee and tea

11.30 *Metabolic profiling of mammalian-microbial interactions*
Prof.dr. Elaine Holmes
Imperial College London, Biomolecular Medicine, UK

12.00 *The health impact of prebiotics: definition, in vitro/in vivo measurement, efficacy, limitations and pitfalls*
Dr. Fred Brouns
Cargill, Belgium and Maastricht University, the Netherlands

12.30 *Probiotics in animal nutrition and health*
Dr. Frederique Chaucheyras-Durand and Dr. Henri Durand
Lallemand, France

13.00 Lunch

Thursday 29 May 2008

Afternoon chair:
Dr. Koen Venema
TNO Quality of Life, the Netherlands

BENEFICIAL MICROBES AND THE EPITHELIUM

14.00 *Commensal and probiotic bacteria that enhance the epithelial barrier function: role for endoplasmic reticulum stress responses*
Prof.dr. Dirk Haller
Technical University of Munich, Nutrition and Food Research Centre, Germany

14.30 *Visceral sensitivity and the modulation of pain receptors by Lactobacillus*
Prof.dr. Pierrre Desreumaux
The French National Institute for Health and Medical Research (Inserm), France

15.00 *Interactions of probiotic bacteria and Escherichia coli in the intestinal tract*
Dr. Alojz Bomba
P.J. Šafárik University in Košice, Institute of Experimental Medicine, Slovakia

15.30 *Epithelium crosstalk with the immune system*
Dr. Iliyan D. Iliev
FIRC Institute of Molecular Oncology Foundation - European Institute of Oncology, Italy

16.00 Networking break: coffee and tea

BENEFICIAL MICROBES AND THE IMMUNE SYSTEM

16.30 *The topography of the intestinal immune system*
Dr. Oliver Pabst
Hannover Medical School, Institute of Immunology, Germany

17.00 *Microbes that shape the development of the immune system*
Dr. June L. Round
California Institute of Technology, Division of Biology, USA

17.30 *Prospects for immunomodulation by beneficial microbes*
Dr. Richard Verbeek
TNO Quality of Life, the Netherlands

18.00 End of day 1

18.15 Informal get-together: boat trip across the Amsterdam canals

20.00 Conference dinner in the 15th century St. Olofs Chapel

Friday 30 May 2008

Morning chair:
Dr. Jan Sikkema
Top Institute Food and Nutrition, the Netherlands

HEALTH APPLICATIONS OF BENEFICIAL MICROBES

08.30 *Probiotics prevent intestinal dysfunction caused by psychological stress*
Dr. Mélanie Gareau
University of Toronto, The Hospital for Sick Children, Canada

09.00 *Can probiotics prevent common cold and maintain health in the workplace?*
Prof.dr. Jürgen Schrezenmeir
Federal Research Institute of Nutrition and Food, Department of Physiology and Biochemistry of Nutrition, Germany

09.30 *Disease-inhibiting potential of probiotics in models of inflammatory bowel disease*
Dr. Lex Nagelkerken
TNO Quality of Life, the Netherlands

10.00 *Prospects for use of functional foods in prevention of allergic diseases*
Dr. Annick Mercenier
Nestlé Research Centre, Nutrition and Health Department, Switzerland

10.30 Networking break: coffee and tea

11.00 *Screening and application of beneficial microbes for infant nutrition*
Dr. Margaret H. Dohnalek
Abbott Laboratories, Abbott Nutrition Division, USA

11.30 *Anti-hypertensive effects of microbially produced bioactives*
Dr. Peter Olesen
Chr. Hansen, Denmark

12.00 *Probiotics in animal feed applications: concept, facts and perspectives*
Prof.dr. Carlos Simões Nunes
DSM Nutritional Products, France

12.30 Lunch

Friday 30 May 2008

Afternoon chair:
Dr. Koen Venema
TNO Quality of Life, the Netherlands

FUTURE DEVELOPMENTS

13.30 *Metagenomics: discovering new functionalities of microbes*
Dr. S. Dusko Ehrlich
INRA, France

14.00 *Future scientific perspectives: the brain-gut axis*
Prof.dr. Robert-Jan M. Brummer
School for Health and Medical Sciences, Örebro University, Sweden and Maastricht University, the Netherlands

14.30 *Vision on beneficial microbes: 2010-2020*
Dr. Nico van Belzen
ILSI Europe, Belgium

15.00 Closing lecture:
Putting microbes to work – in the end, what's really possible?
Dr. Gregor Reid
Canadian R&D Centre for Probiotics, Lawson Health Research Institute and University of Western Ontario, Canada

15.30 End of the **TNO Beneficial Microbes Conference**

LECTURES

Beneficial Microbes: the first 100 years since Metchnikoff and beyond

Ger Rijkers

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Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. The first written documentation of the health promoting effect of probiotics can be found in the Persian bible, in which it reads in Genesis 18, verse 8 that Abraham (Ibrahim) owned his longevity to the daily consumption of fermented milk products. The scientific literature on probiotics starts with Metchnikoff in 1907, which incidentally also dealt with longevity. His hypothesis was that the aging process was fuelled by intestinal microbes (autointoxication) and that lactic-acid producing bacteria could suppress growth of autointoxicating bacteria. At that time, life expectancy at birth in Europe was 51 years for males, 53 years for females. Today, this is 74 for males and 80 for females. The 23-27 years gain in life expectancy in a century is the combined result of improved hygiene, better housing, feeding and clothing, less physical demanding labour and less environmental pollution. Also a better control of infectious diseases, through many of these previous factors, through vaccination and through antibiotics and the general improvement in preventive and curative medicine have contributed to this gain. With all these variables, direct proof for the first part of Metchnikoff's hypothesis is virtually impossible to obtain. As far as the second part of his hypothesis is concerned, probiotic bacteria do have the ability to interfere with growth of other intestinal bacteria and thus certainly can contribute to a better control of infectious diseases. Indeed, probiotics nowadays are successfully used for prevention and treatment of gastrointestinal infections and prevention of respiratory infections. There are many other areas of application including inflammatory bowel diseases, allergic diseases and even hypertension and obesity. Indirectly it can be concluded that probiotics bacteria therefore contribute to longevity, i.e. a long and healthy life.

Research on probiotics is rapidly expanding. On May 14th 2008 the search term 'probiotics' results in 4277 publications in the PubMed database, of which more than 29% (1244) were reviews. For comparison, the search term 'antibiotics' yields 475,910 publications of which 8% (39,873) are reviews. Clearly, the field of probiotics does not suffer from reviews but from original research. What is needed at the start of the second century of probiotics is research into the molecular mechanisms of the interactions between microbes and man. This will learn which bacterial strains and molecules interact with their human host to exert specific beneficial effects.

New approaches to understand microbial fermentation in the gut

Koen Venema[1,2]

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The human colonic microbiota comprises a complex microbial ecosystem vital for human gut health. An essential function of this community is the fermentation of dietary substrates, which escape digestion in the upper digestive tract. Such substrates, in particular dietary fibers, and prebiotics, are known to be important promoters of intestinal health, mainly through their interactions with the human colonic microbiota. However, these interactions are far from being fully understood. In the past, stimulation of activity and growth of microbial species in the colon has generally been investigated by determining the amount of metabolites and the numbers of (a limited set of) species in faecal material, respectively. However, faecal material does not necessarily reflect what happens in the proximal colon, where fermentation of most of the current commercial prebiotics takes place. Therefore, we have used stable-isotope (^{13}C)-labelled carbohydrates to determine which microbial species are involved in fermentation of these substrates, in addition to metabolomics analyses to determine the metabolites that are produced from these substrates. These experiments were performed in TNO's in vitro model of the large intestine (TIM-2). Incorporation of ^{13}C-label into microbial biomass was investigated using stable-isotope probing (SIP). Production of ^{13}C-containing metabolites was followed using LC-MS and NMR. In addition, the HIT-Chip, a microarray platform developed at the Wageningen University, and the I-Chip, a similar platform developed by TNO, were used to study the composition of the microbiota. The data allowed us to develop a model for fermentation of carbohydrates by the collective microbiota based on the metabolomics data. In addition, using SIP we showed concrete evidence of cross-feeding between microorganisms. These results are integrated with clinical studies using amongst others transcriptomics to study 'gut health' in human volunteers. Also, tools are developed to study the interaction between micro-organisms and the host. This contribution will highlight the latest developments in this multi-disciplinary research field, including examples from the gastro-enterology area, new screening methods, and the exciting area of nanotechnology.

Development of intestinal microbiota and the impact of diet and lifestyle

Willem M. de Vos

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Our intestinal tract is colonized since birth by a defined set of microbes that develops into a stable climax community in adult life. There is considerable attention for their role as the intestinal microbiota has been implied in intestinal aberrations and lifestyle diseases such as obesity. Based on information from 16S rRNA gene clone libraries, metagenomic sequencing and phylogenetic microarrays, it can be estimated that there are more than 5000 different intestinal phylotypes. Using a high throughput phylogenetic microarray, the Human Intestinal Tract Chip (HITChip) that covers a significant fraction of the total intestinal diversity, we addressed several methodological issues and microbial concepts.

It was established that DNA extraction protocols together with cloning and sequencing approaches, may introduce significant biases that partly explain the different conclusions of the anecdotal studies reported so far. Similarly, biases were found in an often used intestinal model due to inoculation artefacts that could be prevented by appropriate handling.

The high resolution and throughput analysis offered by the HITChip analysis allowed studying the spatiotemporal dynamics of the intestinal microbiota. Each individual was found to harbour a unique microbiota that shows high dynamics after birth but significant stability in adult life, confirming earlier profiling studies. Moreover, the ileum-specific microbiota differs considerably from the colon microbiota in numbers, diversity and dynamics. In addition, we could address the concept of the existence of a core microbiota of identical phylotypes within adults. It was observed that within a single adult individual, some bacterial groups show a higher stability in time than others. While other effects can not completely be excluded, this is likely to reflect specific interactions with the host, as these stable phylotypes include *Bifidobacterium* spp. that are among the early colonizers in the gut and common between monozygotic twin pairs that were living separately for years.

In conclusion, the present data sets support the assumption that the development of the intestinal microbiota is governed by environmental, genetic and stochastic factors. The application of the HITChip and other high throughput tools for analyzing intestinal microbiota allows for establishing correlations with diet, lifestyle and intestinal aberrations. The first results of these approaches that include double blind placebo-controlled interventions will be discussed.

Metabolic profiling of mammalian-microbial interactions

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Metabolic profiling technologies such as high resolution NMR spectroscopy or mass spectrometry in combination with multivariate statistical analysis and bioinformatics analyses are useful tools in the armory of post genomic strategies for Systems Biology research. The metabolic profiles of biofluids and tissues reflect changes in gene expression and protein activity and can monitor their impact on homeostasis. These profiles also report on genetic and environmental stimuli and can be used to assess the outcomes of gene-environment interactions.

It has become apparent that the metabolic profile of urine contains metabolites originating from many sources including endogenous, dietary, medicinal and gut microbial. Indeed the interaction between the mammalian host and its commensals appears to influence, or be influenced by, an extraordinarily wide range of factors including diet, ageing, obesity and disease. Here the focus will be on characterizing the changes in mammalian-microbial co-metabolites under various physiological and pathological challenges and on introducing various mathematical solutions to integrating and interpreting metabolic and metagenomic data. Specific examples will include rodent and human models of obesity, nutritional interventions with pre-/probiotics and profiling the microbial and metabolic changes associated with intestinal disorders.

The health impact of prebiotics: definition, *in vitro/in vivo* measurement, efficacy, limitations and pitfalls

Fred Brouns [1,2], K. Verbeke[3], N. Delzenne[4] and A. Adam[1]

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Do the data cover the claim?

There are a number of considerations to be made and limitations to be understood when one wants to establish the impact of single food components or bio-actives such as prebiotics and probiotics on metabolic and physiological effects and generalize the observations made to overall benefits of health of the human body. Only a thorough understanding will help to put scientific data in a correct context when defining product benefit statements for consumers. First, the ingestion of the daily food will lead to the intake of a large number of compounds that impact on metabolism, physiology and cell-function. As such, the effect of any dietary intervention is always the effect of a concerted action of all food components ingested and metabolized at that particular moment. This makes the study of the effect of a single food component in terms of health support and disease prevention a delicate one that may have important limitations for generalization towards the effect that may occur when included in mixed meals.

A second consideration is that of studying effects in a healthy population, vs. the study in a population that is more at risk or already diseased. For example, if, in a study addressing the effects of specific food components on the prevention of certain cancers the study population would be composed of healthy individuals it would require maybe a few thousand of subjects and a study duration of >10 years to be able to detect a meaningful effect on the clinical endpoint 'cancer'. For practical and economical reasons this is simply impossible. To avoid such problems the alternative focus of choice is often that of high-risk populations that are more prone to developing the disease, as for example adenoma patients in whom the recurrence of adenoma and the size/growth of tumour tissue is taken as a marker of the efficacy of nutritional test compounds to beneficially impact on prevention. But also this approach has its limitation since the question may be raised whether such a genetically affected patient group is really representative to obtain results that are relevant to aspects of disease prevention in the general healthy population. Thus far this question has not really been answered and the studies that have been done with adenoma patients on the effects of prebiotics generally have produced disappointing results. Alternatively it is possible to expose animals to selected carcinogens or inflammatory substances thereby inducing a diseased and study the effects of food components on either the process of initiation (is a lag phase induced?) and/or progression (is progression to more detrimental states delayed or prevented?). Such studies can be done in short term and may help to unravel mechanistic aspects of the biochemical actions of the food compound studied. Again, also in this respect the question is justified as to whether the observations done in animals after exposure to quite aggressive compounds in conditions that do not occur as such in daily human life is really meaningful in terms of health management. A last alternative often used is the in vitro study of human cell line responses. On the one hand this is ideal to study direct influences that nutrients or non-nutritive compounds can exert on human cells but, on the other hand, many influences that normally are present in the human body at the same time are being eliminated in this situation. For example the concerted action of hormonal and neural

influences and the metabolic effects of a many other compounds that normally circulate with body fluids.

Thirdly, there is one other aspect that should not be overlooked. Since the human gut, especially the colon, is relatively difficult to access *in vivo* for the study of microbiota and their metabolism, science does have to rely largely on animal models and *in vitro* work. Thus, most of the work on the fermentation properties of fibers and prebiotics has necessarily been done *in vitro*, using human inoculate.

It is in the light of these limitations that we have to interpret a significant part of the current knowledge on potential gut health benefits in humans.

Gut-microbiota: can we really handle them?
A large body of data suggests that intestinal microbiota may influence gut metabolism and epithelial physiology and function both positively and negatively. The observation that germ free animals do not develop chronic inflammatory disease is of significant value in this respect. If microbiota are involved in the etiology of gut disease, what make this sometimes happen and what factors play a role when it does not happen?

Available evidence shows us that a more putrefactive microbiota activity results in an increase of carcinogenic and mutagenic compounds and induces cell damage and inflammatory responses. Seen the (ever increasing) number of species that make up the overall gut microbiota (probably >1000) the question 'are some species favourable and others unfavourable' as well as what determines their 'aggressive, defensive and balancing' behaviour is very relevant. Can we really understand what we do if we target to modify the microbiota activity of only a few strains that we can measure, without knowing what the impact is on so many other species that we at present are unable measure. Should we study the 'overall microbiota metabolome'? The latter may challenging and helpful, but will require an appropriate analysis of a huge amount of data allowing to relate the gut microbiota metabolome to key physiological functions.

Is dietary fiber = prebiotic?
'What makes bulking dietary fiber (DF) different from specific oligosaccharides?' Early epidemiological studies indicated that populations that consume a high proportion of non-starch polysaccharide (NSP) dietary fibre in their daily diet suffer less from gastrointestinal diseases, in particular colorectal cancers, than populations that consume diets that are high in fat and protein but low in NSP fibre. In this respect increasing the amount of vegetables and NSP DFs, has been suggested to contribute as much as 25-35% to risk reduction for colorectal cancer. Based on these observations, DFs and substances that are part of the fibre complex such as antioxidants, flavonoids, sulphur containing compounds and folate have been proposed as potentially protective agents against colon cancer. Other differences in fiber functionality and physiology are related to the molecular composition in terms of the carbohydrate types present in the molecule and other compounds present in the side chains. Isolated hull fiber such as bran, which is often used as insoluble bulking agent is relatively low in micronutrients compared to e.g. aleurone fiber, which can be obtained by using special milling conditions and which is particularly rich in micronutrients and antioxidants. The currently increased recommendation to stimulate the consumption of whole grain foods is related to make 'fiber-complexed micronutrients' more available to the body. In this respect it may be questioned whether prebiotic oligosaccharides, which are devoid of any micronutrients and antioxidants, are more beneficial compared to more complex but highly fermentable carbohydrates such as wheat aleurone and fruit fibers.

Are SCFA key to health benefits?
There are recent indications that the regular consumption of certain subclasses of highly fermentable dietary fibre sources results in gut associated immune and flora modulation as

well as a significant production of short chain fatty acids (SCFA). In vitro studies as well as animal studies indicate that SCFA in particular propionate and butyrate have the potential to locally support the maintenance of a healthy gut epithelium and to reduce risk factors that are involved in the development of gut inflammation as well as colorectal cancer. Acetate, the most abundantly produced SCFA and also propionate is only partly taken up by the liver and is also passed into the systemic circulation. It may thus induce metabolic effects that may impact on health in several organs. An example of this is the observation that enhanced insulin sensitivity of adipocytes correlated significantly with the extent to which acetate and propionate were present in the circulation as a result of the consumption of resistant starch (Robertson 2003, 2005). Butyrate is the prime substrate for the energy metabolism of the colonocyte and acts additionally as growth factor to the gut epithelium. In normal cells butyrate has been shown to induce proliferation at the crypt base, enhancing a healthy tissue turnover and maintenance. In inflamed mucosa butyrate stimulates the regeneration of the diseased lining of the gut. In neoplastic cells butyrate inhibits proliferation at the crypt surface, the site of potential tumour development. Moreover, models of experimental carcinogenesis in animals have shown the potential to modify a number of metabolic actions and steps in the cell cycle in a way that early events in the cascade of cancer development may be counteracted while stages of progression may be slowed down.

Local gut- or systemic effects on health?

In this respect it should be understood that the functional-physiological effects of indigestible carbohydrate are always complex and are difficult to isolate. For example the inclusion of RS or viscous DF in a food matrix will impact on total available carbohydrate content and accordingly reduce the overall glycemic and insulinemic response. The latter may help to reduce risk factors associated with insulin resistance, obesity, diabetes and cardiac heart disease. In addition, it is hypothesized that fermentation and SCFA production might be involved as well in the prevention of those disease states. Effects of SCFA, particularly of acetate and propionate, on adipose tissue metabolism have been suggested as a possible mechanism (Robertson 2003, 2005). Moreover, a reduction of high glycemic carbohydrates in the daily diet may also impact favourably on a reduction of colon cancer risks. A viscous intestinal environment will also entrap compounds in the small intestine and favour their transport to the colon where they will either be metabolized or ‘complexed’ and excreted. Some fiber types are particularly good for entrapping bile acids and increasing cholesterol excretion, such as beta-glucan, psyllium, guar gum and pectin. Other observations indicate that gut microbiota and their metabolism in one way or another are related to certain health and disease outcomes such as diabetes, obesity, hyperactivity/ADHD, autism, impaired immunity, food intolerance and allergies. This makes the study of possible interactions very interesting.

Prebiotics: changing definitions ?

Overall, data on the efficacy of prebiotics to increase SCFA production, their molar ratio’s, to enhance the growth of favourable microbiota species, etc always need to be interpreted carefully. Attempts to define what prebiotics in fact are, are related to the question of the relevance of their effect on gut microbiota composition and/or metabolism . The original definition given by Gibson and Roberfroid: “Prebiotics are non-digestible food ingredients that selectively stimulate a limited number of bacteria in the colon, to improve host health” is currently being re-discussed, in order to take into accounts different opinions (Gibson and Roberfroid, 1995; Gibson *et al.*, 2004; FAO, 2007). Results concerning the characterisation of the fermentation profile of fibers are known to differ between experiments depending on the inoculate, duration and in vitro method used (continuous vs. in batch systems)(Fässler *et al.*, 2006a,b). As such it is impossible to directly compare results from different laboratories.

