FINAL PROGRAMME
&
ABSTRACTS OF LECTURES AND POSTERS

2nd TNO Beneficial Microbes Conference

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

15-17 March 2010
Noordwijkerhout, the Netherlands

2nd TNO Beneficial Microbes Conference

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

Advisory Board

Prof.dr. Jerry Wells (chairman)	Wageningen University, the Netherlands
Prof.dr. Dirk Haller	Technische Universität München, Germany
Dr. Kikuji Itoh	University of Tokyo, Japan
Dr. Marjorie Koenen	TNO Quality of Life, the Netherlands
Dr. Annick Mercenier	Nestlé Research Center, Switzerland
Prof.dr. Theo Niewold	Katolieke Universiteit Leuven, Belgium
Dr. Gregor Reid	University of Western Ontario, Canada
Dr. Koen Venema	TNO Quality of Life, the Netherlands

For information on the Beneficial Microbes and/or Gut Health research programmes within TNO, please contact:
TNO Quality of Life
Dr. Koen Venema
P.O. Box 360
NL-3700 AJ Zeist, the Netherlands
T +31 30 6944703, F +31 30 6944075
koen.venema@tno.nl
www.tno.nl

WELCOME AT THE *2nd* TNO BENEFICIAL MICROBES CONFERENCE!

Dear participant,

It is with great pleasure that we welcome you to the ***2nd*** **TNO Beneficial Microbes Conference** to be held on 15-17 March 2010 at the NH Conference Centre Leeuwenhorst in Noordwijkerhout, the Netherlands.

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 ***2nd*** **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 systems biology in this research area will be highlighted. An important aspect is the application of beneficial microbes, both through probiotics and prebiotics (via the endogenous microbiota), for product development in food and feed industry.

The specific topic areas are the interplay between beneficial microbes and nutrition, the crosstalk between microbes and epithelium or immune system, application in the gut and elsewhere in man and animals, 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 new initiatives for the application of beneficial microbes in the food and feed industry.

We wish you an active and fruitful meeting!

On behalf of the Advisory Board,

Koen Venema and Marjorie Koenen

***2nd* TNO Beneficial Microbes Conference**

Secretariat

Bastiaanse Communication
P.O. Box 179
NL-3720 AD Bilthoven
the Netherlands
T +31 30 2294247
F +31 30 2252910
BM@bastiaanse-communication.com
www.bastiaanse-communication.com

CONTENTS

Key to the abstracts of lectures and posters:

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

CONFERENCE PROGRAMME

Monday 15 March 2010

10.30 Opening of the ***2nd*** **TNO Beneficial Microbes Conference**

SESSION 1
SETTING THE SCENE

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

10.45 *Applications of beneficial microbes: state of the art!*
Dr. Mary Ellen Sanders, Dairy & Food Culture Technologies, USA

11.15 *A microbiomic perspective of the commensal microbiota*
Dr. Joël Doré, Institut National de la Recherche Agronomique (INRA), Micalis, France

11.45 *The microbiota in weight management*
Dr. Arthur Ouwehand, Danisco Finland H&N, Finland

12.15 **Lunch break**

SESSION 2
GUT MICROBIOME-HOST METABOLIC CROSSTALK

Chair: Prof.dr. Jerry Wells, Wageningen University, the Netherlands

13.30 *Does the gut microbiota contribute to the development of insulin resistance?*
Dr. Chieh-Jason Chou, Nestlé Research Center, Switzerland

14.00 *The interaction of short-chain fatty acids with the adipose tissue: relevance prevention of type 2 diabetes*
Prof.dr. Roel Vonk, University Medical Center Groningen, the Netherlands

14.30 *Stable-isotope technology to gain knowledge on crosstalk between microbe and host*
Dr. Albert de Graaf, TNO Quality of Life, the Netherlands

15.00 **Contributed paper**
Gut microbes and dietary polyphenols: approaches to study bioconversion and bioavailability
Dr. Doris Jacobs, Unilever R&D, the Netherlands

15.15 **Contributed paper**
Small intestinal segment perfusion test in pigs: future applications in microbe-gut crosstalk?
Dr. Jan van der Meulen, Wageningen University and Research Center, BioMedical Research, the Netherlands