Health aspects of prebiotics

With all limitations, we know today that a regular ingestion of selective strains of lactobacilli, bifidobacteria and certain yeasts (all probiotics) as well as substrates that these species

consume for their metabolism and growth (prebiotics), sometimes in combination (synbiotics), is associated with what we feel are favourable modifications of the gut microbiota composition, its enzyme activities, epithelial barrier function, gut associated immune responses, a reduction of secondary bile acids, fecal ammonia, faecal water toxicity as well as a reduction in potential in DNA damage or enhancement of its repair. Other effects, such as modified fluid secretion and absorption, gut motility, transit, stool frequency and fecal bulk have been observed as well. This has led to understanding that the gastrointestinal tract and in particular the colon is much more than just a 'passing through digestive organ'. Acknowledgement that specific substrates potentially also may enhance the growth of pathogenic microbiota, thereby unfavourable effecting gut metabolism and epithelial function is important. In some studies increased fecal water toxicity, inflammatory responses, impaired gut barrier function and increased DNA damage and tumour growth has been observed after consuming fermentable carbohydrates, although it was hypothesized that is would be less and puts emphasis on the need to intensify research in this area. Do we understand why? And what the implications of these observations are? The current presentation will focus on a selected number of these aspects: (i) a definition of dietary fiber, prebiotic and symbiotic; (ii) *in vitro* and *in vivo* determination of functional and physiological characteristics of indigestible carbohydrates; (iii) impact of prebiotics on gut microbiota associated metabolism and health/disease; and (iv) required scientific substantiation to make consumer benefit claims.

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Probiotics in animal nutrition and health

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The gastro-intestinal tract (GIT) of domestic animals harbours dense and complex microbial communities, which can be composed of bacteria, protozoa, fungi, *Archaea*, and viruses. Considerable research has been devoted during these last 30 years to characterisation of digestive ecosystems in terms of microbial composition and functional diversity, which has led to a better understanding of the major contribution of the gut microbiota to animal nutrition and health. Amongst beneficial effects, GIT microbial communities are involved in digestion and fermentation of plant polymers which is of particularly great importance in herbivore animals. The indigenous gut microflora is also responsible for the synthesis of vitamins, the bioconversion of toxic compounds to non toxic residues, the stimulation of immune system, the maintenance of gut peristalsis and intestinal mucosal integrity and plays a barrier role against colonisation by pathogens. Numerous environmental factors are able to affect the composition and functions of gut microbiota in livestock animals. Indeed, feeding practices, composition of animal diets, farm management, productivity constraints are parameters which can influence the microbial balance in the GIT and consequently affect feed efficiency, digestive welfare and health of the animals. An abrupt shift from forage-based to high readily fermentable diet has been shown, for example, to induce important modifications of the ruminal microbial communities, leading to an increased risk of ruminal acidosis, which is recognised as one of the major digestive disorders in dairy and beef herds. Weaning represents also a critical period during which the still immature gut microflora has to face a brutal change in diet, this leading to increase the susceptibility of the young animals to pathogen colonisation. In this context, the possibility to use feed supplements to achieve better animal health, welfare and productivity through manipulation of the GIT microbial ecosystem has gained considerable attention in the last 25 years. In this goal, growth-promoting antimicrobials, such as ionophore antibiotics, have been widely distributed and are still used in some countries. However, due to increasing safety concerns upon the risk of release of antibiotic resistance in the environment, and of persistence of chemical residues in animal products, other strategies based on supplementation of more 'natural' products such as probiotics, have been developed to improve herd health and productivity. Increasing amounts of scientific data are supporting that these products, which are defined as a source of live (viable) naturally occurring microorganisms, can beneficially affect the balance of GIT microbiota and that they have a real interest in animal nutrition and health.

Probiotics for ruminants and monogastric herbivores

In adult ruminants, probiotics have been mostly selected to target the rumen compartment, which is the main site of feed digestion. The rumen microbial ecosystem is constituted by a wide diversity of strictly anaerobic bacteria, ciliate protozoa, filamentous fungi, and *Archaea* which are responsible for degradation and fermentation of 70-75% of the dietary compounds. The most common marketed products are live yeast (*Saccharomyces cerevisiae*) preparations. In dairy ruminants, live yeasts have been shown to improve performance, the most consistent effects being an increase in dry matter intake and milk production (Jouany, 2006; Sniffen *et al.*, 2004; Stella *et al.*, 2005). Also, in beef cattle or young ruminants, growth parameters (average daily gain, final weight, intake, feed to gain ratio) have been reported to be improved by daily live yeast supplementation (Galvao *et al.*, 2005; Lesmeister *et al.*, 2004). These improvements in performance have been related to greater total culturable ruminal bacterial population densities, stimulated growth and fibre-degrading activities of cellulolytic microorganisms, stimulated growth of lactate-utilising bacteria leading to

stabilization of ruminal pH and decreased risk of acidosis, improved ruminal fermentation (Chaucheyras-Durand *et al.*, 2007). The most significant effects have been reported when yeasts have been included in the diet of animals during particularly stressful periods for the gut microbiota and the animal: at weaning, at the beginning of the lactation period, after a dietary shift from high forage to high readily fermentable carbohydrates. Regarding bacterial probiotics, lactate-producing bacteria (enterococci, lactobacilli), which would sustain a tonic level of lactic acid, thus allowing the lactate-utilising species to flourish (Nocek *et al.*, 2002), or lactate-utilising bacteria (*Megasphaera elsdenii,* propionibacteria) able to utilise lactate as energy source (Klieve *et al.*, 2003; Stein *et al.*, 2006) could represent possible means to limit lactic acidosis in high-concentrate fed animals. A growing interest is to use probiotics to reduce excretion by adult ruminants of human pathogens, such as *Escherichia coli* O157 or *Salmonella*. Certain strains of *Lactobacillus acidophilus* have shown to decrease numbers of *E.coli* O157 in feedlot cattle faeces Younts-Dahl *et al.*, 2005) or *in vitro* in sheep faecal suspensions (Chaucheyras-Durand *et al.*, 2006) and appear also to reduce shedding of *S. enterica* (Stephens et al., 2007). Reducing the environmental impact of livestock, either in terms of mitigating methane or nitrogen pollution, is also an increasing concern and probiotics may represent an interesting ecological tool to achieve this goal (Newbold and Rode, 2006). In young pre-ruminants, bacterial probiotics such as lactic acid bacteria (*Lactobacillus* sp., *Bifidobacterium* sp., *Enterococcus* sp., *Propionibacterium* sp.) or *Bacillus* spores generally target the small intestine, as the rumen is not developed yet, and they represent an interesting means to stabilise the gut microflora and limit the risk of pathogen colonisation. Some products have been shown efficacy to improve weight gain and rumen development in young calves during periods of stress (Abu-Tarboush *et al.*, 1996; Adams *et al.*, 2007). In horses, whose targeted digestive compartment is the caecum-colon, probiotic distribution is particularly relevant in case of transportation stress or during distribution of high concentrate diet. Live yeasts have been demonstrated their efficacy to increase fibre digestibility in the colon and modulate the balance of hindgut bacterial communities, leading to a decreased risk of lactic acidosis (Jouany *et al.*, 2008; Médina *et al.*, 2002).

Probiotics for pigs and poultry

The most common probiotics for monogastric animals are yeasts (*Saccharomyces boulardii*), and bacteria (*Lactobacillus* sp*., Enterococcus* sp*., Pediococcus* sp., *Bacillus* sp.) targeting the hindgut (caecum, colon) which harbours an abundant and very diverse microbial population mainly composed of bacteria and *Archaea*. In gestating sows, distribution of probiotics has shown beneficial effects on feed intake, average live weight (Böhmer *et al.*, 2006) with at the same time a greater size and vitality of the litter (Taras *et al.*, 2005, 2006). The efficacy of probiotics may be related to stabilisation of the gut microflora, as suggested by recent studies using DNA community fingerprinting techniques on faecal samples taken from sows at farrowing and receiving a *Saccharomyces boulardii* based-product (Walker *et al.*, 2008). From birth to post-weaning, piglets are very sensitive to gut colonisation by pathogenic bacteria (*E. coli*, *Clostridium difficile*, *C. perfringens*, *Salmonella, Listeria*) parasites (*Isospora, Cryptosporidium*) or viruses (*Coronavirus, Rotavirus*), which are responsible for growth reduction and diarrhoea. Probiotics are therefore particularly recommended during this period and numerous studies have demonstrated efficacy of several products (Cassey *et al.*, 2007; Lallès *et al.*, 2007; Taras *et al.*, 2006). Performance benefits have also been reported after weaning as for example with a strain of *Saccharomyces boulardii* (Bontempo *et al.*, 2006). In this study, the yeast probiotic promoted a 'healthy' intestine, by encouraging an early restoration of the intestinal mucosal thinning generally occurring at weaning, and would possibly improve local resistance to infection. Similar findings have been reported with *Pediococcus acidilactici*-based probiotic supplementation (Di Giancamillo *et al.*, 2008). Benefits on intestinal IgA secretion and reduction of translocation of enterotoxinogenic *E. coli* have also been observed with*S.boulardii* or *P.acidilactici* given to piglets (Lessard *et al.*, 2008). In fattening pigs, growth performance has been shown to be improved in the presence of probiotics which for

some of them (lactic acid bacteria) are directly added in the liquid feed in order to increase the microbiological and nutritional quality of the feed (Moran *et al.*, 2006; Van Winsen *et al.*, 2001). In poultry, benefits of probiotic supplementation (live yeast or bacteria) are reported on broilers performance and health, with evidence of increased resistance of chickens against *Salmonella* infections (Banjeree and Pradhan, 2006; Higgins *et al.*, 2007, 2008). Probiotics can increase feed efficiency and productivity of laying hens (Kurtoglu *et al.*, 2004; Yörük *et al.*, 2004), and an improvement of egg quality (decreased yolk cholesterol level, improved shell thickness, egg weight) has also been measured in some studies.

Probiotics in aquaculture

When looking at probiotics intended for an aquatic usage, it is important to take into account the intricate relationship that an aquatic organism has with its direct environment, compared to terrestrial animals (Kesarcodi-Watson *et al.*, 2008). Gram-negative facultative anaerobic bacteria are dominant in fish and shellfish digestive tract, but the intestinal microbiota of aquatic animals may change very rapidly with the intrusion of microbes coming from water and food (Gatesoupe, 1999). This is probably a reason to explain that a large number of probiotics developed in aquaculture are bacteria directly originated from aquatic environment. However, more 'traditional' bacterial or yeast species marketed for animal nutrition (*Lactobacillus, Pediococcus, Bacillus, S. cerevisiae*) are also used. They can target fish eggs and larvae, fish juveniles and adults, crustaceans, bivalve molluscs and also live food such as rotifers, artemia, or unicellular algae (Verschuere *et al.*, 2000). Growth promoting effects, through a better feed utilisation and digestion, as well as biological control of pathogen colonisation are the most important expected benefits of probiotic applications. Disease outbreaks caused by *Vibrio* sp. or *Aeromonas* sp. have been recognised as a significant constraint on aquaculture production (Verschuere *et al.*, 2000), particularly in the shrimp subsector, where vibriosis is currently one of the main diseases identified (Castex *et al.*, 2008). Whereas *in vitro*, antagonism to pathogens has been clearly demonstrated for a wide range of probiotic strains (Gatesoupe, 1999), *in vivo* evidence of efficacy is still very scarce. A recent study shows that under pond conditions, the distribution of a *Pediococcus acidilactici*-based probiotic could be an effective treatment for limiting prevalence and load of *Vibrio nigripulchritudo* strains in haemolymph of marine shrimps (Castex *et al.*, 2008).

Modes of action

Several mechanisms have been proposed to explain effects of probiotics and it is likely that the positive results reported in the different animal studies are due to a combination of some, if not all, of these. The metabolic activities of the probiotic strains and a good survival throughout the gut appear to be of great importance for an optimal efficacy. Effects are also greatly dependent on the strain used. In monogastric animals, the production of organic acids (lactic or acetic acid) by bacterial probiotics can help decrease the gut pH, create more favourable ecological conditions for the resident microbiota and decrease the risk for pathogen colonisation. Release of antimicrobial peptides, such as bacteriocins, which inhibit the growth of pathogenic bacteria, or production of enzymes able to hydrolyse bacterial toxins have been demonstrated in several studies. Some strains can competitively exclude pathogenic bacteria through their higher affinity for nutrients or adhesion sites. Certain probiotics produce nutrients and growth factors which are stimulatory to beneficial microorganisms of the gut microbiota. In addition to interacting and stimulating other microorganisms, probiotics also interact with the host, by influencing the immune response, or producing components able to positively affect mucosa development or the metabolism of the host intestinal cells. Some probiotics can also metabolise or aid in the detoxification of certain inhibitory compounds such as amines or nitrates or scavenge for oxygen, which is of great importance in gut anaerobic ecosystems. Most of these mechanisms have also been proposed to explain effects of probiotics in the human gut, where several benefits both in terms of nutrition and health have been demonstrated.

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Commensal and probiotic bacteria that enhance the epithelial barrier function: role for endoplasmic reticulum stress responses

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The genetic predisposition to deregulated mucosal immune responses and the concurrent prevalence of certain environmental triggers in developed countries are strong etiologic factors for the development of chronic immune-mediated intestinal inflammation in patients with inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis. It has become clear from numerous clinical and experimental studies that enteric bacteria are a critical component in the initiation and progression of chronic intestinal inflammation. The hypothesis that enteric bacteria accelerate and aggravate the disease pathologies of IBD was supported by clinical observations and studies in gnotobiotic animal models of experimental colitis. Consistent with the observation that IBD patients fail to maintain immunologic tolerance towards bacterial antigens, the barrier function of the intestinal epithelium is abrogated under conditions of chronic intestinal inflammation. In addition, accumulating evidence suggests that enteric bacteria not only have the ability to induce intestinal inflammation, but also mediate beneficial activities. Several studies in animal models of experimental colitis and human IBD trials show considerable therapeutic relevance for VSL#3 and *Escherichia coli* strain Nissle, supporting the hypothesis that commensal enteric bacteria are a critical component in the development/prevention of chronic intestinal inflammation. Intestinal epithelial cells (IEC) must adapt to a constant changing environment by processing the combined biological information of the intestinal luminal content including enteric bacteria as well as host-derived immune signals, suggesting an important function of this biological interface in maintaining mucosal homeostasis. Although advancing knowledge regarding the cellular mechanisms of innate and adaptive immune signalling has led to a better understanding of the disease pathologies in IBD, still little is known about the molecular mechanisms of enteric bacteria in targeting protective and detrimental cell type-specific signal transduction pathways in the genetically susceptible host. Changes in the homeostasis of bacteria- and host-derived signal transduction at the epithelial cell level may lead to a break in the intestinal barrier function and the development of mucosal immune disturbances. In this context, we demonstrated that bacterial proteases contribute to the loss of barrier function and the development of experimental colitis. An emerging new paradigm suggests that stress response mechanisms in the endoplasmatic reticulum (ER) and mitochondrial dysfunctions may contribute to the loss of tissue homeostasis and the development of chronic intestinal inflammation. The ER stress response requires energy-dependent adaptation mechanisms, including the synthesis of organelle (ER- and mitochondrium)-specific chaperones as well as the induction of ER-associated and proteasome/lysosomal-mediated protein degradation mechanisms. Interestingly, VSL#3 triggers post-translational inhibitory mechanisms for TNF-induced chemokine (IP-10) secretion in IEC through the modulation of ER-associated protein processing mechanisms. In conclusion, the lack of adequate control mechanisms at the level of ER-associated stress mechanisms may shape the extent and duration of inflammatory processes contributing to the loss of epithelial cell homeostasis and disease progression in the genetically susceptible host.

Key publications

Shkoda, A., Ruiz, P.A., Kim, S.C., Daniel, H., Rogler, G., Sartor, R.B. and Haller, D., 2007. IL-10 blocked endoplasmic reticulum stress in the intestinal epithelium: impact on chronic inflammation. Gastroenterology 132:190-207; Clavel, T. and Haller, D., 2007. Bacteria- and host-derived mechanisms to control intestinal epithelial cell homeostasis: implications for chronic inflammation. Inflamm. Bowel Dis. 13:1153-1164.

Visceral sensitivity and the modulation of pain receptors by *Lactobacillus*

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Visceral pain and intestinal discomfort are frequent and invalidating disorders that affect a large part of the general population. These symptoms, hardly relieved by conventional treatments, might be potential targets for the use of probiotics, alive micro-organisms providing health's benefit for the host when administered in adequate quantities. Mechanisms of action of probiotics remain poorly understood. Some studies suggest that they modulate the immune response and intestinal flora, and play a role as barrier against pathogenic bacteria, in particular in the prevention of infectious diarrhoea. Their role in the regulation of abdominal pain and intestinal comfort is hypothetical. Two receptor families play a key role in pain regulation: the opioid and cannabinoid receptors. We demonstrated that μ-opioid receptors (μOR) and type 2 cannabinoid (CB2) receptors were expressed in epithelial colonic cells in response to oral administration of the non-pathogenic bacteria *Lactobacillus acidophilus* NCFM. These bacteria have a dose related effect, inducing rapidly a strong and stable expression of μOR and CB2 receptors in the majority of colonic epithelial cells, the first cells that are intimately in contact with the gut flora.

In rat and mice, daily oral administration of *L. acidophilus* NCFM allows a decrease of abdominal pain threshold value evaluated by colorectal distension. This analgesic effect was inhibited by blocking CB2 receptors. *L. acidophilus* NCFM induced an antinociceptive effect at the same magnitude as 1 mg/kg of morphine administered subcutaneously and enhanced by 65% the suboptimal analgesic effects of morphine used at 0.1 mg/kg.

Treating abdominal pain and discomfort with probiotics is a new concept. This safe treatment might be of particular interest among patients with intestinal discomfort and irritable bowel syndrome, a common disease affecting 20% of the general population. The use of *L. acidophilus* NCFM can be envisaged in co-administration with morphine to reduce the effective doses and side effects. Numerous different probiotics are available, but all these microorganisms do not have the same ability to induce μOR and CB2 receptors. A better understanding of the mechanisms of actions of probiotics, focussing particularly on their ability to induce expression of μOR and CB2 receptors, should allow the selection of microorganisms with analgesic effects. Based on these data, a randomized clinical trial against placebo is now in progress in the USA to evaluate clinically the therapeutic efficacy of *L. acidophilus* NCFM administration in patients with irritable bowel syndrome.

Interaction of probiotic bacteria and *E. coli* in the intestinal tract

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Probiotics could represent an effective alternative to the use of synthetic substances in nutrition and medicine. Probiotics are biopreparations containing living cells or metabolites of stabilised autochthonous microorganisms that optimise the colonisation and composition of gut microflora in both animals and humans, and have a stimulatory effect on digestive processes and the immunity of the host. Lactobacilli are most frequently used for probiotic purposes. Diarrheic diseases present a serious health and economic problems. *Escherichia coli* plays an important role in the etiology of the diarrheic syndrome. The use of lactobacilli containing probiotics seems to be a very efficacious method of prevention and treatment diseases caused by enterotoxigenic *E. coli*. The pathogenicity of *E. coli* is conditional on two factors: the ability to produce enterotoxin and the presence of colonization factors enabling the carrier to colonize the mucosa of the small intestine. The inhibition of *E.coli* adhesion to the intestinal mucosa prevent from occurrence of diarrhoea. Probiotics as natural bioregulators help to maintain the balance of intestinal microflora by means of several mechanisms, such as competition for intestinal mucosa receptors, competition for nutrients, production of antibacterial substances and stimulation of immunity. Despite increasing knowledge gained, the mode of action of probiotics has not been fully explained yet. Our study was aimed at the mechanism of inhibition of *E.coli* adhesion to the intestinal mucosa by application of probiotic bacteria. The inhibition of *E.coli* adhesion by probiotic microorganisms have been observed using gnotobiotic and conventional lambs and pigs. In lambs, *E. coli* CCM 612 (0101:K99) and *Lactobacillus casei* 294/89 have been used. In pigs, *E. coli* 08:K88, *L. plantarum* and *L. paracasei* were included in the study.

In gnotobiotic lambs, inoculation with enterotoxigenic *E. coli* (ETEC) alone resulted in diarrhoea with a typical clinical picture and patho-anatomical findings. *E. coli* adhered to the mucosa of the digestive tract at counts amounting to 5.0 log10 per cm^2. The numbers of mucosa-adherent *L. casei* 294/89 following *E. coli* inoculation varied between 1.9 and 2.7 log10 per cm^2. Preventive administration of *L. casei* inhibited the negative effects of ETEC in gnotobiotic lambs, minimized the clinical signs to those a very moderate diarrhoea in the first 12 h after inoculation and significantly reduced the patho-anatomical findings. Enterotoxigenic *E. coli* counts decreased by 99.1 and 76% on days 2 and 4 after inoculation respectively, and amounted to 3 log10 per cm^2. In gnotobiotic pigs, *E. coli* 08:$K88^+$ inoculated alone colonized the mucosa of both jejunum and ileum at counts 6.41 and 6.08 log10 per cm^2, respectively. In experimental groups, the counts of adhered *E. coli* in the identical section of the small intestine, following the inoculation by *Lactobacillus* spp., amounted to 6.35 and 6.43 log10 per cm^2, respectively. The used strain *Lactobacillus* spp. showed an inhibition of 2.1 mm against *E. coli* 08:$K88^+$ under *in vitro* conditions. Two to five days after *E. coli* inoculation, *Lactobacillus* spp. counts adhered to the jejunal mucosa ranged from 5.4. to 6.5 log10 per cm^2 and adhered to the ileal mucosa ranged from 6.1 to 6.8 log10 per cm^2. In the third experiment, the effect of the inoculation of three *L. plantarum* strains upon lactic, acetic, acetoacetic and propionic acid levels in the mucosal film and the jejunal and ileal contents has been investigated in gnotobiotic pigs. In the jejunum of the inoculated animals, the mucosal film revealed significantly increased levels ($p<0.01$) of lactic, propionic and acetoacetic acids when compared to the contents (25.3 vs. 10.8 mmol/l; 18.5 vs. 5.0 mmol/l and 29.7 vs. 11.2mmol/l, respectively) as well as insignificantly increased acetic acid levels (11.0 vs. 5.8 mmol/l).

In the ileum of gnotobiotic pigs, propionic acid levels of the mucosal film were significantly higher than those of the contents (21.2 vs. 9.5 mmol/l; $p<0.05$). In another experiment, the effect of short-term and continual preventive application of *L. casei* subsp. *casei* upon the adhesion of *E. coli* 08:K88 to the jejunal mucosa in gnotobiotic pigs was investigated. After short-term application *L. casei* subsp. *casei* no inhibitory effect upon the adhesion of *E. coli* 08:K88 to the jejunal mucosa was observed. On the contrary, the number of *E. coli* 08:K88 adhered to the jejunal mucosa in gnotobiotic piglets continual inoculated with *L. casei* subsp. *casei* (5.6 log10 per cm^2) was significantly lower ($p<0.05$) in comparison to the group inoculated only with *E. coli* 08:K88 (7.2 log10 per cm^2).

The efficacy of probiotics may be potentiated by several methods: the selection of more efficient strains, gene manipulation, the combination of several strains, and the combination of probiotics and synergistically acting components. From the practical point of view combination with synergistically acting components of natural origin seems to be the best way of potentiating the efficacy of probiotics. By this method, more effective probiotic preparations – potentiated probiotics are developed. In the last experiment, the influence of the administration of *L. paracasei*, maltodextrin Maldex 150 and Raftifeed IPX fructo-oligosacharides on the inhibition of adhesion of *E. coli* 08:K88 to the mucosa of the jejunum, ileum and colon as well as on the organic acid levels was investigated in 33 conventional piglets. Combined administration of *L. paracasei*, Maldex 150 and Raftifleed IPX decreased the numbers of *E. coli* 08:K88 adhering to the jejunal mucosa by 2.0, 0.6 and 1.0 log10 when compared to the groups receiving only *L. paracasei*, Maldex 150 and *L. paracasei* or Raftifeed IPX and *L. paracasei*, respectively. Comparison of organic acid levels in the individual experimental groups revealed acetic acid levels to be significantly higher in the ileum of piglets administered by lactobacilli, maltodextrin and fructo-oligosaccharides in comparison to the other groups. The combination of *L. paracasei*, maltodextrin Maldex 150 and Raftifeed IPX proved to be the most effective one to inhibit the counts of *E. coli* 08:K88 adhering to the intestinal mucosa of the jejunum and colon of conventional piglets.

The results of our experiments showed that: (i) the preventive inoculation of lactobacilli with low adhesion ability significantly decreased the number of *E. coli* adhered the mucosa of the digestive tract in gnotobiotic lambs; (ii) the preventive inoculation of lactobacilli with high adhesion ability did not decrease the number of *E. coli* adhered to the intestinal mucosa in gnotobiotic pigs; (iii) the inoculation of lactobacilli significantly increased the levels of organic acids in the intestinal mucosal film in comparison to the contents in gnotobiotic pigs; (iv) the continual preventive application of lactobacilli was more effective upon the adhesion of *E. coli* to the intestinal mucosa in gnotobiotic pigs in comparison to the effect of short-term application; and (v) the combination of lactobacilli, maltodextrin and fructo-oligosacharides was the most effective upon the adhesion of *E. coli* to the intestinal mucosa in gnotobiotic pigs. Summing up the results of our experiments, the following conclusions can be drawn:

- The competition for adhesion receptors on the intestinal mucosa likely did not play a decisive role in the mechanisms of inhibition of enterotoxigenic *E. coli* adhesion to the intestinal mucosa by lactobacilli. More likely, it seemed to be a metabolite-mediated inhibition.
- The significantly higher levels of the lactobacilli produced organic acids in the intestinal mucosal film in comparison to the intestinal content may present an efficient barrier inhibiting the adhesion of digestive tract pathogens to the intestinal mucosa
- The continual preventive application of lactobacilli upon the adhesion of enterotoxigenic *E.coli* to the intestinal mucosa is more effective in comparison to the short-term application.
- The inhibitory effect of lactobacilli upon the adhesion of *E. coli* to the intestinal mucosa can be significantly potentiated by combination with maltodextrin and fructo-oligosacharides.

Epithelium crosstalk with the immune system

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The gut is the most extended organ that interfaces with the external environment, whose major task is to digest ingested food. It is aided in this function by beneficial microorganisms that colonize the whole intestinal tract with a greater representation in the terminal ileum and the colon. Even though there is an abundance of bacteria at mucosal surfaces, exaggerated immune responses are avoided because commensal bacteria and food antigens are physically separated from the direct contact with mucosal immune system by epithelial barrier. Accordingly, bacterial uptake is localized in the Peyer's patches (PP) where specialized epithelial cells (ECs), called M-cells, would be able to transport them through the EC barrier and make them available for antigen presentation.

However, this model has been questioned by recent findings, suggesting that bacteria can be taken up throughout the intestine by dendritic cells (DCs) which extend protrusions between the EC tight junctions. These findings raised a number of important questions such as

- How intestinal tolerance is achieved if any bacteria can be ingested by DCs throughout the entire intestinal tract?
- Are gut resident DCs different from the conventional one in their ability to induce inflammation?
- Why commensal bacteria have a reduced ability to activate antigen presenting cells at mucosal surfaces?

To answer these questions and to understand better how the complicated cellular network in the intestine maintains the mucosal balance, we concentrated our study on 3 major players in the intestinal homeostasis: epithelial cells, dendritic cells and T cells, and their interaction with the microflora.

Intestinal ECs are the first barrier between the mucosal immune system and the external environment. As mucosal DCs are in close contact with epithelial cells, we investigated the possibility that intestinal ECs controlled the function of underlying DCs and rendered them refractory to activation. We then explored the ability of EC-conditioned DCs to induce immunosuppressive T cell responses. In order to study EC-DC interactions, we have developed a two partner co-culture system, that mimics the *in vivo* spatial distribution of two important players that regulate mucosal immune responses: polarized monolayers of epithelial cells and DCs. Using this system, we found that both in mouse and humans intestinal ECs drove the differentiation of tolerogenic DCs with decreased IL-12 and IL-23 production, but elevated IL-10 in response to LPS and bacterial stimuli. Interestingly, EC-conditioned DCs were able to induce T regulatory cell (Treg) differentiation, non-inflammatory Th2 response, and contracted Th1 and Th17 development. In order to evaluate if EC conditioning takes place also *in vivo*, we confronted the ability of DCs isolated from mucosal sites (MLN DC) versus splenic DCs to induce Treg differentiation. Only MLN DCs, but not spleen DCs, were able to induce Treg development. Notably, spleen DCs acquired this ability after conditioning with intestinal ECs. Thus tolerogenic DC conversion might take place in the gut and EC-derived factors are most likely involved in this process. In order to explore this possibility, we employed a number of techniques to block the accessibility of different EC-derived factors to DCs. Using this approach, we identified that EC-derived TGF-β and retinoic acid, and in humans also thymic-stromal lymphopoietin (TSLP), were all required for DC conversion. Soluble factors released by ECs were involved in conferring DCs with 'non-inflammatory' phenotype, but only DCs in close contact with ECs upregulated the

expression of CD103, a marker that characterizes MLN tolerogenic DCs coming from mucosal sites. To understand the role of EC-DC induced Treg cells *in vivo* we employed a mouse model of DSS-induced colitis. Using this model, we found that Treg cells isolated from EC-conditioned DC co-cultures were extremely potent in suppressing colitis in mice and homed to the gut. Thus ECs can induce tolerogenic DCs with Treg polarizing properties by both, contact dependent and contact-independent mechanisms, and this process takes place in the gut.

Our findings give insights on how tolerance in the gut is achieved, but it remains unclear if ECs discriminate between 'beneficial' and 'dangerous' signals or if this is a property to underlying APC. To answer to this question we first analyzed the response of ECs to different bacteria. We stimulated polarized Caco-2 monolayer with invasive *Salmonella*, non-invasive *Salmonella* or probiotic bacteria and one hour later bacteria growth was blocked with antibiotics. We found that ECs released MIP3α and IL-8 only in response to invasive *Salmonella* which was able to penetrate the monolayer. We then conditioned human monocyte DCs (MoDCs) with ECs supernatants form each of the treatments and co-cultured them with naïve T cells. In our control treatments MoDCs were directly stimulated with each of the strains. We found that only supernatants from invasive *Salmonella* treated ECs were able to activate MoDCs. Supernatants from ECs treated with non-virulent strain or probiotic bacteria did not activate DCs and did not induce inflammatory immune responses, demonstrating that ECs respond to bacterial stimuli and induce immunity only when PAMPs (pathogen-associated molecular patterns) penetrate the EC barrier. In addition, direct activation of MoDCs with *Salmonella* resulted in two times stronger IL-12p40 and IL-6 production, compared to probiotic treatment.

It is now evident that intestinal ECs are not simply a barrier between mucosal immune system and the external environment. Our findings add new insights and present a mechanism by which ECs play active role in tolerance induction or immune activation in the gut by controlling DC function.

The topography of the intestinal immune system

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The intestinal immune system has to survey a large surface area which is exquisitely sensitive to environmental determinants. This is owed to the endeavour to constantly admit transit of nutrients while that of micro-organisms, in particular pathogens, needs to be prevented or at least contained locally once an infection is installed. The most impressive visible sign of immunological activity in the gut are Peyer´s patches, that are easily spotted already by the naked eye as aggregated follicles bulging out of the intestinal tube. However, besides Peyer´s patches, solitary intestinal lymphoid tissue (SILT) provides a structural platform to efficiently initiate immune responses in the murine small intestine. SILT consists of dynamic lymphoid aggregates that are heterogeneous in size and composition, ranging from small clusters of mostly lineage-negative cells known as cryptopatches to larger isolated lymphoid follicles rich in B cells. Here we report a novel technique that allows monitoring the dynamic behaviour of individual SILT over time. We demonstrate that colonization of germ free mice with commensal bacteria provokes an adjustment of the spectrum of SILT to that observed under specific pathogen free conditions by the conversion of pre-existing lymphoid structures into larger-sized SILT. Further enhanced microbial stimulation by means of oral infection with the enteropathogen *Salmonella* yields SILT that exceed the size spectrum of structures observed under pathogen free conditions. *Salmonella* directly infects SILT triggering a vigorous inflammatory response and immunopathology that leads to enlargement and morphological destruction of SILT. Dissemination of *Salmonella* from infected small intestinal tissue into the periphery depends on dendritic cell migration. Dendritic cells constitutively traffic from the intestine to the gut draining mesenteric lymph nodes (MLN) in a chemokine receptor CCR7-dependent mechanism that is essential to induce tolerance to food proteins. Salmonella exploit this pathway and utilize migrating dendritic cells to reach the MLN. However, under normal conditions the MLN prevent spreading of intestinal dendritic cells and thereby *Salmonella* beyond these lymph nodes. Surgical removal of the MLN breaks this vital barrier and results in fatal systemic infection. This indicates that the MLN do not only serve as important site for the induction of intestinal immune responses but also limit the dissemination of enteropathogens.

Microbes that shape the development of the immune system

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Colonization of humans with multitudes of commensal species creates an ecosystem harbouring members of five of the six kingdoms of life. Surprisingly however, the gut is stably colonized by both beneficial and potentially pathogenic microorganisms. Moreover, imbalances in the composition of the bacterial microbiota, known as dysbiosis, are postulated to be a major factor in human disorders such as inflammatory bowel disease (IBD). We report herein that the prominent human symbiont, *Bacteroides fragilis*, protects animals from experimental colitis induced by *Helicobacter hepaticus,* a commensal with pathogenic potential. Most importantly, this beneficial activity requires a single bacterial molecule (polysaccharide A or PSA). Animals harbouring *B. fragilis* not expressing PSA, along with *H. hepaticus*, develop disease and produce pro-inflammatory cytokines in colonic tissues similar to *H. hepaticus* colonization alone. Purified PSA administered to animals protects from experimental colitis, intestinal pathology and wasting disease through anti-inflammatory, interleukin 10–producing $CD4^+$ T cells. Furthermore, PSA suppresses IL-23-driven IL-17 production in an animal model of intestinal inflammation, directing an IL-10-dependent protective response in the host. These results reveal the first molecule of symbiotic bacteria that networks with the immune system to mediate the critical balance between health and disease. Harnessing the immunomodulatory capacity of symbiosis factors such as PSA may ultimately provide therapeutics for human inflammatory disorders based on entirely novel biological principles.

Prospects for immunomodulation by beneficial microbes

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The probiotic properties of commensal bacteria including lactobacilli and bifidobacteria are likely to be determined at least in part by their effects on dendritic cells (DC). DC play a key role in mounting innate responses as well as in instructing adaptive immune responses in the gastrointestinal tract and elsewhere in the human body. Like traditional immune stimulants such as LPS, probiotic bacteria promote maturation of cultured human DC by inducing elevated expression of MHC class II and co-stimulatory molecules. Different effects have been reported on cytokine induction, especially of major regulatory cytokines such as TNF-alpha, IL-12 and IL-10. Yet, these previous analyses have failed to reveal consistent differences between such effects of probiotics on the one hand, and of LPS on the other. Selective response markers for probiotics, however, would be important for our understanding of their biological properties and for a rational selection of strains for *in vivo* studies.

To study the impact of probiotics on cultured human DC, several probiotic strains were supplied to cultures of immature human DC. After 48 h of culture, these DC were analyzed in detail for surface markers and cytokine secretion, defined as 'early effects' on DC. In addition, gene profiling was performed on a large group of genes which encoded mediators of inflammation, growth factors and development. These were defined as 'late effects'. The effects of probiotics on DC were compared to unstimulated DC or DC stimulated with LPS as danger signal. In the early stages of stimulation, all probiotic bacteria induced qualitatively very similar responses in DC at the level of surface markers and secretion of cytokines and chemokines. All probiotic bacteria led to markedly increased levels of the surface markers HLA-DR, CD40, CD80, CD86 and increased secretion levels of the cytokines and chemokines relative to unstimulated DC. LPS induced very similar effects on DC as the probiotics. The only difference found was a markedly lower immune stimulatory effect by *B. animalis* BB-12, the only *Bifidobacterium* tested, as compared to lactobacilli. Late responses of the DC, on the other hand, tended to diverge. Microarray transcript profiling for 268 cytokines, chemokines, growth factors and their receptors after 48 h of culture revealed various transcripts to be selectively induced by certain probiotics but not LPS. Among these gene markers are markers that are known to exert an anti-inflammatory effect *in vivo*.

These data indicate that late rather than early DC responses may be helpful to clarify the divergent biological effects of probiotics on human innate immune responses and may be helpful in selecting beneficial probiotics for *in vivo* use.

Probiotics prevent intestinal dysfunction caused by psychological stress

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Stress is defined as any threat or disturbance in homeostasis, which includes physical or psychological stressors that can occur acutely, chronically, and during early life. The timing and duration of exposure to stressors impacts on the extent of damage observed in the intestinal mucosa, as well as the persistence of such changes following recovery. Exposure to acute stresses, as assessed, for example, following exposure to 1 h of water avoidance stress (WAS), limits intestinal damage to the surface epithelium and includes both an increased secretory state and barrier dysfunction. These effects are maintained, in part, by corticotrophin-releasing factor (CRF) and enteric cholinergic nerves. Prolonged exposure to stressors, such as occurs with chronic stress, leads to more extensive gut damage, including altered bacterial-host interactions, mucosal inflammation, and epithelial cell apoptosis that are mediated via activation of mast cells. Administration of probiotics for the duration of the stress reverses the changes in bacterial-host interactions, but not the affected permeability.

Due to the ability of CRF to mimic acute stress, we assessed the role of chronic CRF administration on parameters affected by chronic stress. Using osmotic mini-pumps to infuse CRF peptide, similar to chronic stress, mast cells are responsible for increasing the secretory state, enhancing both paracellular and macromolecular permeability, as well as altering bacterial-host interactions.

Early life stress, in contrast to psychological stress, has long-term effects on colonic physiology that persists into adulthood. Maternal separation of pups causes colonic barrier dysfunction, altered bacterial host interactions, activation of the hypothalamus-pituitary adrenal axis, and altered cholinergic innervation in weanling aged rats. Daily treatment with *Lactobacillus*-containing probiotics for the duration of the separation prevents these changes in colonic function, a feature which persists into adulthood by preventing WAS-induced barrier defects. Bacterial infection was employed as a different type of stressor to determine whether neonatal mice are more susceptible to pathogens than adult animals. Infection with *Citrobacter rodentium* caused epithelial cell hyperplasia and colonic inflammation in neonatal mice, as previously established in adults, but the neonates also lost body weight and succumbed to the infection whereas adult mice do not. Daily treatment with the probiotics starting one week prior to infection reduced hyperplasia, prevented weight loss and prevent death in neonatal animals challenged with the enteric bacterial pathogen.

Taken together, these studies provide evidence that probiotics have beneficial effects in a variety of well established and complementary models of stress. Therefore probiotics could serve as a novel intervention for humans, both in the adult and pediatric populations, undergoing periods of excessive stress.

Can probiotics prevent the common cold and maintain health in the workplace?

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There are several controlled human trials on the effect of probiotics on winter infections and the common cold, respectively (De Vrese *et al.*, 2005, 2006; Turchet *et al.*, 2003; Tubelius *et al.*, 2005; Hatakka *et al.*, 2001; Weizman *et al.*, 2005). In these trials different strains of LAB were used. In 2 trials the administration of probiotics resulted in a lower duration of infections and lower severity of symptoms (De Vrese *et al.*, 2005, 2006; Turchet *et al.*, 2003). In 3 trials a lower incidence of episodes due to winter infections was reported (Tubelius *et al.*, 2005; Hatakka *et al.*, 2001; Weizman *et al.*, 2005). This, however, was not significant when respiratory tract infections alone were evaluated. Sick leave was lower (10.6%) in the probiotic group compared to the placebo group (26.4%) ($p<0.01$) and 0% compared to 33% in a shift-worker subset (Tubelius *et al.*, 2005).

At present a meta-analysis on the effect of probiotics on respiratory infections is lacking.

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Disease-inhibiting potential of probiotics in models of inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract, with a prevalence of up to 0.2 % in the Western countries. IBD comprises two major forms - Crohn's disease (CD) and ulcerative colitis (UC) – which are distinct from a histopathological point of view. CD is characterized by transmural inflammation that can affect the entire gastrointestinal tract, whereas UC is rather characterized by superficial inflammation confined to the colon. A dysregulated mucosal immune response to the intestinal microbiota is considered as a prime cause of IBD. In this regard, activation of innate cells through Toll-like receptors (TLR), in conjunction with impaired regulatory T cells, might lead to an exaggerated inflammatory response. In CD this can in part be attributed to impaired NOD2 function and increased NF-κB activation, eventually leading to excess IL-23 and the development of T cells secreting IL-17.

Most if not all animal models of chronic inflammation are dependent on disruption of immunological tolerance due to the activation of TLR on immature dendritic cells (DC), resulting in their maturation, the activation of NF-κB and the expression of pro-inflammatory cytokines. Mature DC are well-equipped to induced polarized T cell responses, which may give rise to chronic inflammatory disease in the absence of control by regulatory T cells. Interestingly, however, ligand-binding to C-type lectins receptors (CLR) may keep DC in a tolerogenic state, thereby preventing the development of an unwanted T cell response. Peptide-targeting to CLR has indeed shown disease-inhibiting potential in models of autoimmunity. Likewise, intestinal immune homeostasis is probably dependent on a delicate balance between triggering of the innate immune system via TLR and tolerization via CLR; perturbation of this balance may result in chronic inflammation of the gut.