15.30 **Networking break**

Monday 15 March 2010

SESSION 3
HOST-MICROBE CROSSTALK

Chair: Prof.dr. Jerry Wells, Wageningen University, the Netherlands

16.00 *Life in the slime*
Prof.dr. Gunnar Hansson, University of Gothenburg, Department of Medical Biochemistry Sweden

16.30 *The role of beneficial microbes in the gut-brain axis: reduction of pain perception*
Prof.dr. John Bienenstock, McMaster Brain-Body Institute, St. Joseph's Healthcare, Canada

17.00 *Early life stress: beneficial microbes as a helper against psychiatric illnesses?*
Dr. Siobhain O'Mahony, University College Cork, Alimentary Pharmabiotic Centre, Ireland

17.30 **Contributed paper**
Exopolysaccharides of the probiotic Lactobacillus rhamnosus GG shield against innate immune factors in the intestine
Dr. Sarah Lebeer, K.U.Leuven, Centre of Microbial and Plant Genetics, Belgium

17.45 **Contributed paper**
Identification of genetic loci in Lactobacillus plantarum that modulate the immune response of dendritic cells
Dr. Marjolein Meijerink, Top Institute Food and Nutrition and Wageningen University, Animal Sciences, Host-Microbe Interactomics, the Netherlands

---

18.00 - 19.30 **TNO's Lounge Party**   **Poster presentations**

Tuesday 16 March 2010

SESSION 4
MICROBIAL INFLUENCE ON THE IMMUNE SYSTEM

Chair: Prof.dr. Dirk Haller, Technische Universität München, Germany

08.30 *Regulation of adaptive immune cell differentiation by intestinal commensal bacteria*
Dr. Kenya Honda, University of Tokyo, Graduate School of Medicine, Department of Immunology Japan

09.00 *Probiotic-derived lactocepin degrades the proinflammatory chemokine IP-10: impact on chronic intestinal inflammation*
Dr. Gabriele Hörmannsperger, Technische Universität München, Research Center for Nutrition and Food Science (ZIEL), Germany

09.30 *Probiotics and the immune system: an industrial perspective*
Dr. Joost van Neerven, FrieslandCampina, the Netherlands

10.00 **Contributed paper**
Oligosaccharides synergize with Toll-like receptor ligands to enhance a TH1 type immune response when applied to human intestinal epithelial cells in vitro
Sander de Kivit, Utrecht University, Utrecht Institute Pharmaceutical Sciences (UIPS), the Netherlands

10.15 **Networking break**

SESSION 5
BENEFICIAL MICROBES IN EARLY LIFE

Chair: Dr. Annick Mercenier, Nestlé Research Center, Switzerland

10.45 *'Microbe-driven' immune development in early life*
Prof.dr. Johan Garssen, Danone Research - Centre for Specialised Nutrition and Utrecht University, Department of Pharmaceutical Sciences, the Netherlands

11.15 *Beneficial microbes for premature babies and children with malignancy on chemotherapy*
Prof.dr. Yuichiro Yamashiro, Juntendo University, Japan

11.45 *Probiotics and allergy: a clinical perspective*
Dr. Jon Vanderhoof, Mead Johnson Nutrition, USA

12.15 **Contributed paper**
Impact of oral administration of probiotics, Bifidobacterium breve M-16V, in early infancy on the development of gastro-intestinal microbiotia
Dr. Jiro Nakayama, Kyushu University, Department of Bioscience and Biotechnology, Japan

12.30 **Lunch break** (offered by Danone, the Netherlands)

Tuesday 16 March 2010

SESSION 6
THERE'S MORE TO LIFE THAN THE GUT!