It is hypothesized that probiotics contribute to intestinal immune homeostasis by the induction of tolerogenic DC in conjunction with regulatory T cells. This presentation will review the disease-inhibiting potential of probiotics and underlying mechanisms based on experimental animal models of IBD.

Prospects for use of functional foods in prevention of allergic diseases

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Allergy is defined as an over-reaction of the immune system - accompanied or not by increased levels of IgE - to a specific antigen (called allergen) that occurs in a portion of naturally exposed individuals. Of note, intolerance to lactose or gluten, and hypersensitivity reactions to histamine or tryptamine are not considered as food allergies but correspond to adverse reactions to foods. Allergy is a syndrome covering a collection of manifestations at the level of the gastro-intestinal tract, respiratory airways and/or skin. Allergic diseases have increased substantially over the last decades and are recognized today as epidemics by WHO. Overall, food allergies affect 5-6% of children and 1-3% of adults, while the frequency of respiratory allergies is as high as 20-30%. However, the prevalence of different types of allergy varies within different countries and age populations. Cow's milk allergy is the first food allergic manifestation that occurs in suckling infants. While it often resolves by itself, some children rather develop subsequent allergies to a variety of foods such as cereals, eggs and peanut. Atopic infants are at very high risk (30-60%) to next evolve towards allergic rhinitis and later on towards allergic asthma. The predisposition of atopic subjects to develop successive allergic manifestations is called the 'atopic march'. Atopy is defined as personal and/or familial tendency to become sensitized and produce IgE in response to ordinary exposure to allergens.

The steep increase in allergic manifestations can evidently not be attributed to a change in the genetic background. The currently investigated 'Hygiene Hypothesis' (see Schaub *et al.*, 2006) postulates that environmental changes linked to the Westernized conditions of life may explain this phenomenon. In particular, the lack of exposure to protective microbial stimulus in urban as opposed to rural areas may be responsible for a Th2-bias of the host immune system that is characteristic of the allergic status. This hypothesis was raised as result of cross-sectional or longitudinal epidemiological studies. Even though the Hygiene Hypothesis does not solve all open questions, recent papers have reported that life on the farm or farm milk consumption may protect against onset of certain respiratory allergies, at least in populations with a specific genetic background (Lauener *et al.*, 2002). The 'farming effect' seems to be especially effective in early life. The conclusions of these studies underline the importance of key factors impacting on allergy such as the genetic background of the host, the routes and doses of exposure to allergens, the importance of intervention windows, the protective role of certain microbes and the quite likely role of the intestinal microbiota. The Hygiene Hypothesis is at the basis of the rationale for using probiotics as nutritional intervention to fight allergies. Other ingredients such as flavonoids, vitamins, polyphenols etc. have also been investigated for their potential role in preventing allergies but probiotics remain high on the list of candidate protective agents.

Since the first clinical trial in 1997 (Majamaa and Isolauri, 1997), over 20 randomized double-blind placebo-controlled intervention studies enrolling over 2,800 subjects (including placebo groups) have been conducted. Three types of trials were performed that aimed at achieving primary prevention of atopic diseases, treatment of atopic eczema or secondary prevention of allergic rhinitis, respectively. Globally, these studies conducted to sometimes conflicting results, which reflects the variety of possible confounding factors that may affect clinical outcomes. Typically, the clinical trials dealing with probiotics and allergy vary substantially in

their design (duration of treatment and intervention window), the targeted population (age and sub-type of allergy), the number of enrolled subjects, the environmental factors, and the nature of the probiotic supplement. However, comprehensive reviews (Prescott and Björsten, 2007; Betsi *et al.*, 2008; Caramia *et al.*, 2008) and one meta-analysis (Lee *et al.*, 2008) focused on atopic disease/dermatitis have recently been published. Even though they partly diverge in their conclusions, the consensus is that the evidence is stronger for prevention of atopic disease than for treatment of atopic dermatitis, and that the probiotic approach certainly deserves to be further explored. There is indeed, in the case of food allergy, a need to find alternative solutions to the currently recommended eviction diet (allergen avoidance). Concerning respiratory allergies, the positive studies reported a reduction of symptoms and an improvement of quality of life of the patients suffering for allergic rhinitis.

In parallel to the human trials, active research is pursued in the selection of well performing anti-allergy probiotic strains and the design of combinations of protective food ingredients. Efforts are also invested to better understand the mechanisms underlying modulation of the allergic reactions and the role played by the intestinal microbiota in protection or onset of this disease. Finally, the discovery of new biomarkers of tolerance or allergy would be of great help. To this end, a variety of *in vitro* assays and animal models are currently in use or under development. To move the field forward, gaps or points to consider have been identified by an expert group participating to the ongoing ILSI Probiotics Task Force. Their conclusions will be published as a peer-reviewed article in the coming months and will be briefly presented during the conference.

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Screening and application of beneficial microbes for infant nutrition

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Numerous research findings continue to be published that support the addition of probiotic strains to infant nutritional products. Commercial products exist today, focusing mainly on use of probiotic strains for children over the age of one year. The application of probiotics to infant nutritional products will continue to gain acceptance from the health care professional, from the consumer, and from regulatory agencies if the justification for the selection of the probiotic strain or strains is based on key scientific assessments, and involves screening processes that establish a clear scientific basis for the benefit to the infant.

There are several assessments that support the selection of probiotic strains for infant nutritional products. These include survival in the upper gastrointestinal (GI) tract, establishing dose, an assessment of variables related to safety, and establishing efficacy, or more generally, the benefit for the infant. Designing a research program for screening of probiotic strains must take in to account not only the expected benefit, but also the level of the benefit to the infant. Understanding the mechanism of action for probiotic strains that leads to the intended outcome, is also relevant.

Using *in vitro* models, it is possible to show that not all probiotic strains transit the upper GI tract to the same extent. Cumulative survival by different strains of lactobacilli, or for lactobacilli vs. bifidobacteria, can be significantly different. Manufacturing conditions for the cultures likely play a role in impacting survival. These findings influence the fortification rate for the probiotic, to insure an adequate dose reaches the colon.

The colon is typically viewed as a major site for the role of the probiotic. The ability to influence the dynamics of the microbiota, and provide benefit to the host, has been fundamental for much of the early research establishing the relevance of probiotic application to infant nutrition. Colonization of the infant colon with the probiotic strain, or strains, can be transient; higher doses of probiotics delivered to the colon still may not result in more benefit to the host (i.e. production of a better profile of short chain fatty acids). Colonization, dose-related responses, and aspects to safety are strongly influenced by the individual strain of the probiotic. Each probiotic strain, even if closely related genetically, can behave uniquely when introduced into the dynamic and competitive environment of the colon.

Safety parameters are key to the selection of probiotic strains for infant nutritional applications. Evaluating the impact of probiotics on short chain fatty acid production in the colon can provide a key safety assessment. Using short chain fatty acids as a marker, it is also possible to evaluate the influence of D-lactate vs. non D-lactate producing Lactobacillus strains on the total D-lactate level within both the small intestine and colon. Data show that strains of lactobacilli that produce D-lactate, but are not sole D-lactate producers, do not alter the balance of total D-lactate present in the colon; however, the presence of a prebiotic, either alone or in conjunction with probiotic strains, can alter the proportion and amount of D-lactate that is present.

Screening of probiotic strains should include use of models that can evaluate the benefit of different strains to the host. For example, promotion of immune system regulation, and the ability to influence the development of the immune system, can be evaluated with

established *in vitro* and preclinical models. Some of these models provide more relevance to the human situation, and thus give more indication of possible clinical outcome. Outcomes such as reduction in episodes of diarrhoea, defense against other types of infection, prevention of allergy, and influence on development and function of the immune system can all be evaluated with models. Use of multiple outcome models can provide insights into key aspects of mechanism of action, and help add to the predictive nature of the *in vitro* and preclinical trials prior to efficacy studies with infants.

Addition of probiotics to infant nutritional products should not be viewed outside of the context of other components in the formulation that might impact the probiotic strain, notwithstanding the critical issue of water activity of the product. Screening of probiotic strains should take into account other components in a formulation that might affect the probiotic – such as the presence of prebiotics, and of other factors such as nucleotides and zinc. Data from *in vitro* studies have shown that the presence of the a prebiotic, and dose of that prebiotic, can negatively or positively impact the ability of the probiotic to survive transit thru the upper GI tract, and may significantly influence the microflora and dynamics in the colon in a very different way than the probiotic alone.

Screening probiotic strains for use in infant nutritional products allows for the selection of the most appropriate strain. The data can also benefit the marketing of the product, by providing scientific credibility to claims associated with the specific attributes or outcomes associated with the probiotic-containing product. Screening is also important to address the safety aspects of probiotics, and is likely needed to support regulatory review prior to commercial sale. Using established models and a thorough scientific assessment of probiotic strains could add value to the product and help insure acceptance by both the health care professional and the consumer.

Anti-hypertensive effects of microbially produced bioactives

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Atherosclerotic cardiovascular disease (CVD) is the leading cause of premature deaths in Western populations and is – together with obesity, hypertension, and T2 diabetes – part of the metabolic syndrome. Atherosclerotic progression, plasma cholesterol and elevated blood pressure (hypertension) are among the generally accepted major CVD risk factors and , therefore, also validated biomarkers for animal studies and human clinical studies. Large epidemiological studies (such as the NHANES study in the USA) suggest that consumption of milk and milk products is inversely related to the risk for hypertension. And several intervention studies have reported that diets rich in low-fat dairy products have significant anti-hypertensive effects (decrease in mean systolic and diastolic blood pressure). It is therefore expected that to a certain degree CVD and other chronic lifestyle diseases can be prevented by modification of the habitual diet (WHO, 2002) – and in recent years a role of milk-derived bioactive compounds in maintaining cardiovascular health by regulating blood pressure has received high attention. Most focus has been on milk-derived peptides that in a wide variety of *in vitro* and *in vivo* experiments have been associated with an inhibitory effect on the angiotensin I converting enzyme , ACE. This enzyme plays a crucial role in the function of the rennin-angiotensin system where it converts angiotensin I to angiotension II . The latter is a strong vasoconstrictor and the anti-hypertensive effect of ACE-inhibitors is believed primarily to be a vasodilation effect caused by reduced angiotensin II concentrations.

Basically, two routes of research and product innovation has been taken – i.e. (i) by enrichment or supplementation with antihypertensive peptides produced by (*ex vitro*) enzymatic hydrolysis of precursor dairy protein fractions, or (ii) by natural (*in situ*) fermentation of milk products where proteolytic activities of lactic acid bacteria (LAB) hydrolyse caseins and whey proteins into a wide population of larger and smaller peptides that may be further modified within the product matrix as well as during gastric and intestinal passage.

This presentation will focus on the fermented milk (FM) approach which offers functional and perceptional advantages as a preferred technology for future natural functional dairy products within a dietary prevention scenario. For the production of FM products with hypotensive and/or ACE-inhibitory activity an increasing number of LAB species have been used, primarily from the genus *Lactobacillus* (*L. helveticus, L. casei, L. plantarum, L. rhamnosus, L. acidophilus, L. lactis* ssp. *Lactis* and ssp. *cremoris,* and *L. delbrueckii* ssp. *bulgaricus)* but examples are also known from *Streptocoocus thermophilus* and *Enterocoocus faecalis*. However, *L. helveticus* has by far been the preferred fermenting organism in the pursuit of more efficient ACE-inhibitory dairy products not only due to its generally higher proteolytic activity but also to the specificity of the proteolytic enzymes resulting in more active peptides. Due to their apparent ability to be absorbed in the intestine and transported across cell layers, a lot of focus and experimental data are related to dipeptides and tripeptides (such as Ile-Pro-Pro, IPP and Val-Pro-Pro, VPP). However, as to the precise nature and physiological mechanism(s) of the peptides causing the measured ACE-inhibitory and hypotensive effects, much is still to be learned.

FM-based consumer products containing bioactive peptides and being marketed for their

ability to lower blood pressure (and backed up by human clinical trials) have been pioneered by Calpis in Japan and by Valio in Finland and are now slowly being introduced in a number of European countries by major players such as Unilever and Danone. Although the efficacy of such products are generally at the low end, levels of 3-6 mm Hg (comparable to the use of certain anti-hypertensive drugs) have been reported. So far the market penetration has not been impressive which may be related to several perceptional and factual uncertainties. Being targeted towards a well-validated biomarker such as hypertension which is sometimes considered a disease itself, these products may be perceived more as a treatment of a disease rather than prevention – which may add to the confusion about differences between food and medicine. And it still remains to be seen how these products will qualify for future health claims under the more strict regulatory schemes put into action in the European Union as well as in many other regions.

Probiotics in animal feed applications: concepts, facts and perspectives

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Probiotics are here defined as generally done by WHO and FAO - live micro-organisms, which, when administered in adequate amounts, confer a health benefit to the host. For some time, they were called 'microbial probiotics' to distinguish them from the 'chemical probiotics' and in the USA they are yet designated by direct-fed microbials. Probiotics are an old and a renewed human knowledge. For example, fermented milks are known for several thousand years whereas the demonstration of the anticarcinogenic activity of some lactic acid bacteria strains is less than 30 years old. The first question arising is related to the definition of 'health benefit for the host'. How can this be measured? The classical experiment of Collins and Carter, performed thirty years ago, using *Salmonella enteritidis* infection in germ-free and in conventional mice is one of the clearest demonstrations ever obtained of the protective role of the gastrointestinal microflora against microbial diseases of gut origin. Nowadays, it is clear that humans and animals have in their gastrointestinal tract a very large population of microorganisms protecting them against some diseases that start in the gut. Taking that into account, why do we need probiotics?

The use of probiotics for farm animals is based on the knowledge that the gastrointestinal microflora is involved in host health and in resistance to disease. The stressful conditions experienced, particularly, by the young animal cause changes in the composition and/ or activity of the gastrointestinal microflora. Probiotic supplements attempt to repair the resulting disequilibrium and should provide the type of bacterial population having a similar profile and activity to that existing in animals not excessively influenced by modern farm husbandry methods. This practice would not create anything that is not present under natural environmental conditions, but just reinforce the gastrointestinal microflora to its normal balance and protective role.

Already available probiotic products are of varying composition and efficacy but the concepts behind them are intellectually sound. Under the right conditions the claims made for some probiotics can be realized. The ban of antibiotic growth promoters in animal husbandry within the EU and their potential ban in other areas of the world have led to a large interest in the use of alternative concepts. Probiotics as replacing agents in animal production are one of the most studied alternatives. Can they fill the gap effectively? Will they be able to do it in a similar level of effects or in a smaller one? More scientific knowledge is needed about probiotics, their mechanism(s) of action, their effects, their safety and their appropriate use. Despite scientific doubts as to their real validity, probiotic products are flourishing and the interest in establishing scientific credibility has became important for many scientists and industrial companies. The global probiotic market is growing every year. Considering human and animal applications for bacterial probiotics the world market for 2008 is estimated to be about 200-250 Mio €. It is foreseen that it will be, most probably, of more than 350 Mio € within five years.

The competitive exclusion of the allochthonous micro-organisms entering the gut by the autochthonous ones (also called barrier effect, bacterial interference or colonization resistance) represent the main function devoted to the gastrointestinal microflora. Among the mechanisms proposed for explanation of the phenomenon the production of antagonist

compounds seems to be an important factor. The production of antimicrobial compounds is often considered as one of the main beneficial mechanisms of probiotics. A very large number of antimicrobial compounds (bacteriocins and bacteriocin-like inhibitory substances) produced by several strains of the gastrointestinal microflora have already been identified. The modulation of the gastrointestinal microflora is thus one of the potential interests of the use of probiotics. The modulation of the immune functions is another area of high interest in the development of probiotics.

In animal farming, probiotics generally produces highly variable effects. A product which is effective in one place may be not efficacious in another. There are a number of possible reasons for this variation. Among the microbiological reasons, it can be cited: the source of the probiotic bacteria, the stability of the host gastrointestinal microflora, the diet used and the microbism of the farm. Also, in spite of a careful selection of microbial strains, it seems not probable that a probiotic micro-organism colonize definitively the gastrointestinal tract and thus repetitive administration is necessary. The use of any probiotic would raise questions on safety since the product has to be consumed in large amounts on a regular basis. The main concern is whether using probiotics in animal feed increases the risk of inter-species transfer of antibiotic resistance genes. Safety remains an important issue as *a priori* safe species cannot always be taken for granted. Every product must therefore be considered on a case by case basis.

The study of the gastrointestinal microflora is a fundamental aspect of probiotic research and development. Comparative studies using germ-free and conventional animals, strictly anaerobic culture methods and microscopy have provided the knowledge on which current concepts of the ecology of the gastrointestinal microflora are based. It is known that the large majority (60 to 70 %) of the gastrointestinal micro-organisms is not cultivable by the available techniques. It must be assumed, however, that they play an important role in the whole ecosystem. Recent application of molecular methodologies will enhance knowledge on the very complex microbial ecosystem and enable the development of a modern concept of the microflora-host relationships, which needs to be established. Such knowledge will also be important for the development of products based on micro-organisms under a new scientific basis.

Metagenomics: discovering new functionalities of microbes

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A detailed understanding of human biology will require not only knowledge of the human genome but also of the human metagenome, defined here as the ensemble of the genomes of human-associated microorganisms. Our project focuses on the microorganisms of the gut, which are particularly abundant and complex and have an important role for human health and well-being. We shall implement and integrate the following activities:

- creation of a reference set of genes and genomes of intestinal microbes, using high fidelity metagenomic sequencing and full genome sequencing of selected bacterial species;
- creation of the generic tools, based on the high density DNA arrays and novel ultra-high throughput re-sequencing techniques, to study the variation of the human gut microbiota;
- use of the tools to search for correlations between genes present in the gut microbiota and disease, focusing on the inflammatory bowel disease and obesity, two pathologies of increasing social relevance;
- study of the genes correlated with the disease, both in terms of their function in microbes and their effect on the host, with a focus on host-microbe interactions;
- development of an informatics resource to store and organize the heterogeneous information generated within the project, such as gene and genome sequences, gene frequencies in healthy and sick individuals or gene functions and also enriched by information relevant to the human gut microbiota generated outside of the project;
- creation of the bioinformatics tools to carry out the meta-analysis of the information; and
- creation of an interface with the stakeholders, including an international board to promote cooperation and coordination in the human metagenome field, and general public.

Our project should give an unprecedented view of the gut microbiota and their variability, identify microbial signatures of prognostic and diagnostic value, lay grounds for organization and interpretation of metagenomic information and open avenues to modulate human gut microbiota in a reasoned way, enabling to optimize the health and wellbeing of any individual.

Future scientific perspectives: the brain-gut axis

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According to the brain-gut axis model, the brain and the gut are highly integrated and interact through bi-directional communication pathways connecting the enteric nervous system (ENS), the central nervous system (CNS), the autonomic nervous system (ANS), neuroendocrine centres and the (gut) immune system. According to this model not only intestinal function is affected by the brain, but also brain function may be influenced by intestinal factors including those connected to its microbial community.

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder affecting about 10-15% of the adult Western population and generally considered now as a disorder primarily involving the brain-gut axis. A multicomponent conceptual model of IBS has been postulated integrating physiologic, affective, cognitive and behavioural factors. Although this concept of the brain-gut axis is commonly accepted among researchers and gains support also among physicians, assessment of integrated brain-gut function is still in its infancy. However, this integrated approach is pivotal to gain better insight in its regulation and to achieve new breakthroughs in optimalisation of brain-gut functioning by life style, dietary or pharmacologic means. Given the high prevalence of sensory abnormalities in IBS, and the correlation of visceral sensitivity with symptoms, altered colorectal perception has been considered to be a biological hallmark of IBS. Enhanced visceral perception (hypersensitivity) and motor responses (compliance, contraction, accommodation) may result both from peripheral (e.g. inflammation, infection) or central (e.g. attention, anticipation, mood) sensitisation mechanisms. The presence of (low-grade) mucosal inflammation in a substantial proportion of subjects with IBS-like abdominal complaints plays probably also an important role in afferent gut-brain signalling via the enteric nervous system (ENS). Evidence is rapidly increasing that luminal factors, such as associated with the microbial community, play a pivotal role in modulation of this signalling. Hence, in-depth knowledge of brain-gut interaction may also facilitate better understanding of the (patho)physiology of intestinal ageing, allergy, innate and adaptive immune function, as well as aberrations in brain function such as in case of certain autistiform and mood disorders.

The lecture will start with a presentation of the brain-gut concept with its various communication pathways and its implication for both intestinal and brain functioning. Available biomarkers of integrated brain-gut function at the level of the gut, the brain, and mediators of brain-gut signalling, respectively, will be highlighted. Potential ways to affect brain-gut signalling will be reviewed. As serotonin (5-HT) is one of the key denominators of brain-gut interaction, some examples of serotonergic brain-gut signalling and its pharmacologic as well as dietary modulation paradigms will be illustrated.
The role of the intestinal microbial community in brain-gut interaction is still poorly understood and discrimination of small intestinal and colonic microbial community, respectively, is necessary in this respect. However, a number of potential mechanisms how intestinal microbes can beneficially affect gut-brain signalling will be hypothesized. Available evidence as well as future perspectives will be presented.

Vision on beneficial microbes: 2010-2020

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The main item of the presentation will be to report on the ILSI Europe workshop 'Guidance for assessing the probiotics beneficial effects: how to fill the gap', 22-24 May 2008, Montreux, Switzerland. In addition, the presenter will comment from his personal perspective.

Background of the workshop
Both research into the physiological effects of probiotics as well as availability of probiotic products on supermarket shelves are increasing rapidly. Claims on specific probiotics are currently accepted in the USA and Japan, and also in some European countries. There is a need for comprehensive guidelines on the assessment of the characteristics of probiotics, and of the foods containing them, to ensure claims are scientifically substantiated.
The ILSI Europe Probiotics Task Force in association with the International Dairy Foundation (IDF) has hosted the workshop with the aim to assess the physiological efficacy of probiotics. The results of this work could guide the scientific substantiation of health claims on probiotics.

Aim and objectives of the workshop
The overall aim of this workshop was to review a 'good practice' document prepared by an ILSI Europe Probiotics Expert Group. They have evaluated the existing evidence related to the effect of probiotics in prevention or treatment of inflammatory bower diseases, allergy, risk of infections and metabolism, aiming to define guidelines for further human trials in these areas. This document builds on the work done in the Gut Health and Immunity Expert Group of PASSCLAIM, on work of the ILSI Europe Nutrition and Immunity in Man Task Force and on existing FAO/WHO guidelines. It was not intended to reach consensus at the workshop but to build bridges on central issues and reach a joint vision for future perspectives for probiotic research: the next generation of studies and the role of probiotics in health promotion. The workshop objectives were to:

- provide input to a good practice guide for demonstrating functionality of probiotics through guidelines and recommendations from interpretation of the available data; and
- scoping future research and next generation studies .

Next steps
Following the workshop the expert group will take the discussions into consideration and finalise the above manuscript. This paper, authored by the expert group, will be published in a peer-reviewed journal.

Putting microbes to work – in the end, what's really possible?

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As this conference has highlighted, many advances are being made in understanding the role of indigenous microbes in health. This has led to the development of probiotic products designed to confer general and specific benefits on the host. The use of the term 'probiotic' is still not properly used by too many products, and adherence to the FAO/WHO Guidelines must become a goal for all companies. In short, without properly acquired human studies showing a physiological benefit, a product should be referred to as something other than a probiotic. Nevertheless, there is mounting evidence that microbes play a critical role in the health of humans. Efforts to use probiotic approaches to improve mood, cognition, nasopharyngeal, respiratory, gastrointestinal, urogenital and vascular health. Arguably, the distant site effects are the most intriguing. The ability of probiotic organisms to reduce the recurrence of bladder cancer, and the duration of the common cold, are two examples that would suggest the mechanisms involved are not simply immune modulation or inhibition of pathogen growth, as so often probiotic mode of action is alluded to.

In twenty years from now, we will look back and consider our current efforts in probiotics and prebiotics to be quite primitive. At present, we advocate ingestion of varying amounts of prebiotic in the hope of manipulating beneficial microbes, yet we have not conclusively identified how these microbial foods alter species, strains and the milieu. Likewise, we ingest one or more billion 'probiotic' microbes and are not very sure what they do, how they do it, and why they do not stay inside us. This makes the clinical end-point so important, and emphasizes the need to identify relevant biomarkers. Thus, what's possible is not limited by the microbes themselves, but by our ability to select and deliver the 'right' strains, and monitor the effects they have on the host in the short and long term. As research in probiotics accelerates, along with commercial product availability, there are indications that products are being tested at the limits of their potential, before we truly understand which ones are the optimal choice and under which circumstances their chance of success is realistic. Animal models may to some extent reduce the risk of failure, but ultimately we have to face ethical dilemmas, such as what are the long term effects of treating newborns, or if there are no other options should we apply probiotics to severely ill patients? Due to lack of funding, and perhaps a hesitation to tackle complex randomized, placebo-controlled clinical studies, we have not yet shown in a conclusive manner, that regular probiotic or prebiotic use lead to better long term health or longevity. Thus, we move forward lacking some fundamental information on the place of probiotics and prebiotics in human evolution and long term well-being. For now, we mostly focus on the application of these products to every day life conditions and prevention of illness. Whilst this is laudable, our efforts, mostly in food and dietary supplement applications are catching the regulatory authorities somewhat off guard, and causing some concerns in medical circles, reiterating the need to perform more studies proving that microbial manipulation provides tangible benefits to humans.

Deciphering the microbiota in various bodily niches, assessing their genetic potential, understanding the effects of diet and host factors over time, and identifying the complex signalling and other interactions that occur between the indigenous and probiotic microbes and the host, will allow us to design a multitude of probiotic and prebiotic products that not only help to restore and maintain health, but potentially lead to longer life itself, as Metchnikoff once predicted.

POSTERS

P1 *Human proteolytic enzymes – digestion of milk and milk proteins and the effect on gut related bacteria*
H. Almaas[1], T.G. Devold[1], H. Holm[2], M. Jacobsen[3], R. Flengsrud[1], T. Langsrud[1] and G.E. Vegarud[1]
[1]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Norway, [2]University of Oslo, Department of Nutrition, Norway and [3]Regional Hospital of Østfold, Fredrikstad, Norway

P2 *Lactobacillus plantarum AGR1526 enhances intestinal epithelial tight junction integrity via the production of an active compound*
R.C. Anderson, K.M. Armstrong, W.C. McNabb and N.C. Roy
AgResearch Grasslands, Food, Metabolism & Microbiology Section, Food & Textiles Group, New Zealand

P3 *Don't celiacs really digest gliadin? Gut bacteria involved in the degradation pattern of gliadin in celiac disease small intestinal mucosa*
D. Bernardo[1], J.A. Garrote[1,2], I. Nadal[3], A.J. León[1], C. Calvo[1,4], L. Fernández-Salazar[5], A. Blanco-Quirós[1], Y. Sanz[3] and E. Arranz[1]
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P4 *Automated testing of the antimicrobial effect of Weissella and Leuconostoc species isolated from Boza against pathogens*
M. Borcaklı and T. Öztürk
Tübitak MRC, Food Institute, Turkey

P5 *Gut fermentation products of wheat aleurone suppress cell growth and survival of human adenocarcinoma cells*
A. Borowicki[1], K. Stein[1], D. Scharlau[1], M. Glei[1], K. Scheu[2], G. Brenner-Weiss[2], M. Gusko[3] and B.L. Pool-Zobel[1]
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P6 *Analysis of [U-^{13}C]starch conversion by a colonic microbiota using NMR*
A.A. de Graaf[1,2], K. Venema[1,2], A. Maathuis[1,2], P. de Waard[3] and N.E.P. Deutz[4]
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P7 *How healthy is a gluten-free diet? Effects on gut microbiota and immune function*
G. De Palma, I. Nadal, M.C. Collado and Y. Sanz
Institute of Agrochemistry and Food Technology (CSIC), Microbial Ecophysiology Group, Spain

P8 *Prebiotic effects of chickpea as determined by 16S rRNA gene libraries and terminal restriction fragment length polymorphism*
U. Fernando[1], J. Hill[1], G. Zello[2], R. Tyler[1], W. Dhal[2,3] and A. Van Kessel[1]
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P9 *Probiotic strain Lactobacillus paracasei CNCM I-2116 decreases skin reactivity*
A. Guéniche[1], J. Benyacoub[1], I. Bureau[1], I. Castiel[2], S. Blum[1] and J. Leclaire[2]
[1]Nestlé Research Center, Switzerland and [2]L'Oréal Recherche, France

P10 *A novel ingredient for skin aging*
A. Guéniche, P. Bastien, L. Breton and I. Castiel
L'Oréal Recherche, France

P11 *Oral Skin Probiotic™ facilitate early recovery of cutaneous immune homeostasis after UV exposure in humans*
A. Guéniche[1], C. Dezutter-Dambuyant[2], J. Leclaire[3] , T. Buetler[1], H. Smola[4], S. Blum[1] and J. Péguet Navarro[2]
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P12 *Crosstalk of E. coli LF82 - isolated from a Crohn's disease patient - with Caco2 cells*
C. Hübner[1], I. Petermann[1], J.-Y. Jang[1], J. Sutherland[2] and L.R. Ferguson[1]
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P13 *In vitro bioconversion of polyphenols in tea and wine by the intestinal microbiota*
D.M. Jacobs[1], G. Gross[1,2], S. Peters[1], J. van Duynhoven[1], E.E. Vaughan[1] and T. van de Wiele[2]
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P14 *Beneficial effects on caecal microbiota of broilers with essential oils*
S. Lahtinen[1], A. Ouwehand[1], K. Tiihonen[1], M.H.L. Bento[2], H. Schultze[3] and N. Rautonen[1]
[1]Danisco Finland Oy, Finland, [2]Danisco Animal Nutrition, England and [3]Danisco Animal Nutrition, the Netherlands

P15 *Colonization of the human intestinal tract by potential probiotic strains of Lactobacillus*
N. Larsen[1], F. Kvist Vogensen[1], K. Fleischer Michaelsen[2], A. Pærregaard [3] and M. Jakobsen[1]
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P16 *Digestibility and prebiotic potential of nondigestible carbohydrate fractions from novel maize-based fibers in a dynamic in vitro model of the human intestine*
A. Maathuis[1], K. Venema[1], A. Evans[2], A. Hoffman[2] and L. Sanders[2]
[1]TNO Quality of Life, the Netherlands and [2]Tate & Lyle, USA

P17 *Dietary fibre and gut microbiota affect post-weaning intestinal physiology in conventional and gnotobiotic pigs*
G. Malik[1], M.D. Drew[1], D. Hoehler[2] and A.G. Van Kessel[1]
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P18 Modulation of *in vitro Candida albicans* infection by *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14
R.C.R. Martinez[1], S. Mifflin[2], K.L. Summers[2], A. Nomizo[1], E.C.P. De Martinis[1] and G. Reid [2,3,4]
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P19 *A potential probiotic strain Bacillus smithii TBMI12 administrated as endospores and used as competitive exclusion agent against Salmonella enteritidis*
T. Michelson[1], I. Suitso[2], E. Talpsep[2], E. Jõgi[2], P. Naaber[3] and A. Nurk[2]
[1]University of Tartu, Institute of Molecular and Cell Biology, Estonia, [2]University of Tartu, Institute of Technology, Estonia and [3]Tartu University Hospital, Estonia

P20 *The effect of feed raw material on the composition of intestinal microbiota, acidity of the gut and performance in broilers*
E. Munukka[1], J. Vaahtovuo[1], M. Korkeamäki[1], J. Valaja[2], E. Valkonen[2], J. Vuorenmaa[3] and E. Helander[3]
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P21 *Shifts in clostridia, bacteroides and immunoglobulin-coating faecal bacteria associated with weight loss in obese adolescents*
I. Nadal[1], A. Martí[2], C. Azcona[2], M. Martin-Matillas[3], C. Campoy[3], M. Garcia-Fuentes[4], C. Redondo-Figuero[4], M. Delgado[5], O.L. Veiga[6], J. Warnberg[9], J.M. Garagorri[7], L. Moreno[8], A. Marcos[9] and Y. Sanz[1]
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P22 *What do gut microbes have to do with obesity?*
K. Nones, G. Paturi, J. Monro, M. Suman, E. de Guzman, C. Butts, S. Martell and J. Sutherland
New Zealand Institute for Crop & Food Research, Nutrition & Health Science Group, New Zealand

P23 *Distribution and function of different species of the Bacteroides fragilis group in individuals with Japanese cedar pollinosis*
T. Odamaki, J.-Z. Xiao, M. Sakamoto, S. Kondo, T. Yaeshima, K. Iwatsuki, T. Enomoto and Y. Benno
Morinaga Milk Industry Co. Ltd., Japan

P24 *Probiotics reduce the incidence and duration of cold and flu-like symptoms in children*
A.C. Ouwehand[1], S. Li[2], M. Mubasher[3], C. Reifer[4] and G. Leyer[1]
[1]Danisco Cultures Division, Finland, [2]Tongji University, Medical College, Department of Preventive Medicine, China, [3]University of Texas at Houston, Department of Biostatistics, School of Public Health, USA and [4]SPRIM, USA

P25 *Improvement of symptoms of bloating in patients with functional bowel disorders by probiotic strains Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07*
Y. Ringel[1], O. Palsson[1], G. Leyer[2], S. Causey[1], S. Yeskel[1], S. Lahtinen[3], S. Faber[4] and T. Ringel-Kulka[5]
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P26 *Reduced diversity and increased virulence-gene carriage in intestinal enterobacteria of celiac children*
E. Sánchez, I. Nadal[1], E. Donat[2], C. Ribes-Koninckx[2], M. Calabuig[3] and Y. Sanz[1]
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P27 *Molecular analysis of the porcine gut microflora in response to feeding with probiotics and prebiotics*
V.A. Sattler[1], G. Wegl[1], C. Plitzner[2], S. Nitsch[3], G. Schatzmayr[3] and V. Klose[1]
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P28 *Modification of an in vitro model simulating the whole digestive process to investigate cellular endpoints of carcinogenesis and chemoprevention*
K. Stein[1], A. Borowicki[1], M. Glei[1], D. Scharlau[1], K. Scheu[2], G. Brenner-Weiss[2] and B.L. Pool-Zobel[1]
[1]Friedrich-Schiller-University Jena, Institute for Nutrition, Department of Nutritional Toxicology, Germany and [2]Research Centre Karlsruhe, Institute for Technical Chemistry, Group for Water- and Geotechnology, Germany

P29 *A cell based assay to detect the effect of food extracts on human defensin levels*
J. Sutherland[1,4], C.E. de Guzman[1,4], H. Martin[2,4], C. Huebner[3,4] and I. Petermann[3,4]
[1]New Zealand Institute for Crop & Food Research, New Zealand, [2]Horticultural Research Institute, New Zealand, [3] University of Auckland, Faculty of Medical and Health Sciences, Department Nutrition, New Zealand and [4]Nutrigenomics New Zealand

P30 *Fecal microbiota in early rheumatoid arthritis*
J. Vaahtovuo[1], E. Munukka[1], M. Korkeaäki[1], R. Luukkainen[2] and P. Toivanen[3]
[1]CyFlo Ltd, Finland, [2]Satakunta Central Hospital, Department of Rheumatology, Rauma, Finland and [3]Turku University, Department of Medical Microbiology, Finland

P31 *Immuno-biological effects of different Escherichia coli preparations and common bacterial metabolites on the cells of the human immune system tested in a highly complex human organo-typical co-culture model*
K. Venema[1] and M.W. Schmolz[2]
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P32 *Use of a dynamic, computer-controlled in vitro model of the stomach and small intestine (TIM-1) to study survival of probiotics in a chewable tablet*
K. Venema[1], A. Maathuis[1], I. Giesbrecht[2], P. Bohnhorst[2] and A. Christ[2]
[1]TNO Quality of Life, the Netherlands and [2]Merck Selbstmedikation GmbH, Germany

P33 Comparative metabotyping and metagenomic analysis of two lean rat strains
A. Waldram[1], Y. Wang[1], E. Holmes[1], I.D. Wilson[2], G. Gibson[3] and J.K. Nicholson[1]
[1]Imperial College London, Faculty of Medicine, SORA Division, Biological Chemistry, UK, [2]AstraZeneca, UK and [3]Reading University, School of Food Biosciences, UK

P34 *Gene expression in Lactobacillus acidophilus during passage through an in vitro gastro-intestinal tract model*
G. Weiss and L. Jespersen
University of Copenhagen, Faculty of Life Sciences, Department of Food Science, Denmark

P35 *Is icecream a suitable carrier for probiotics?*
D. Wolvers, I. Mohede, E. Meynen, Y. Dommels, R. Shadid, N. Johnson, J. Ueckert and R. Albers
Unilever Food and Health Research Institute, the Netherlands

P1

Human proteolytic enzymes – digestion of milk and milk proteins and the effect on gut related bacteria

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An *in vitro* digestion model to degrade milk proteins using human proteolytic enzymes was developed in order to mimic human digestion. The breakdown of proteins was performed by a two-step digestion assay, using human gastric juice (HGJ) at pH 2.5, and human duodenal juice (HDJ) at pH 8. The human proteolytic enzymes were obtained by collecting gastric and duodenal juice according to Holm *et al.*(1988). A modified digestibility assay, AOAC Official Method 982.30 (Rasco, 1994), was used to measure the digestion of milk. Protein breakdown was studied by SDS-PAGE and IEF after each digestion step and peptides analysed by LC-MS/MS. Commercial enzymes, pepsin (Sigma) and Corolase PP containing trypsin and chymotrypsin (Røhm) were studied as a comparison. Raw skim milk from cow and goat were digested in addition to pasteurized and sterilized milks. Caprine milk proteins from goat were degraded significantly faster than the proteins from cow milk. Differences were observed between raw, pasteurized and sterilized milks. The caseins were degraded in all milk samples. However, β-LG in cow milk seems to be more resistant to degradation than β-LG from goat milk. Peptides in the hydrolysates showed antibacterial effect on some selected Gram-negative and Gram-positive bacteria, among them *Listeria monocytogenes* (Almaas *et al.*, 2006)

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P2

*Lactobacillus plantarum AGR*1526 enhances intestinal epithelial tight junction integrity via the production of an active compound

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Tight junctions are protein complexes that form a physical barrier between epithelial cells to limit the passage of undesirable molecules into the body. The preservation of the integrity of the tight junctions between intestinal epithelial cells is critical in the maintenance of wellness. There is evidence that probiotic bacteria may play an important role in the maintenance of intestinal barrier integrity. The hypothesis of this study is that the potential probiotic bacterium *Lactobacillus plantarum* AGR1526 can enhance intestinal barrier integrity affecting the tight junction proteins. A human colon cancer epithelial cell line (Caco-2) was used as an *in vitro* model of the intestinal barrier because these cells spontaneously form tight junctions between adjacent cells. Caco-2 cells were grown in M199 supplemented with 10% foetal bovine serum, 1% non-essential amino acids and 1% penicillin-streptomycin at 37°C with 5% CO_2. The effects of *L. plantarum* AGR1526 and AGR1526 supernatant on the integrity of tight junctions between adjacent intestinal epithelial cells were determined by monitoring the trans-epithelial electrical resistance (TEER) across confluent Caco-2 cells. AGR1526 supernatant was prepared by incubating the potential probiotic strain in M199 (without foetal bovine serum and penicillin-streptomycin) at an initial 600 nm optical density of 0.9 for 8 h, then removing the bacterial cells by centrifugation and filter sterilisation. AGR1526 (OD_{600nm} 0.9) and AGR1526 supernatant were added to Caco-2 cells grown for 7 days on collagen membranes (Cellagen™ Discs CD-24, MP Biomedicals, OH, USA) and the TEER were measured two hourly (n=4 per treatment). Additionally, the effect of AGR1526 and AGR1526 supernatant on tight junction integrity was determined by immuno-staining the tight junction protein zona occuldin 2 (also called tight-junction protein 2). Caco-2 cells were grown on Permanox™ coated slides (In Vitro Technologies) for 7 days until confluent. Caco-2 cells were treated with live AGR1526 (OD_{600nm} 0.9) and AGR1526 supernatant for 4 and 8 h (n=4 per treatment per time point). After treatment, the Caco-2 cells were rinsed with PBS, fixed in 4% (w/v) paraformaldehyde for 20 min, quenched with 50mM NH4Cl (in 1x PBS) for 15 min and blocked with blocking buffer (2%, v/v) foetal bovine serum, 1% BSA, 0.1% Triton X-100, 0.05% Tween 20 in 1x PBS, pH 7.2) for 20 min. The Caco-2 cells were then immuno-stained with the primary antibody, rabbit anti-ZO-2 (1:100), in blocking buffer for 1 h, followed by a PBS wash to reduce non-specific staining (0.1% Triton X-100, 0.05% Tween 20 in 1x PBS), and the secondary antibody, Alexa Fluor 488 goat anti-rabbit IgG (1:200), in blocking buffer for 1 h. The slides were imaged with a confocal microscope (Leica TCS SP5) with the Argon laser excitation emissions between 500 and 540nm. The images were viewed using LAS AF Lite (Leica Application Suite) v1.8.2 software. AGR1526 caused a 60% increase in TEER across Caco-2 cells compared to the untreated controls from 2 to 8 h ($P<0.05$). AGR1526 supernatant caused a similar increase in TEER to live AGR1526 from 2 to 6 h ($P<0.05$), but after 8 h the active compound appeared to be used up. Both the live AGR1526 and AGR1526 supernatant caused a visible increase in immuno-stained ZO-2 compared to the untreated controls, with the affect being more pronounced after 4 h than 8 h. This study shows that AGR1526 can affect intestinal barrier integrity via the tight junction proteins, and that is effect may be mediated by compounds produced by the bacterium.