Chair: Dr. Marjorie Koenen, TNO Quality of Life, the Netherlands

13.30 *Beneficial microbes in the oral cavity*
Dr. Jeremy Burton, BLIS Technologies, New Zealand

14.00 *Beneficial microbes in the urogenital tract*
Ruben Hummelen, M.Sc., Lawson Health Research Institute, Canada and Erasmus MC-University Medical Center Rotterdam, Department of Public Health, the Netherlands

14.30 *Microbes on the surface: molecular analysis of the skin microbial flora*
Dr. Bart Keijser, TNO Quality of Life, the Netherlands

15.00 **Contributed paper**
The axillary microbiome – defining the microbial ecology of the human underarm
Dr. David Taylor, Unilever R&D, UK

15.15 **Networking break**

SESSION 7
BENEFICIAL MICROBES IN FEED APPLICATIONS, AN UPDATE

Chair: Prof.dr. Theo Niewold, Katholieke Universiteit Leuven, Belgium

15.45 *Dietary manipulation of rumen microbiota to reduce methane production*
Dr. Diego Morgavi, Institut National de la Recherche Agronomique (INRA), Herbivores Research Unit, France

16.15 *New topics and limits related to the use of beneficial microbes in pig feeding*
Prof.dr. Paolo Bosi, University of Bologna, Department Agri-food Protection and Improvement, Italy

16.45 *Applications of beneficial microbes in poultry production: an overview*
Dr. John Patterson, Purdue University, Department of Animal Sciences, USA

17.15 *Probiotics in companion animals: a brief review.*
James Kyffin, Probiotics International, UK

17.45 **Contributed paper**
Establishing beneficial bacteria in the gut of chicks prior to hatching
Dr. Jean E. de Oliveira, Provimi Research and Innovation Centre, Belgium

18.00 - 19.30 **Poster presentations**   **Drinks**

Wednesday 17 March 2010

SESSION 8
MORE PROBIOTICS APPLICATIONS THAN YOU IMAGINED

Chair: Dr. Jeremy Burton, BLIS Technologies, New Zealand

08.30 *Pre- and probiotics for human skin*
Prof.dr. Jean Krutmann, Heinrich Heine University, Environmental Health Research Institute (IUF), Germany

09.00 *Pathophysiological basis of immune dysfunction in liver failure: strategies for novel therapeutic approaches*
Prof.dr. Rajiv Jalan, University College London, UK

09.30 *Probiotics may help women regain their figures after pregnancy*
Dr. Kirsi Laitinen, University of Turku and Turku University Hospital, Finland

10.00 *Can probiotics affect flu and immunity against H1N1?*
Prof.dr. Juergen Schrezenmeir, Germany

10.30 **Contributed paper**
Microbial healthcare cleaning
Dr. Robin Temmerman, Chrisal, Belgium

10.45 **Networking break**

SESSION 9
FUTURE DEVELOPMENTS: SYSTEMS BIOLOGY

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

11.15 *Systems biology strategies to uncover hidden host-microbe interactions: current insights and future applications*
Dr. Peter van Baarlen, Wageningen University, Animal Sciences, Host-Microbe Interactomics, the Netherlands

11.45 *The new science of metagenomics: bioprospecting the secrets of microbial communities*
Dr. Daniel van der Lelie, Brookhaven National Laboratory, Biology Department, USA

12.15 *How to measure health improvement in apparently healthy people?*
Prof.dr. Renger Witkamp, Wageningen University and TNO Quality of Life, the Netherlands

12.45 Closing of the ***2nd*** **TNO Beneficial Microbes Conference**

LECTURES

Applications of beneficial microbes: state of the art!

Mary Ellen Sanders
Dairy & Food Culture Technologies, USA
mes@mesanders.com

There is an upsurge in applications of beneficial microbes. With probiotics, the scientific advances march steadily forward, the marketplace continues to expand, consumers are getting more sophisticated and healthcare professionals are taking notice. But with this growth and development, numerous challenges have arisen. One clear challenge is translating science into product claims that are understood by consumers in a way that accurately reflects the scientific substantiation. Another is developing scientific dossiers that convince regulators that a causal relationship has been established between the product and the health benefit. Too often claims are being made with no or unconvincing data, which results in a marketplace with too many unvalidated products, and disappointed consumers. Claims must also be compatible with the category of product. In general, claims that a product cures, prevents or treats disease cannot be used on food or nutritional supplements, even if supported by credible research. Designing human trials that are scientifically and clinically meaningful and consistent with regulatory framework to support claims can be very difficult, as well as costly. The public registration of human trials will certainly improve the credibility of industry-funded research and provide a more balanced understanding of positive and negative human trials. Importantly, products marketed as foods or nutritional supplements have not been evaluated for safety in patient populations. Therefore 'off label' use of probiotic products in patient populations must be done so with a careful eye to appropriate and safe use.