P3

Don't celiacs really digest gliadin? Gut bacteria involved in the degradation pattern of gliadin in celiac disease small intestinal mucosa

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It has been suggested that Celiac Disease (CD) might be associated to an enzyme deficiency that would lead to an incomplete digestion of gliadin and the development of the disease. This study aimed to identify possible differences in the pattern of gliadin digestion between CD and non-CD patients, and to characterize the nature and functional properties of gliadin-digesting proteases in the duodenum. Gliadin zymograms were performed on whole protein extracts of duodenal explants from CD patients, both treated (9 patients) and untreated (20 patients), and non-CD controls (18 individuals). Zymograms were also performed using extracts from peripheral blood mononuclear cells (PBMCs), intraepithelial lymphocytes (IEL) and lamina propria mononuclear cells (LPMCs), and also from explants after 24 hours culture with antibiotics and with cell culture fractions of the duodenal biopsy-associated microbiota from 5 untreated CD patients. A CD-specific and reproducible gliadin-degrading protease pattern of 7 bands (92, 82, 35, 33, 26, 24, and 20 kDa) was found in CD patients, both in untreated and treated patients without duodenal alterations, but not in non-CD individuals. All of these bands were of metalloprotease nature, and were not found neither in the IEL nor LPMCs compartments, nor in PBMCs extracts from CD patients. A complete depletion of CD-specific gliadin-degrading proteases was found after 24 hours biopsy culture with antibiotics. Moreover, some of these proteases were detected in the biopsy-associated microbiota from untreated CD patients, confirming its bacterial origin. The specific pattern of bacteria gliadin-degrading metalloproteases detected in the duodenal mucosa of CD patients provides a new pathogenic insight, which allows us to propose new prophylactic and therapeutic alternatives to restore the composition of the gut microbiota and its metabolic activity.

P4

Automated testing of the antimicrobial effect of W*eissella* and *Leuconostoc* species isolated from Boza against pathogens

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In recent years a growing interest has been seen in the health enhancement effects of lactic acid bacteria. Therapeutic and preventive beneficial effects of probiotics have been proved by many research studies. Boza is a traditional Turkish cereal based fermented beverage. W*eissella* and *Leuconostoc* species play an important role in the traditional Boza fermentation. Metabolic substances generated by lactic acid bacteria such as organic acids, hydrogen peroxide, acetoin and bacteriocins have growth inhibitory effects on other microorganisms. The antimicrobial effect of 5 W*eissella* and 13 *Leuconostoc* species was investigated on *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 14579) and *Staphylococcus aureus* (ATCC 25923). An automated turbidometer Bioscreen C system (Labsystems, Finland) was used in the screening of the antibacterial potential of lactic acid bacteria strains. Pathogens and lactic acid bacteria were cultivated in BHI and MRS broths, respectively. First, the standard curves of pathogens were obtained against plate count results by preparing a serial dilutions. Antimicrobial extracts were used in the test system without dilution and regression analysis was done to show the linear relationship between values measured and log CFU/mL counts of pathogens. None of the *L. mesenteroides* and *W. confusa* strains revealed antimicrobial activity against *S. aureus* , only one *L. lactis strain* showed an inhibitory effect on *S. aureus*. Antagonistic activity of *W. confusa* and *L. lactis* against pathogens was detected on *E. coli* and *B. cereus*.

P5

Gut fermentation products of wheat aleurone suppress cell growth and survival of human adenocarcinoma cells

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Wheat grain and particularly aleurone contain high amounts of dietary fibre. Fermentation by human gut flora may enhance level of short-chain fatty acids (SCFA) which are potentially chemoprotective by suppressing the growth of tumour cells. Wheat aleurone, whole meal wheat flour and wheat bran were digested and fermented *in vitro.* Fermentation supernatants (fs) were analysed for SCFA and bile acid contents. Corresponding mixtures of SCFA and desoxycholic acid (DCA) were prepared. HT29 adenocarcinoma cells were treated for 24-72 h with individual substances, mixtures or complex fs. Cell survival was determined by quantifying fluorescence of DAPI-labelled DNA. Fs of wheat samples contained 2-3 fold higher concentrations of SCFA than the fs control, but reduced levels of DCA. Fs of aleurone and of whole meal flour had equal growth suppressing activities and were significantly more effective than the fs control. Fs bran was of intermediary activity. EC_{50} activities ranged from 10% (whole meal), 12% (aleurone) to 14% (bran) and 19% (control) after 48 h treatment. Fs inhibited cell growth more than the corresponding SCFA mixtures. Growth inhibitory activity of SCFA was mostly due to butyrate and was not affected by DCA. In conclusion, gut flora-mediated fermentation of wheat aleurone results in reduced level of tumour promoting DCA but higher levels of SCFA especially butyrate, which inhibits growth of human adeno-carcinoma cells. The higher activities of fs point to the involvement of additional growth inhibitory effects of other bacterial metabolites. Most of the activities of whole meal and of bran are due to their contents of aleurone.

P6

Analysis of [U-^{13}C]starch conversion by a colonic microbiota using NMR

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The colon is one of the body's metabolically most active organs. While insight in colonic microbial diversity increases, little is known of the metabolic function of the microbes, the way diet affects metabolic fluxes, and how produced metabolites affect human health.
Short chain fatty acids (SCFA) and especially butyrate, produced by the colonic microbiota, are important mediators of health and disease. Linking the biosynthesis of SCFA to specific gut microbial pathways holds promise for the development of prebiotics that specifically modulate these pathways *in vivo*. Therefore, ^{13}C stable isotope labeling, NMR, and mathematical modeling of isotopomer distributions were employed to investigate colonic microbial metabolic pathway activities. The TNO *in vitro* model of the colon (TIM-2) was inoculated with a standardised adult human fecal microbiota. [U-^{13}C]starch was added at t=0, and lumen as well as dialysate samples were taken at various time points for analysis. GC-MS, enzymatic methods and 1- and 2-dimensional NMR techniques at 500 MHz were used to identify microbial metabolites and to analyze their ^{13}C contents. The SCFA acetate, propionate and butyrate were determined as the principal starch degradation products. Quantitative insight in bacterial metabolic routes was obtained from analysis of ^{13}C-^{13}C coupling patterns in 2D HSQC spectra that resulted from incorporation of intact ^{13}C glucose backbone fragments in SCFA. These indicated that e.g. propionate was almost exclusively formed via the succinate pathway. In conclusion, this stable isotope study underlines the importance of colonic bacterial SCFA metabolism.

P7

How healthy is a gluten-free diet? Effects on gut microbiota and immune function

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The type of diet influences the composition and function of the gut microbiota and, thereby, the host's health. Dietary strategies are also part of the therapeutic approaches used for the management of specific diseases. Celiac disease (CD) is an intestinal inflammatory disorder caused by a permanent intolerance to dietary gluten. The only therapy for CD patients is to adhere to a long-life strict gluten-free diet (GFD). Nevertheless, the consequences of this dietary practice on the gut microbiota have not been determined. Herein, the effects of a gluten-free diet on the composition of the gut microbiota and immune stimulatory properties have been analysed. Then healthy human adults (mean age: 30.3 years; range: 23-40 years) were submitted to a GFD for 1 month. The microbial composition of faecal samples was analyzed before and after the introduction of a GFD by real-time PCR. The ability of faecal bacteria to stimulate cytokine production by peripheral blood mononuclear cells (PBMCs) was also determined by ELISA. *Bifidobacterium*, *Lactobacillus* and *B. longum* counts significantly decreased (P=0.011, P=0.006 and P=0.024, respectively), while those of *Escherichia coli* increased (P=0.031) as a result of the GFD. The production of TNF-α, IFN-γ, IL-10 and IL-8 by PBMCs upon stimulation of faecal bacterial extracts was also significantly reduced (P=0.000, P=0.028, P=0.006 and P=0.000, respectively) after the diet. Thus, the GFD resulted in reductions of beneficial gut bacterial populations and ability of faecal extracts to stimulate host's immunity. The GFD constitutes an important environmental variable to be considered in treated CD patients for its detrimental effects on beneficial bacteria levels and their possible immunoregulatory roles.

P8

Prebiotic effects of chickpea as determined by 16S rRNA gene libraries and terminal restriction fragment length polymorphism

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Changes in the composition of gastrointestinal microbiota by dietary interventions using prebiotics provide an opportunity for improving health and preventing disease. The primary objective of this study was to assess the prebiotic potential of chickpea and its main oligosaccharide component, raffinose by examining their effects on human intestinal microbiota. Twelve healthy adults (18-65y) consumed their usual diet supplemented with soups and desserts that were unfortified, or fortified such that subjects consumed 200g/d canned chickpeas or 5 g/d raffinose for 3 week periods in a random crossover design. Three stool samples were collected from each subject at the end of each period and pooled by subject to permit microbial profiling using 16S rRNA based terminal restriction fragment length polymorphisms (T-RFLP) and quantitative real-time polymerase chain reaction (qPCR). Stool samples were further pooled by diet for preparation of three 16S rRNA clone libraries comprising 576 randomly sequenced clones per library.

Preliminary classification of the clone libraries revealed that the predominant members of the fecal microbiota were of *Clostridiaceae* followed by *Lacnospiraceae* and *Eubacteriaceae* and the pattern was common in all 3 libraires. Analysis of Msp1, Hha1 and HaeIII T-RF patterns indicated *Clostridium* clusters XIVa, IV/XVIII were present in a majority of subjects from all three diet groups. The characteristic TRFs for Msp1 and HaeIII digests for *Bifidobacterium* species were detected in all 3 diet groups and quantitative real-time PCR analysis showed a higher abundance of bifidobacteria in the raffinose diet compared to the other two treatments ($P>0.05$). Furthermore T-RFLP analysis showed that Msp1 TRFs ranging 570-580bp representing *Lactobacillus casei/L.salivaris* group was present in 9%, 8% and 22%, of individuals from the control, raffinose and chickpea diets respectively. The number of individuals showing TRFs for *Clostridium histolyticum/C. lituseburense* groups (Clostridium clusters I/II and XI) which include several pathogenic bacteria species and putrefactive bacteria were lower in the chickpea diet compared to the other two treatments. Protein fermentation in the human distal colon is known to produce toxic metabolites such as ammonia, amines and phenolic compounds but the availability of carbohydrates for fermentation in the large intestine has shown to reduce protein deamination leading to less ammonia production. Diet appeared to affect colonization by selected high ammonia producing bacterial isolates such an isolate closely related to *C. thermocellum* was detected in 83%, 92% and 42% of individuals whereas a *Eubacterium* sp., was detected in 16%, 42% and 16% of individuals in the control, oligosaccharide and chickpea groups, respectively. Overall our results indicate that chickpea and its main oligosaccharide component raffinose may have a potential to modulate the intestinal microbial composition to promote intestinal health in human.

P9

Probiotic strain *Lactobacillus paracasei* CNCM I-2116 decreases skin reactivity

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Over the last decades the prevalence of persons presenting reactive skins has increased in industrial countries. Reactive skin is characterized by a marked sensitivity of the skin to physical (heat, cold, wind) or chemical (topical product application) stimuli and impaired skin barrier repair ability. Probiotics are defined as live microorganisms that when ingested in sufficient amount positively balance the host microbiota and beneficially improve health. Beyond the effect on the intestinal microbiota, some probiotic strains display potent immune-modulatory properties including at the skin level. The aim of this study was to evaluate the effect of probiotics on skin reactivity. For that purpose, a preparation of *Lactobacillus paracasei* CNCM I-2116 powder was tested in a randomized double-blind vehicle-controlled trial. 66 female volunteers with reactive skin received probiotics (n=33) vs. placebo (N=33) powders suspended in drinking water at a daily dose of approximately 10^{10} CFU for two months period. Skin sensitivity was assessed by stinging test (capsaicin test) and skin barrier function recovery was determined by the evaluation of the trans-epidermal water loss following disruption by repeated tape-stripping. These evaluations were performed at initiation (day 1), day 29, day 43, at the end of the supplementation (day 57) and during a follow-up period at days 64 and 78. The results showed that the volunteers who received *L. paracasei* CNCM I-2116 presented a significant decrease in skin sensitivity over the treatment period. Moreover the barrier function recovery, following tape-stripping disruption, was significantly faster for the volunteers having received *L. paracasei* CNCM I-2116 compared to the volunteers taking control powder. These effect was also observed during the follow up period. Key physiological parameters associated with skin homeostasis as well as gut microbiota balance were assessed as secondary outcomes during the supplementation period. Cutaneous moisturizing factors such as urea and lactate remained unchanged throughout the study in the volunteers receiving probiotics whereas they were decreased in the placebo group. Finally, in the group of volunteers having received probiotics, the strain *L. paracasei* CNCM I-2116 was detected in 70% of the stool samples, reflecting the ability of the bacterium to remain viable throughout the gastrointestinal tract. Moreover, gut microbiota analysis have shown a significantly higher number of lactobacilli in the probiotic group compared to placebo group. The results of the study demonstrate that oral supplementation with the probiotic *L. paracasei* CNCM I-2116 has a beneficial effect on reactive skin. This finding supports new strategies based on a nutritional approach for the treatment and/or prevention of the symptoms related to reactive skin.

P10

A novel ingredient for skin aging

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Skin aging is characterized by wrinkles, flaky skin, xerosis and by an impaired of skin barrier repair ability. The aim of this study was to evaluate the effect of a novel bacterial extract in aqueous solution on some symptoms of skin aging. For this purpose, a topical cream containing a *Bifidobacterium* lysate was tested in a randomized double-blind placebo-controlled trial. Sixty six female volunteers with reactive skin where randomly given either the cream with the bacterial extract at 10% (n=33) or control cream (n=33). The volunteers applied twice a day the cream to the face, arms and legs for two months. Skin barrier repair ability, leg dryness, facial roughness and natural moisturing factors amounts in the stratum corneum and were assessed by skin barrier recovery evaluated by measuring trans-epidermal water loss following barrier disruption induced by repeated tape-stripping, clinical assessment and biochemical measurement at day 1, day 29 and day 57. The results showed that the treatment led to increase skin resistance against physical and chemical aggression compared to group of volunteers who applied control cream. Noteworthy, the stripping number to obtain barrier function disruption was significantly increased for volunteers treated with the active ingredient compared to control treated group ($p=0.0044$) at the end of the treatment (day 57). Clinical and self-assessment revealed a significant decrease in skin dryness after 29 days for volunteers treated with the cream containing the 10% bacterial extract ($p=0.028$) and skin urea level for the active extract treated group increased while this natural moisturizing factor decreased in the control group. The results of this study demonstrate that this specific bacterial extract may have a beneficial effect on skin. Indeed, the findings suggest that this specific lysate may improve dryness and skin protection against environmental potentially irritating agents, external aggressions or stressed psychological conditions and prevent skin appearance associated with skin aging.

P11

Oral Skin Probiotic™ facilitate early recovery of cutaneous immune homeostasis after UV exposure in humans

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There is now strong evidence that probiotic bacteria can modulate immune responses in many infectious and inflammatory conditions. In the present study, we analyzed whether oral supplementation with *Lactobacillus johnsonii* (Skin Probiotic™) could interfere with down-regulation and/or recovery of normal skin immune status after UV exposure. The probiotic bacterial strain *L. johnsonii* was tested in a randomized, double-blind, placebo controlled clinical trial with 54 healthy volunteers. Half the volunteers received Skin Probiotic™ the other half a placebo during 6 weeks prior to exposure to solar-simulated UV irradiation (2 x 1.5 MED). Blister roofs and skin biopsies were recovered 1, 4 and 10 days after UV exposure from unirradiated and irradiated skin sites and used for mixed epidermal cell lymphocyte reaction (MECLR) and immunohistochemical analysis. Skin Probiotic™ supplementation did not prevent UV-induced phenotypic alterations and maturation of Langerhans cells (LCs) or the decrease in allostimulatory function in irradiated skin samples 18 h post-UV exposure. Interestingly, 4 days post UV exposure the allostimulatory capacity of epidermal cells was totally recovered in the Skin Probiotic™ group correlating with normalization of CD1a expression within epidermis. In contrast, MECLR was still decreased in the placebo group with paralleled reduction in the CD1a LC marker in irradiated epidermis at 4 and 10 days after UV. Moreover $CD36^+$ monocytic cells colonized epidermis as soon as 18 h post irradiation and disappeared faster in the Skin Probiotic™ supplemented group. For the first time, these results provide evidence that ingested probiotic bacteria accelerate the recovery of cutaneous immune homeostasis after UV exposure.

P12

Crosstalk of *E. coli* LF82 - isolated from a Crohn's disease patient - with Caco2 cells

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Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic relapsing inflammation of the digestive tract. The current pathophysiologic concept of IBD suggests a combination of environmental (bacterial flora, nutrition), immunological and genetic factors in the development of the disease. In particular there is strong evidence of a role for the indigenous flora in driving inflammation in genetically predisposed individuals and that an imbalance between 'protective' and 'aggressive' bacteria can lead to IBD in these subjects. So far little is known about the complex interplay between the different bacteria with the gut epithelium, food components and with each other in a healthy state. The picture gets even more complex in patients suffering from IBD. We infected the gastrointestinal cell line Caco2 with a mucosal *Escherichia coli* strain isolated from a patient with Crohn's disease. The cytokine profile was analysed over a certain time period using a human cytokine array and real time PCR. The bacterial contact results in an increased secretion of neutrophil chemotactic factors (IL8 and Gro-α), and the cytokines TNF-alpha and IL32. The latter induces the expression of TNF-alpha, and IL8 in monocytic cells. Our results provide insights into the fine- tuned crosstalk between a pathogenic bacterium and its host.

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P13

In vitro bioconversion of polyphenols in tea and wine by the intestinal microbiota

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Epidemiologic data as well as experimental studies suggest beneficial health effects of natural polyphenols that are abundantly present in foods such as tea and wine. However, the complex underlying mechanisms have not been identified completely yet. After ingestion, some phenolic compounds can be absorbed by the gut epithelium, whereas others pass to the large intestine and are metabolized by the colonic microbiota, followed by absorption of bacterial degradation products. Therefore, potential positive effects of polyphenol-rich foods might be dependent on biotransformation by intestinal bacteria and would therefore vary between individual persons due to differences in endogenous microbiota composition. At the same time, specific phenolic compounds have also been shown to influence microbial community structure by regulation of bacterial growth. These complex interactions are addressed in the EU-Transfer of Knowledge project 'Gutsystem', with the present study particularly focussing on in vitro fermentation models to simulate intestinal microbial bioconversion of polyphenols. In particular, in vitro batch fermentation models were carried out to assess the inter-individual variability of microbial polyphenol metabolism. The bioconversion of tea and wine polyphenols was monitored using NMR-based metabolite profiling and GC/MS-based profiling of phenolics. In contrast to targeted techniques focusing on isolated substances, the profiling methods presented allow to detect a wide range of potentially active breakdown products from complex polyphenol extracts. Preliminary results demonstrate the usefulness of combined analytical profiling techniques to shed light on the colonic metabolism of dietary polyphenols.

P14

Beneficial effects on caecal microbiota of broilers with essential oils

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Healthy intestinal microbiota plays a key role in the development of broilers. In particular, the effects of microbiota on nutrition, immune function and pathogen resistance are critically important. The restrictions on the use of antibiotic growth promoters in poultry farming have increased the interest in alternative compounds capable of beneficially modulating intestinal microbiota. Essential oils (EO) are aromatic compounds with antimicrobial properties. We examined the effect of an EO blend added to broiler diets on the development of the caecal microbiota of male Ross broilers within two independent studies. Microbiota composition was assessed culture-independently by %G+C profiling and by quantitative PCR. Beneficial changes in the composition of the caecum microbiota of the broilers were observed as a result of EO supplementation. The %G+C profiling of the total caecal microbiota revealed reduced ($P<0.05$) relative abundance of bacteria with %G+C ca. 50%. Within this %G+C range reside the opportunistic pathogens *Escherichia coli* and *Salmonella*. The observations of the %G+C profiling assays were supported by the results of the qPCR analysis, which suggested a trend for reduced levels of *E. coli* in the broilers supplemented with EO ($P<0.10$). In addition to *E. coli*, the %G+C profiling suggested beneficial changes in the relative amounts of *Lactobacillus* and *Bifidobacterium* species, but these changes did not reach statistical significance in qPCR analysis. Nevertheless, the ratio of the *Lactobacillus*/*E. coli* determined by qPCR was higher ($P<0.05$) in the EO group compared to the controls. The current results demonstrate the ability of essential oils to beneficially modulate the intestinal microbiota of broilers. The EO blend selectively reduced the relative quantity of *E. coli* without having harmful effects on *Lactobacillus* and *Bifidobacterium* species. The results suggest that EO may offer an effective alternative for antibiotic growth promoters used in poultry industry.

P15

Colonization of the human intestinal tract by potential probiotic strains of *Lactobacillus*

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The probiotic bacteria must possess a number of functional characteristics including the ability to survive and colonize the human gastrointestinal tract. Since the functional properties of probiotic bacteria are strain-specific, selection and assessment of the potential probiotic isolates are important for development of new efficient probiotic preparations. We investigated the ability of potential probiotic strains *Lactobacillus casei* D12, *L. paracasei* Q85, *L. paracasei* Z11 and *L. plantarum* Q47 to colonize the intestinal tract in the human intervention studies. The strains have been isolated from human biopsies and feces and in previous research reported as highly adhesive *in vitro* and able to modulate immune response. Well-characterized probiotic bacteria *L. rhamnosus* 19070, *L. reuteri* 12246 and *L. paracasei* F19, were used as reference strains. The intervention studies were performed with 8 healthy adults who consumed bacterial preparations twice a day, during 12 days. The preparations contained either the new isolates of *Lactobacillus* or known probiotic strains (10^{10} CFU of each strain per serving). Biopsies from ascendens, transversum, and descendens colon and fecal samples were collected at the last day of intervention. The recovery of bacterial strains from human intestine was evaluated by microbiological analysis of biopsies and feces followed by identification of the isolates by Rep-PCR. As expected, the probiotic strains 19070 and F19 were reisolated in high numbers confirming their ability to survive and colonize the intestinal tract. Among the new isolates, the recovery of Z11 and Q85 was the highest and comparable to that of probiotic strains 19070 and F19, indicating their potential to colonize the human intestine and possibility of their application as probiotics in future.

P16

Digestibility and prebiotic potential of nondigestible carbohydrate fractions from novel maize-based fibers in a dynamic *in vitro* model of the human intestine

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A well balanced intestinal microbiota can offer numerous potential health benefits to the host. Some nondigestible dietary fibers act as prebiotics by stimulating the growth of beneficial – or minimizing the growth of undesirable – microbes. In this study, the prebiotic potential of novel fibers were evaluated using an *in vitro* model of the human intestine (TIM-2). Resistant starch (RS), resistant maltodextrin (RM), soluble corn fiber (SCF), soluble fiber dextrin (SFD) and biogum (BG) were pre-digested and monosaccharides separated by chromatography. The remaining fraction was presented to the model of the large intestine (TIM-2; inoculated with microbiota of American origin) at a rate of 10 g/24 h for a 72 h period. Samples were obtained from the lumen of the model every 24 h and short chain fatty acids (SCFA), branched chain fatty acids (BCFA), lactate and ammonia measured. DNA from luminal samples were hybridized to DNA arrays printed with probes to detect group level and individual species of microbes. Compared to a low fiber carbohydrate mixture, all novel fibers stimulated the growth of certain bifidobacteria species at least 2 fold. SCF, SFD and BG treatments decreased several *Bacteroides* species by a factor of 2 or more. Butyrate generation was greatest and ammonia production was least in the BG and SCF treatments as compared to cellulose, a non-fermentable control. Lactate accumulation was lowest in the RS and RM treatments. Overall, these novel fibers show different yet favourable fermentation profiles and prebiotic potential in this dynamic *in vitro* system of the human intestine.

P17

Dietary fibre and gut microbiota affect post-weaning intestinal physiology in conventional and gnotobiotic pigs

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Mechanisms by which dietary fibre and commensal microbiota influence post-weaning intestinal physiology were studied using conventional and gnotobiotic pigs. Caesarean-section derived gnotobiotic pigs (n=24) were reared in HEPA-filtered isolator units (4 pigs/unit) according to an established protocol. Pigs were fed sterilised sow colostrum (120 mL/pig) followed by Similac® (2:1 with water) *ad libitum*. At 14 d of age gnotobiotic pigs were weaned to irradiated corn or wheat/barley-based diet supplemented with DL-methionine or 2-hydroxy-4-methylthiobutanoic acid on equimolar basis. Conventional (CV) pigs (n=32) were sow-reared and weaned to the same non-sterilized 4 experimental diets. At 24 d of age, all pigs were killed and digesta and tissue collected at 75% of small intestinal (SI) length. Gnotobiotic pigs were contaminated such that 8 pigs (2 isolators) were monoassociated (MA) with a bacterium closely related to *Providencia* spp. and 16 pigs (4 isolators) were monoassociated with *Enterococcus faecium.* Viscosity and pH were estimated in digesta, villus height and crypt depth were measured in hematoxylin and eosin stained tissue cross-sections. As a measure of proliferative and apoptotic activity, proliferating cell nuclear antigen (PCNA) expression and caspase-3 activity were measured by quantitative PCR and ELISA, respectively. Digesta viscosity was higher ($P < 0.001$) in wheat/barley-fed and monoassociated pigs. Crypt depth was increased ($P<0.001$) in CV pigs. A significant interaction was observed between diet and microbial status, wheat-barley diet reduced ($P<0.001$) villus height and increased ($P<0.05$) PCNA expression in CV pigs only. Caspase-3 activity was increased ($P<0.001$) in CV pigs. For aminopeptidase, transcript abundance and activity were higher ($P<0.05$) in MA compared to CV pigs. In contrast, sucrase transcript abundance was lower ($P<0.05$) and activity was higher ($P<0.05$) in MA compared to CV pigs. Species of bacterial contaminant and source of methionine did not affect the parameters studied. Results suggest that fibre composition of the postweaning diet influences intestinal mucosal physiology mediated indirectly by gut microbiota.

P18

Modulation of *in vitro Candida albicans* infection by *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14

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Vulvovaginal candidiasis (VVC) is a significant problem affecting 75% of all women at least once during their lifetime. The condition is mainly due to *Candida albicans*, a commensal dimorphic fungal organism of the gastrointestinal and lower female reproductive tract. Previous work from our research group indicated daily oral intake supplementation with capsules containing probiotic strains (*Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14) improved the treatment of Brazilian patients diagnosed with VVC who received a single dose of fluconazole. However, the host defence mechanisms against VVC are not well established yet. The objectives of this study were to evaluate the profile of cytokines constitutively produced by epithelial vaginal cells in comparison with (i) co-culture with *C. albicans* 3153A (simulating an infection *in vitro*); (ii) vaginal cells infected with *C. albicans* and challenged with probiotics (*L. rhamnosus* GR-1 and *L. reuteri* RC-14). Vaginal epithelial cell line VK2/E6E7 was cultured alone ($7.5x10^4$ cells/mL) and co-cultured with *C. albicans* (ca. $2x10^4$ CFU/mL) for 6 h at 37°C, in 24-wells plates. After that time-point, both were challenged (alone or in combination) with *L. rhamnosus* GR-1 (ca. 10^7 CFU/mL), *L. reuteri* RC-14 (ca. 10^7 CFU/mL) or their respective spent culture supernatant (SCS) [1:10 (v/v)] for an extra 6 h co-incubation period. At each point, samples were aspirated from the wells, the populations of *C. albicans* were determined and the supernatant was essayed for quantification of IL-6, IL-8, IL-1a, IP-10, RANTES and VEGF by ELISA. VK2/E6E7 constitutively produced IL-8, IL-1a and VEGF (15.33 pg/mL, 107.39 pg/mL and 52.71 pg/mL, respectively). In the presence of *C. albicans*, the vaginal cells increased to 380pg/mL the production of IL-1a, a potent pro-inflammatory cytokine with a broad spectrum of action. When *L. rhamnosus* GR-1 or *L. reuteri* RC-14 were in contact with VK2/E6E7, the cell line increased several times the production of IL-8, a chemotactic factor for neutrophiles. The highest level of IL-8 was observed with SCS of *L. reuteri* RC-14. Interestingly, when *C. albicans* and the vaginal epithelial cells were co-cultured, the challenge with either *L. rhamnosus* GR-1 or *L. reuteri* RC-14 (culture or SCS) no increase in the production of IL-1a was observed. In challenge studies with the addition of SCS from *L. reuteri* RC-14, a decrease in production of IL-1a was observed. After 24h of co-incubation, the combination of both cultures from *L. rhamnosus* GR-1 and *L. reuteri* RC-14 was the most effective in decreasing *C. albicans* population. In conclusion, co-culture with probiotics presented an anti-*Candida* effect and the production of cytokines by vaginal cells was influenced by probiotic strains or SCS.

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P19

A potential probiotic strain *Bacillus smithii* TBMI12 administrated as endospores and used as competitive exclusion agent against *Salmonella enteritidis*

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Traditionally antibiotics are used to protect animals against *Salmonella* infections. To avoid spreading of antibiotic resistant bacteria European Union have banned all antibiotics in animal feed since 2006 (EC, 2003). According to the above mentioned regulation vaccines, probiotics and competitive exclusion agents have achieved paramount importance in protection of animals against infections. Probiotic bacteria are able to prevent colonization of a gastrointestinal tract by pathogens by blocking mucosal receptor sites of host, secreting antimicrobials, producing by-products of fermentation, stimulating immune system of a host or competing with pathogens for essential nutrients (Fuller, 1991). Probiotic lactic acid bacteria which are in addition sporogenous include members of the genus *Bacillus* found in the gastrointestinal tract of humans and animals (Hong et al., 2005). The present study is related to a potential probiotic bacterium *Bacillus smithii* TBMI12 (*B*. TBMI12) isolated from human gut. The aims of the current study were to test: (i) whether *B.* TBMI12 endospores are able to colonize and form stabile population in gastrointestinal tract of mice without causing any harm to the host animal; and (ii) whether mice which are previously colonized with *B*. TBMI12 spores will be protected against pathogenic *Salmonella enteritidis* wt. In mouse-model we used BALB/c mice obtained from the Institute of Molecular and Cell Biology (University of Tartu, Estonia). Each mouse was housed in separate cage. In total 25 mice in three groups were intragastrically inoculated: (i) only with *S. enteritidis*; (ii) *S. enteritidis* and *B.* TBMI12; and (iii) only with *B.* TBMI12. Results obtained showed that *B.* TBMI12 spores were able to colonize intestinal tract of mice. 40% Of mice colonized previously with *B.* TBMI12 spores were not infected with pathogenic *S. enteritidis* wt. Therefore, we suggest *B.* TBMI12 endospores as a potential competitive exclusion agent against *S. enteritidis*. We conclude that the use of spores of lactic acid bacterium *B. smithii* TBMI12 isolated from human gut could be used potentially as a probiotic bacterium. Furthermore, spore-based products have advantages such as facilitated production and transporting, long-term survival during maintenance as well as after administration in gastrointestinal tract.

P20

The effect of feed raw material on the composition of intestinal microbiota, acidity of the gut and performance in broilers

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The objective of this study was to compare the effect of different feed raw materials on the status of the gut, performance and composition of the cecal microbiota in broilers. Cecal samples from 21-day-old (n=96) and 35-day-old (n=96) 3,200 Ross 508 broilers were collected. The broiler chickens had got the same starter feed for the 7 days. After the starter period 4 pelleted trial feeds based on barley, wheat, oat or dehulled oat were used until the age of 35 days. All feeds contained also soy and vegetable oil and were supplemented with limestone, monocalciumphosphate, sodium chloride, amino acids, vitamins and minerals. All diets were similar in content of ME (11.9 MJ/kg). Daily weight gain (DWG), feed conversion ratio (FCR) and pH of the contents of crop, gizzard, ileum and cecum were determined. From 21-day-old broilers the viscosity of the contents of cecum was measured. Bacterial compositions of the cecal samples (21 and 35 days) were analyzed with a rapid machine method based on flow cytometry (FCM), 16S rRNA hybridization and DNA-staining. Bacterial cells were separated from non-bacterial material, fixed and 16S rRNA hybridized with oligonucleotide probes. A set of five 5'-end labelled oligonucleotide probes hybridizing bacteria belonging to common genera and groups in the cecal microbiota of broilers was used. After hybridization, the bacteria were DNA-stained and analyzed with FCM. The counts of the total bacteria and the hybridized bacteria were determined and Microbial Balance Index (MBI) values were calculated with a patented in-house algorithm. MBI is an index developed earlier for a simplified description of a complex intestinal microbiota and has been associated with good intestinal health, animal performance and FCR. Clear growth differences between the trial groups were observed. The highest DWG (1-35 days) 65 g was achieved with the feed based on wheat compared to 55 g with barley, 57 g with oat and 56 g with dehulled oats ($P<0.05$). Feed raw materials had also significant effect on the viscosity of the content of ileum. The highest viscosities were measured from the broilers fed with the feeds based on barley or dehulled oats. pH of ileum of 21-day-old broilers were different in the trial groups, oat group having the highest pH and barley correspondingly the lowest. Also pH values of the cecum of 35-day-old broilers were different, barley group having the highest pH and oat group correspondingly the lowest. FCM analyses revealed statistically significant differences in the cecal microbial contents between the feeding groups. Feeding with wheat or oat based feeds resulted in the highest total bacteria counts. Also the increases in the total bacteria counts between the two sampling points (21 and 35 days) were the highest with the wheat and oat based feeds. The MBI values describing the composition of the microbiota comprehensively were essentially the same in all trial groups at the age of 21 days. However, at the age of 35 days there were significant differences in the MBI values between the feeding groups. The highest MBI values were again obtained with the feeds based on wheat and oat. In conclusion, the current trial clearly demonstrated that the different feed raw materials have different kinds of effects on the composition of intestinal microbiota, intestinal circumstances such as pH and viscosity of the intestinal content, feed consumption, growth and FCR. The last 2 weeks in the trial turned out to be essential for the development of cecal microbiota, and interestingly the observed differences in the composition of microbiota developed during the last two trial weeks as well.

P21

Shifts in clostridia, bacteroides and immunoglobulin-coating faecal bacteria associated with weight loss in obese adolescents

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Gut microbial imbalances have been linked to obesity in small-scale human studies. The objective of this study was to evaluate the effects of a multidisciplinary obesity treatment programme on faecal microbiota composition and immunoglobulin-coating bacteria in overweight and obese adolescents and their relationship to weight loss. A longitudinal intervention study based on both a calorie-restricted diet (calorie reduction=10-40%) and increased physical activity (calorie expenditure=15-23 kcal/kg body weight/week) was carried out for 10 weeks. Thirty-nine overweight and obese adolescents (BMI mean 33.1 range 23.7-50.4; age mean14.8; range 13.0-16.0) were included in the study. BMI and BMI z-scores were measured before and after the intervention. Faecal microbiota was analyzed by fluorescent *in situ* hybridization and flow cytometry and immunoglobulin-coating bacteria were detected using fluorescent-labelled F(ab´)2 antihuman IgA, IgG and IgM. Reductions in *Clostridium histolyticum* and *Eubacterium rectale-C. coccoides* proportions significantly correlated with weight and BMI z-score reductions in the whole adolescent population. Proportions of *C. histolyticum, C. lituseburense* and *E. rectale-C. coccoides* dropped significantly while those of the *Bacteroides-Prevotella* group increased after the intervention in those adolescents that lost more than 4 kg. IgA-coating bacterial proportions also decreased significantly in subjects that lost more than 6 kg after the intervention and these changes were parallel to reductions in *C. histolyticum* and *E. rectale-C. coccoides* populations. *E. rectale-C. coccoides* proportions also correlated with weight loss and BMI z-score reduction in subjects whose weight loss exceeded 4 kg. Therefore, specific gut bacteria and an associated IgA response were related to body weight changes in adolescents. These results support a role for the gut microbiota in obesity that may involve interactions with both host metabolism and immunity.

P22

What do gut microbes have to do with obesity?

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500 Million adult humans in the world are overweight and 250 million are obese which contributes to the fact that metabolic syndrome is one of the fastest growing global health problems. In a normal healthy adult human the number of microbial cells is approximately an order of magnitude higher than the number of cells making up the entire body, comprising 2-5% of their total body mass. Rather than being considered merely as colonising residents, scientists are discovering the contribution some of these microbes make to various metabolic process and the population of microbes are increasingly being referred to as the 'microbiome'. In fact there is evidence in the literature that obese and lean people have different bacterial populations. There is also speculation that gut microbes interact with fat metabolism via bile acids. The current study investigated the hypothesis that the health implications of diet induced obesity may be negated by including a fermentable vegetable fibre in the high fat diet. The theory behind the mechanism being that caecum microbes ferment the vegetable fibre which then affects a shift in microbial populations or microbial metabolism which has a knock on effect on host fat and energy metabolism via the bile acid pathway. Rats were randomised to receive either high-fat diet with cellulose (HF-C), high-fat diet with broccoli (HF-B), low-fat diet with cellulose (LF-C) or low-fat diet with broccoli (LF-B) with a sample size of n=16 per group. Two trials were run for different lengths of time (1 month and 4 months) to look at both acute and chronic effects. Early results concerning fat deposition, cholesterol and triglycerides will be presented as well as some histology and microbial analysis and their implications will be discussed.

P23

Distribution and function of different species of the *Bacteroides fragilis* group in individuals with Japanese cedar pollinosis

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Japanese cedar pollinosis (JCPsis), an IgE-mediated type I allergy caused by exposure to Japanese cedar pollen, represents a public health issue affecting over 16% of the Japanese population, with increasing prevalence over recent decades. We have previously reported that some intestinal bacteria such as the *Bacteroides fragilis* group significantly fluctuate during the pollen season in JCPsis patients (Odamaki *et al.*, 2007a,b). In addition, intake of the probiotic *Bifidobacterium longum* BB536 strain suppressed fluctuations, alleviated subjective symptoms and affected blood markers (Xiao *et al.*, 2006a,b). The present study investigated associations of the *B. fragilis* group at the species level with JCPsis. After designing 16S rRNA gene-targeted species-specific primer pair sets for *B. caccae, B. coprocola, B. coprophilus, B. dorei, B. eggerthii, B. finegoldii, B. fragilis, B. intestinalis, B. ovatus, B. plebeius, B. stercoris, B. thetaiotaomicron, B. uniformis* and *B. vulgatus* and validating for specificity, fecal DNA from 44 JCPsis and 14 non-JCPsis subjects were quantified by real-time PCR using these primer pair sets. Cell numbers of *B. fragilis* and *B. intestinalis* were significantly higher in JCPsis subjects than in non-JCPsis subjects before the pollen season. These two species increased significantly only in the placebo group at the end of pollen season, not in the non-JCPsis group or JCPsis group administered BB536. Significant positive correlations were found for cell numbers of these two species before and after the pollen season with both composite symptom scores and JCPsis-specific IgE levels. These results imply that prevalence of *B. fragilis* and *B. intestinalis* might represent risk factors for JCPsis. These species might play an exacerbating role in symptom development of JCPsis. Furthermore, intake of BB536 appears to exert positive effects in suppressing fluctuations of these bacteria.

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P24

Probiotics reduce the incidence and duration of cold and flu-like symptoms in children

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Early research, evaluating the benefits of probiotic bacterial consumption by humans, was focused on determining their benefits in a variety of immunological and gastrointestinal maladies. Many fewer studies have been performed evaluating the benefits of probiotic bacteria in healthy subjects. In this study, the impact of probiotic consumption on the incidence and duration of upper respiratory tract infection symptomology in children, was conducted during the winter season by way of a randomized, double-blind, placebo-controlled three-arm study. Two hundred forty eight children, aged three to five years old, were supplemented twice daily for six months with either placebo or one of two probiotic treatments (*Lactobacillus acidophilus* NCFM™ or *L. acidophilus* NCFM™ in combination with *Bifidobacterium animalis* subsp. *lactis* Bi-07). Probiotic intake had a significant impact; reducing both the incidence and duration of fever, coughing and runny nose, and the use of antibiotics. There was a trend for a stronger protective effect for the combination of *L. acidophilus* NCFM™ and *B. lactis* Bi-07. Additionally, children on the probiotic arms had a significant reduction in the number of sick days. Daily supplementation of the diet with these probiotics was a safe and effective way to increase the number of illness-free days.