In addition to compliance with regulatory statutes, claims are scrutinized by other audiences, such as consumers, healthcare professionals, media professionals, consumer watchdog organizations, advertising watchdog organizations, and litigious elements in society. In the USA, the filing of lawsuits that allege fraudulent claims have targeted successful companies, resulting in costly litigation processes.

Another challenge is the lack of legal definition of the term 'probiotic', although progress is being made on this front in some countries. In many regions, it is impossible for consumers to differentiate among the many products on the market, only some of which are scientifically validated. For the probiotic industry to advance, there is a need for independent, scientific assessments of efficacy and content data, with communication of these results to consumers and healthcare providers. This is being tackled by regulators in Europe, but in countries without such activity should consider this process by independent organizations or frameworks.

The range of applications for externally applied beneficial microbes continues to expand. Data are emerging on the impact of probiotic on metabolic conditions such as diabetes and weight gain, skin inflammation, improved growth status in nutritionally compromised children, dental caries, recurrence of vaginal infections, as well as recombinant therapeutic uses. Global efforts on understanding the human microbiome in health and disease will certainly contribute greatly to understanding these effects, and mechanisms of effects, going forward.

A microbiomic perspective of the commensal microbiota

Joël Doré
Institut National de la Recherche Agronomique (INRA), Micalis, France
joel.dore@jouy.inra.fr

The human intestinal tract harbours a complex microbial ecosystem which plays a key role in nutrition and health. The key interactions between food constituents, microbes and the host organism derive from a long co-evolution, resulting in a mutualistic association. The rate of discovery of new bacteria of the microbiota remained low as long as culturing was the only method available. Culture-independent molecular tools have resulted in a dramatic increase in the number of gut microbes identified, allowing a complete reassessment of the microbial diversity of the human gut.

Although the human faecal microbiota is diverse in its composition, and appeared essentially subject-specific, its functionality is expected to be homogeneous between individuals. However it is not yet clear at which level, metagenome, metaproteome or metabolome, this functional homogeneity can be identified. These different levels of integration have recently become accessible for global exploration of the microbiota.

At present, each dominant faecal microbiota is viewed as composed of an average 1000 species, 80 % of which will be specific of their host and a limited fraction (approx. 2% of all species) will be conserved among healthy individuals, being altogether more prevalent and also more represented in relative numbers. They constitute a phylogenetic core. At the metaproteomic level, that translates into a high proportion of conserved proteins between microbiota (50-60%). The dominant mucosa associated microbiota appears different from the faecal microbiota and at the same time highly conserved for the different segments of the colon and the terminal ileum.

The knowledge gathered from these high throughput molecular assessments allows to define the normal microbiota based on both static and dynamic parameters, related to composition and functions. The qualification of eubiosis – the homeostatic context of healthy individuals' microbiota – allows in turn to explore distortions thereof, hence defining dysbiosis. This rather novel situation opens perspectives for both mechanistic exploration into the role of dysbiosis in diseases as well as strategies for reverting dysbiosis to the homeostatic state of eubiosis.

The current status of investigations into the human faecal metagenome will deliver an extensive gene repertoire representative of functional potentials of the human intestinal microbiota. A reference set of micro-organisms is also currently analysed as part of a concerted effort to sequence the human metagenome, the ensemble of the genomes of human-associated micro-organisms. Beyond sequence-based human intestinal microbiome explorations, metagenomic libraries will facilitate functional studies. For example, functional screening of the metagenomic libraries allows for the identification of unexplored genomic resources some of which are likely to be highly relevant to our understanding of host-microbe interactions.

The International Human Microbiome Consortium aims to give an unprecedented view of the gut microbiota and validate microbial signatures of prognostic and diagnostic value. These approaches promise to identify the most redundant genomic traits of the human intestinal microbiota, thereby identifying the functional balance of this organ. Ultimately it will support the concept of a functional core within the intestinal microbiome.

References

1. Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, S., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.-M., Hansen, T., Le Paslier, D., Linneberg, A., Bjørn Nielsen, H., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Zhang, X., Li, S., Yang, H., Wang, J., Brunak, S., Doré, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., MetaHIT Consortium, Bork, P., Ehrlich, S.D. and Wang, J., 2010. A human gut microbial gene catalog established by deep metagenomic sequencing. Nature 464:59-65 (doi:10.1038/nature08821).
2. Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J.-P., Ugarte, E., Muñoz-Tamayo, R., Le Paslier, D., Nalin, R., Doré, J. and Leclerc, M., 2009. Towards the human intestinal microbiota. phylogenetic core. Environmental Microbiology (doi: 10.1111/j.1462-2920.2009.01982.x).
3. Sokol,H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L.G., Gratadoux, J.-J., Blugeon, S., Bridonneau, C., Furet, J.-P., Corthier, G., Grangette, C., Vasquez, N., Pochart, Ph., Trugnan, G., Thomas, G., Blottière, H.M., Doré, J., Marteau, P., Seksik, P. and Langella, P., 2008. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn's diease patients. Proceedings of the National Academy of Sciences of the United States of America (doi: 10.1073/pnas.0804812105).

The microbiota in weight management

Arthur C. Ouwehand
Danisco Finland H&N, Finland
arthur.ouwehand@danisco.com

Although malnutrition is still a major global problem, at the same time more than 1 billion adults are overweight and at least 300 million of them are obese. Throughout evolution our predecessors had to live with the threat of scarcity. Being able to store extra energy during the rare times of excess, improved survival during the ever present risk of famine. In present affluent societies, however, food is always plenty and the risk of obesity substantial. Because obesity is accompanied with increased health risks (e.g. cardiovascular disease, type 2 diabetes, and certain forms of cancer), weight management strategies are important for successful health care.

The intestinal microbiota is estimated to provide 5-10% of our energy needs through fermentation of undigested food components. Differences in microbiota composition may therefore influence the amount of energy that can be harvested from consumed food. This is indeed what research suggests. Analysing the intestinal microbiota composition of obese and lean subjects, it was found that these two populations differ in their faecal microbiota. This was, however, analysed on a very limited number of subjects. Obese subjects harboured more microbes of the phylum *Firmicutes*, while lean subjects harboured more *Bacteroidetes*. It is possible that the *Firmicutes* are more effective in harvesting energy from consumed non-digestible food components then *Bacteroidetes*. In addition to energy harvest, the intestinal microbiota seems to be involved in inducing the storage of energy in the form of body fat by suppressing the hormone fasting induced adiposity factor (Fiaf) which leads to increased fat storage. Other researchers have, however, not always been able to replicate these findings. Also the oral microbiota has been implicated in obesity. But, it is not always easy to differentiate cause and effect in the case of the microbiota and overweight. Thus, although the intestinal microbiota is involved in energy harvesting and possibly in obesity, it will remain difficult to pinpoint which groups of are the main microbial players. Modulating the microbiota may, nevertheless, seem an interesting to aid weight management

Prebiotics usually aim at increasing the levels and/or activity of genera such as *Bifidobacterium* and *Lactobacillus*. New prebiotic targets could be the increase in *Bacteroidetes* and concomitant reduction in *Firmicutes*. It would, nevertheless, remain to be determined that such a strategy would lead to improved weight management.

As for probiotics, they usually aim at directly mediating health benefits, not necessarily through modulation of the intestinal microbiota. But, of course a change in the *Bacteroidetes/Firmicutes* balance could be a new target, changing the efficacy of microbial colonic fermentation. *Bacteroides* probiotics would be another option. However, also for the traditional probiotics (usually bifidobacteria and lactobacilli) there may be targets to aid in weight management. Probiotics could aim at reducing Fiaf and thus reduce fat storage. Probiotics could aim at influencing the efficacy of digestion. This is risky as it interferes with a very basic function and could cause deficiencies. Finally, satiation and satiety could be a target to influence, by targeting the release of satiating hormones. For these potential targets, varying levels of *in vitro* and animal data exist. However, it remains to be shown that they work in humans as well.

Instead of trying to manage weight with pre- or probiotics, one could consider managing the consequences of overweight. This might not be a cosmetically appealing option for the individual, but it may benefit individual and public health. Prebiotics have been shown to influence markers of metabolic syndrome in animal models and human studies indicate

indeed a relation between the intestinal microbiota composition and type-2 diabetes.