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P25

Improvement of symptoms of bloating in patients with functional bowel disorders by probiotic strains *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07

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Functional bowel disorders are the most common gastrointestinal disorders seen in primary care and GI clinics; however, their etiology remains unknown. Intestinal bacteria have been suggested to play a role in the pathophysiology and symptomatology of these disorders. Therefore, modulation of intestinal microflora by antibiotics or probiotics may be beneficial in the treatment of patients with these disorders. In the current study we investigated the effect of probiotic bacteria *Lactobacillus acidophilus* NCFM (NCFM) and *Bifidobacterium lactis* Bi-07 (Bi-07) in patients with non-constipation IBS, functional diarrhea, or functional bloating. Total of 57 patients patients with FBD who met the Rome II criteria of non-constipation-IBS, or functional diarrhea, or functional bloating were enrolled in a prospective double-blind, placebo-control clinical trial. Patients (72% females, 84% whites, mean age 37 years) were randomized into a placebo arm (n=27) and an active arm (n=30) of oral probiotic bacteria containing equivalent amounts of NCFM and Bi-07, 10^{11} CFU of each probiotic bacteria in each dose. The placebo and probiotic products were administrated as capsules for 8 weeks. Patients were evaluated for the following endpoints: global relief of GI symptoms (GSA), specific functional GI symptoms, overall symptoms severity (IBS-Severity Index), satisfaction with treatment, overall well being, and health related quality of life (IBS-QOL). Baseline demographics were similar among the two groups. Bloating and distention scores (measured on a 10 points scale) improved significantly in the probiotics group compared to the placebo group at 4 weeks (4.10±2.6 vs. 6.17±2.9; p=0.009, respectively) and showed a strong trend of improvement at 8 weeks (4.26±2.6 vs. 5.84±3.4; p=0.06, respectively). Secondary analyses using only the IBS subgroup (n=33) showed similar results with significant improvement in bloating and distention in the probiotics group (n=17) compared to the placebo (n=16) group (4.24±3.0 vs. 6.73±3.0, p=0.03, respectively). In conclusion, supplementation of diet with NCFM and Bi-07 capsules significantly improved symptoms of bloating and distention in patients with FBD. This data support the role of intestinal bacteria in the pathophysiology of FBD and suggest an important role for these probiotic bacteria in the management of patients with these disorders.

P26

Reduced diversity and increased virulence-gene carriage in intestinal enterobacteria of celiac children

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Celiac disease (CD) is the commonest immune-mediated enteropathy, characterized by chronic inflammation of the small intestinal mucosa. The ingestion of gluten is responsible for the symptoms of CD but other environmental factors associated with its presentation are unknown. In previous studies, *Escherichia coli* proportions were significantly higher in duodenal biopsies from active CD patients than in controls, and a similar trend was detected in faeces although the differences were not so remarkable. Nevertheless, little is known about the possible association between enterobacterial population and CD pathogenesis. In this study, the diversity and prevalence of virulence-associated genes were investigated in faecal enterobacteria isolated from children with active and non-active coeliac disease (CD), and healthy controls. A total of 155 isolates from 31 subjects were identified at species level. *E. coli* clones were classified into four phylogenetic groups A, B1, B2 and D and prevalence was determined of the virulence-associated genes: type-1 fimbriae (fimA), P fimbriae (papC), S fimbriae (sfaD/E), Dr haemagglutinin (draA), haemolysin (hlyA), capsule K1 (neuB), capsule K5 (Kfic) and aerobactin (iutA). Non-*E. coli* clones were more commonly isolated in healthy children than in CD patients. The four phylogenetic *E. coli* groups were equally distributed in healthy children, while in both types of CD patients most commensal isolates belonged to group A. Within the virulent groups, B2 was the most prevalent in active CD children, while D was the most prevalent in non-active CD patients. *E coli* clones of the virulent phylogenetic groups (B2+D) from active and non-active CD patients carried a higher number of virulent genes than those from healthy individuals. Prevalence of P fimbriae, capsule K5 and haemolysin genes was higher in active and non-active CD children, active CD children, and non-active CD subjects, respectively, when compared with controls. Therefore, this study has demonstrated that virulence microbiota-associated features are linked to CD, opening the door to novel dietary strategies based on pro- and prebiotics to restore gut health in CD patients.

P27

Molecular analysis of the porcine gut microflora in response to feeding with probiotics and prebiotics

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Natural feed additives like pro- and prebiotics do not only influence performance standards of farm animals (e.g. swine) but also change their microbial flora. Due to limitations in classical culture-based techniques, the use of molecular methods allows the gain of more detailed information about community composition and effects of different feeding strategies and host factors on it. Aim of this study was to develop and evaluate denaturing gradient gel electrophoresis (DGGE), a qualitative method to display the genetic diversity of complex microbial community structures to detect possible changes in the microbiota of piglets in response to different feeding practices. Test animals of a feeding trial were sacrificed at day 28 of the feeding trial, and contents of ileum, caecum and colon taken under anaerobic conditions. The experimental groups, each comprised of 12 individual piglets were: (i) control group fed untreated feed; (ii) test group fed with probiotics; (iii) test group fed with pro-and prebiotics; and (iv) test group fed with prebiotics. DNA extraction was performed and the 16S rDNA V3 region was amplified by PCR using eubacterial primers to monitor the complex bacterial community. In a nested PCR approach *Bifidobacterium*-specific primers were used to enhance detection of the low abundant bifidobacterial population. Eubacterial PCR products were separated based on the GC content of sequences by DGGE. Similarity and bacterial diversity of banding patterns of various intestinal samples were analyzed using cluster analysis, Shannon diversity indices and number of bands (species richness) supported by a gel software program (GelComparII, Applied Maths, Belgium). Comparisons between DGGE fingerprints of universal bacterial PCR fragments of ileum, caecum and colon samples (n=8) within and between dietary groups showed first important insights. Results revealed a higher eubacterial diversity in colon and caecum samples compared to ileum samples and also significant differences ($P<0.05$) between the pro- and prebiotic feeding groups and the control group, indicating a pro- and/or prebiotic effect of the microbial and the prebiotic feed additives. This effect was also displayed in the cluster analysis. Based on these findings it can be concluded that the DGGE is a suitable tool to display and detect differences in the bacterial community composition of the animals' gut in response to dietary treatments. However, it is still not very clear to what extent pro- and prebiotics contribute to these changes, since a number of other influencing factors, including environment, variations in genetics, stress, health status within the animals herd, have to be considered. In future feeding trials application of quantitative molecular techniques (e.g. FISH and qPCR) will allow us to monitor changes of specific bacterial populations (e.g. bifidobacteria), with the aim to better characterize the effects on microbiota of piglets.

P28

Modification of an *in vitro* model simulating the whole digestive process to investigate cellular endpoints of carcinogenesis and chemoprevention

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In vitro models simulating the digestive tract are commonly used to investigate degradation of nutrients or development of gut flora. Aim of this work was to modify an established model (Aura *et al.*, 1999) to allow studying mechanisms of carcinogenesis and chemoprevention using human adenocarcinoma cells *in vitro*. Inulin was digested and fermented *in vitro* using two models simulating the whole digestive tract varying in ox bile concentration (established: 41.6 g/l; modified: 0.6 g/l). Fermentation supernatants (fs) were analysed for bile acid concentrations with LC-MS/MS. Cell growth/survival of HT29 cells treated with different concentrations of fs was determined by quantifying fluorescence of DAPI-labelled DNA. Fs control of the established model contained high concentrations of cholic acid (CA: 1562 µM) and desoxycholic acid (DCA: 502 µM), whereas fs inulin contained CA and DCA in lower concentrations (CA: 500 µM, DCA: <0.5 µM). Reduction of ox bile concentration to 0.6 g/l resulted in considerable lower amounts of CA (control and inulin: <0.5 µM) and DCA (control: 26 µM, inulin: <0.5 µM). Fs control obtained from the established model was most cytotoxic (20% fs after 72 h: 99 ± 1% reduction of cell number), thus unsuitable for further cell culture experiments. Both fs obtained from the modified model were less cytotoxic, whereas fs inulin was more effective in reducing cell growth than fs control. In conclusion, by decreasing ox bile concentration secondary cytotoxic side effects of fs are reduced. Moreover fermentation products of inulin suppress growth of HT29 cells. Fs obtained from the modified model can be used for *in vitro* investigations on chemopreventive properties of complex food ingredients.

References

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P29

A cell based assay to detect the effect of food extracts on human defensin levels

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Defensins are an ubiquitous class of antimicrobial peptides found across the plant and animal kingdoms. In humans, two main sub-families exist; alpha and beta defensins which are expressed in multiple tissues but predominantly at epithelial surfaces. Both single nucleotide polymorphisms (for alpha defensins) and copy number variants (for beta defensins) exist in people with inflammatory bowel disease (Crohn's disease and ulcerative colitis). As a result they have lower defensin expression and protein levels in their intestinal mucosa and less anti-microbial activity. The aetiology of Crohn's disease is poorly understood but the involvement of microbial/host gut epithelial interactions is increasingly being accepted as central to pathology development. Whilst bacteria are known to stimulate defensin production, very few other stimulants of defensin expression are known. The aim of this work was to develop an assay to test food components and their effects on defensin production. A Crohn's genotype colon cancer cell line HCT8 was incubated with various food extracts alone and with pathogenic bacteria (*Escherichia coli* LF82). Slot blots were performed to semi-quantitatively determine changes in the production of beta-defensin protein. Slot blots clearly show differences in the amount of protein produced by the cells in response to incubations with food and pathogenic bacteria. In conclusion, the assay has been optimised to detect changes in protein expression. Screening of food extracts, and ultimately their fractions, will follow to identify those that increase defensin production and potentially have a beneficial effect on the symptoms of Crohn's disease.

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Fecal microbiota in early rheumatoid arthritis

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The aim of this study was to compare the composition of faecal microbiota between patients having early rheumatoid arthritis (RA) and patients with fibromyalgia (FM). Fifty-one patients with early RA (mean age 56.7 years, 42 females and 9 males) and 50 patients with FM (mean age 50.5 years, 45 females and 5 males) were included in the study. Inclusion criteria for the patients were: no previous disease modifying antirheumatic drug medication (non-steroidal anti-inflammatory drugs were allowed), no glucocorticoids, antibiotics, or gastroenteritis for at least 2 months prior to sampling and the duration of disease for RA patients not more than 6 months. All RA patients fulfilled the RA criteria of American College of Rheumatology. Patients with extreme diets such as vegans were excluded from the study and only non-hospitalized patients from outpatient care were included. All faecal samples were collected at the same specialist's practice and the individuals of the both patient groups were living in the same geographic region. Bacterial composition of the fecal samples was analyzed with a method based on flow cytometry (FCM), 16S rRNA hybridization and DNA-staining. Bacterial cells were separated from non-bacterial material, fixed and 16S rRNA hybridized with oligonucleotide probes. A set of 8 oligonucleotide probes hybridizing fecal bacteria belonging to common genera and groups in the human faecal microbiota was used. After hybridization, the bacteria were DNA-stained and analyzed with FCM. Results: Four of the 8 oligonucleotide probes indicated statistically significant differences between RA and FM patient groups. RA patients had statistically significantly less bifidobacteria (7.5 % vs. 10.0%, P=0.025) and bacteria of *Bacteroides-Porphyromonas-Prevotella* group (6.9 % vs. 9.8 %, P=0.021), *Bacteroides fragilis* subgroup (4.4 % vs. 6.2 %, P=0.044) and *Eubacterium rectale – Clostridium coccoides* group (10.4 % vs. 13.7 %, P=0.026). Of the bacterial groups studied, *C. leptum* subgroup bacteria were the most common in both patient groups (14.8 % vs. 15.9 %), and the bacteria of *Eubacterium rectale – C. coccoides* group were the second most common. Comparison of the RA and FM groups using summarised results of the all 8 probes yielded a significant difference between the groups (P = 0.039), indicating widespread microbial differences. Over half of the faecal bacteria were detected group specifically with the probes used. It is concluded that the faecal microbiota of patients having early RA and FM patients are different. Since faecal microbiota reflects the microbial composition in the other parts of the alimentary tract, the microbiota of the RA patients will possibly be different on a general level. Our findings support the hypothesis that intestinal microbes may participate in the etiopathogenesis of RA.

P31

Immuno-biological effects of different *Escherichia coli* preparations and common bacterial metabolites on the cells of the human immune system tested in a highly complex human organo-typical co-culture model

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Reliable testing of intestinal bacteria or metabolites produced by them for immuno-biological activities depends largely on the set up of the culture model. Adding bacteria directly to cells of the immune system inevitably leads to strong responses, representing artefacts rather than therapeutically relevant activities. To avoid such artificial findings, we developed a co-culture-model (EDI-Co gut) which separates the bacteria from the immune cells (whole-blood cultures) by a highly differentiated, functional layer of gut epithelial cells. Thus, the change of immune cell activities in this cell culture model can occur only in two ways:

- immunologically active components access the immune cell compartment via regulated absorption by the gut epithelium; or
- active substances cause epithelial cells to secrete own metabolites or mediators that cause a secondary modulation of immune cell activities.

Different strains of *Escherichia coli* as well as some bacterial metabolites were tested in the co-culture model. Despite the fact that there was no direct contact of the bacteria or the metabolites with the leukocytes, clear-cut changes in the response of the immune cells towards subsequent experimental activation could be demonstrated. Surprisingly, strong lot-to-lot differences in the activities of one of the *E. coli* strains elicits the question of standardisation of the manufacturing process for bacterial preparations used in microbial therapy. It was concluded that reliable testing of probiotic bacteria and bacterial metabolites for meaningful results can be done in human organo-typic cell culture models. Yet this requires a minimum of prerequisites:

- organo-typic culture set up, preventing the bacteria to access immune-cells directly;
- tight and functional intestinal epithelium;
- standardised performance of the culture-model; and
- standardised manufacturing process of the samples to be tested.

P32

Use of a dynamic, computer-controlled *in vitro* model of the stomach and small intestine (TIM-1) to study survival of probiotics in a chewable tablet

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The aim of this study was to study survival of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, and *B. bifidum* MF 20/5 present in a chewable tablet during transit through the upper GI-tract of children, and to evaluate the best moment of intake in terms of survival of the probiotics. A validated, dynamic, computer-controlled *in vitro* model of the stomach and small intestine (TIM-1) was used, which accurately simulated the dynamic physiological conditions in the upper GI-tract of children 4-12 years of age. Experiments were performed simulating (i) intake of the chewable tablets during a meal, and (ii) one hour after a meal. Samples were taken after the gastric compartment and at the end of the small intestine. The chewable tablets were pre-treated using a mouth-model. Survival is expressed as percentage of intake of viable cells. After the gastric compartment, when the tablet was taken during a meal, survival was 57% for the sum of the two bifidobacterial strains and 51% for *L. gasseri*. Survival upon ingestion of the tablet 1 h after a meal was 35% and 44%, respectively. Survival after passage through the complete TIM-1 system with intake during the meal was 6% for the bifidobacteria and 8% for *Lactobacillus*. With intake 1 h after the meal, survival was 0.6% and 0.8%, respectively. Use of the predictive, validated *in vitro* model shows that highest survival is obtained when the tablets are taken during/immediately after a meal, which has a protective effect (e.g. buffering capacity in the gastric compartment) and results in a ~1.5-fold higher delivery of viable cells to the small intestine and a 10-fold higher amount of viable cells present within the small intestine.

P33

Comparative metabotyping and metagenomic analysis of two lean rat strains

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The role of gut microbiota on host systems has been shown to be important over recent years in both health and disease. There can be massive variations in microbiome composition from individual to individual, relating to environment and diet, resulting in slightly different metabolism. This potentially alters the breakdown of drugs or calorie uptake and has consequences for host metabolic phenotypes (metabotypes). Recently, we have shown large differences in both microbiome and metabotypes between normal and (fa/fa) obese strains of Zucker rat, probably reflecting differences in food intake and host biochemistry. Here we look at two lean strains of rat (Zucker (-/-), n=8; Alderely-Park Wistar-derived, n=8) to assess the more subtle differences in both metabotype and microbiome composition for normal animals reared in the same facility. Metabolic profiles of urine and plasma were generated using ^{1}H NMR spectroscopy whilst the composition of fecal gut microbiota of all animals was characterized using denaturing gradient gel electrophoresis. These data were then statistically modelled using multivariate data analysis techniques (PCA, OPLS-DA). For each medium that was tested, a clear discrimination could be observed between the two strains, with the Alderley-Park Wistar-derived strain showing higher levels of urinary citrate, N-methylnicotinamide, plasma LDL, VLDL and less urinary creatinine, plasma trimethylamine, methylamine. The bacterial profile also revealed a complete separation between the two strains despite sharing a constant environment and diet throughout the study. Collectively, these data revealed differences between two 'control' rat strains allowing us to see the complexity and highlighted the possible problems that will be encountered in future system biology studies such as control selection and comparability over similar studies.

P34

Gene expression in *Lactobacillus acidophilus* during passage through an *in vitro* gastro-intestinal tract model

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In the present study, an *in vitro* model simulating the physical-chemical events arising in the stomach, upper intestine and lower intestine was used to identify putative genes potentially involved in survival and inhabitation of *Lactobacillus acidophilus* NCFM in the gastrointestinal tract of humans. In this model, *L. acidophilus* NCFM inoculated in reconstituted skimmed milk was applied, and the expression of 3 genes related to stress (GroEL, DnaK and ClpP) and 3 genes related to adhesion (mucin binding protein, fibronectin binding protein and surface layer protein) was analysed by real-time PCR. The digestive juices were prepared artificially, digestion steps were performed at 37°C and to simulate peristaltic contractions, the mixtures were rotated head-over-heels. Gastrointestinal transit times were defined as 5 min in the mouth (saliva), 2 h in the stomach (gastric juice) and 2 h in the intestine (duodenal juice and bile), and samples were taken during the passage in the different compartments. Gene expression analysis revealed that the genes coding for the chaperones GroEL and DnaK were continuously upregulated during incubation of *L. acidophilus* NCFM in gastric juice, with a maximum upregulation after 2 h (10-12 fold). This upregulation declined completely during incubation in duodenal juice and bile. The ClpP protease gene was upregulated in a similar manner during incubation in gastric juice; however, in contrast to the chaperones GroEL and DnaK, the maximum gene expression was reached after 15 min incubation in duodenal juice and bile (20 fold) and then constantly declined. An upregulation of genes related to adhesion (mucin binding protein and fibronectin binding protein) was first detected during incubation in duodenal juice and bile. These gene expressions increased continuously and reached a significant maximum after 2 h of incubation, whereas only a slight upregulation of the gene encoding the surface layer protein was determined. These results indicate that the upregulation of the stress related genes DnaK, GroEL and ClpP possibly enables *L. acidophilus* NCFM to survive the stringent conditions present in the stomach, whereas mucin binding protein and fibronectin binding protein are upregulated during the passage of the duodenal tract in order to potentially enhance the attachment of *L. acidophilus* NCFM to intestinal epithelial cells.

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Is icecream a suitable carrier for probiotics?

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The objective of this study was to investigate the suitability of ice cream (IC) as a carrier for probiotics. A DBRPC parallel study was performed with healthy 25-55 year old volunteers divided in 3 groups of 30. During four weeks, each group consumed either: (i) a daily IC control product, (ii) IC with 1x10E9 CFU *Bifidobacterium lactis* HN019 every day, or (iii) IC with 5x10E9 CFU *B. lactis* Bb-12 every *other* day alternating with control IC. Effects on the intestinal microflora and on phagocytosis, NK cell activity and fecal IgA were investigated. During the four intervention weeks, the product stability of *B. lactis* Bb-12 was similar under laboratory controlled- and home storage conditions, but loss of probiotic viability was 2-3 fold higher for *B. lactis* HN019 under home storage conditions. Consumption of IC with *B. lactis* Bb-12 every-other-day resulted in the presence of a significant number of *B. lactis* in the feces of study subjects compared to controls ($p=0.0003$), whereas every-day consumption of IC with *B. lactis* HN019 did not increase numbers of the strain in feces compared to control subjects ($p=0.58$). Sixty percent of subjects consuming the *B. lactis Bb-12* product had the probiotic in their feces. This is well within the range described for other product formats. In contrast, only 13% of HN019 consumers had detectable *B. lactis* in their feces, whereas 10% of controls showed *B. lactis* positivity, which is similar to what has been described in literature. No shifts were detected in selected genera of the fecal microflora. Besides a very small but significant decrease in NK cell activity after consumption of either test product ($p<0.04$), no changes in immune markers were observed. The lack of beneficial effects on immune markers can be attributed to the fairly healthy study population and the fact that bifidobacteria are currently more and more positioned around gut and digestive health instead of immune health. In conclusion, IC can be a suitable carrier for some, but not for all probiotics. *B. lactis Bb-12* is compatible with IC delivering detectable amounts of live probiotics through the consumers intestine even in an every-other day consumption pattern.