It would seem unrealistic to assume that the use of probiotics or prebiotics will result in weight loss, they could maybe support other means of weight loss and could possibly play a role in alleviating the risks of overweight.

Does the gut microbiota contribute to the development of insulin resistance?

Chieh Jason Chou
Nestlé Research Center, Switzerland
chieh-jason.chou@rdls.nestle.com

Recent evidence indicates that the faecal microbiota profile is different in obese and diabetic subjects when compared with that in lean and non-diabetic subjects, respectively. This association in combination with results from previous rodent studies suggests that the composition of the intestinal microorganisms might contribute to the aetiology of obesity and insulin resistance. However, the causal effect of the gut microbiota on metabolic diseases, especially on insulin resistance, has not been clearly demonstrated.

We answered this question by inoculating different profiles of the gut microbiota to germfree mice. In the first study, donors of caecal microbiota were selected based on the weight gain of mice on a high-fat diet. Caecal microbiota from a high weight gainer with severe hyperglycemia (responder) and from a low weight gainer with mild hyperglycemia (non-responder) was inoculated to two groups of germfree mice. After the inoculation, recipients were put on a high-fat diet for 10 weeks. In contrast to body weight of the donors, both groups gained a similar amount of weight regardless of which profile of microbiota was introduced. The food intake was lower in the group receiving the responder caecal content (RR) than in mice receiving the non-responder caecal content (NR). Interestingly, the NR group showed improved glucose tolerance suggesting that insulin resistance is a transmissible trait via gut microbiota inoculation.

Previously, results in our lab showed that intervention with polymyxin B and neomycin for 2-wk slightly improved fasting glycaemia of diet-induced obesity (DIO) mice, but glycaemia and oral glucose tolerance were much improved after 4-wk washout. The antibiotic treatment also significantly altered the caecal microbiota from a *Firmicutes*-dominant to a *Bacteroidetes*-dominant profile. The association between the persisting *Bacteroidetes*-dominant caecal microbiota and enhanced insulin sensitivity suggests that the gut microbiota can influence the severity of diet-induced insulin resistance.

To test this hypothesis, the untreated *Firmicutes*-dominant or antibiotics-treated *Bacteroidetes*-dominant caecal microbiota was inoculated to mice harbouring a simple background (BG) gut microbiota. Both complex microbiota rapidly and successfully replaced the simple BG bacterial community in the recipient DIO mice. At 7 weeks after the inoculation, mice receiving the *Bacteroidetes*-dominant caecal microbiota from an antibiotic-treated donor were more glucose tolerant than the mice receiving the *Firmicutes*-dominant caecal microbiota from an untreated donor.

Results of two inoculation studies support the argument that the gut microbiota is a credible modulator of diet-induced insulin resistance in mice. The profile of the gut microbiota influences the susceptibility of mice to develop insulin resistance on a high-fat diet.

References

1. Membrez, M., Blancher, F., Jaquet, M., Bibiloni, R., Cani, P.D., Burcelin, R.G., Corthesy, I., Macé, K. and Chou, C.-J., 2008. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. The FASEB Journal 22: 2416-2426 (doi: 10.1096/fj.07-102723).

The interaction of short-chain fatty acids with adipose tissue: relevance for prevention of type-2 diabetes

Roel J. Vonk[1], M. Priebe[1], S. Al-Lahham[1], K. Meijer[1], W. Hongwei[1], K. Venema[2] and H. Roelofsen[1]
[1]University Medical Center Groningen, the Netherlands and [2]TNO Quality of Life, the Netherlands
r.j.vonk@umcg.nl

Short-chain fatty acids (SCFA) are the main bacterial metabolites of the colonic fermentation processes. The retention of the produced SCFA inside the colonic lumen and/or epithelial cells is high in the range butyrate > propionate > acetate. The physiological relevance of the SCFA for the host outside the gastrointestinal tract is getting increased attention. In this presentation we will focus on the effect of SCFA on inflammation processes of the host in relation to insulin resistance. It has been reported that SCFA have an effect on inflammation and immune cells. Butyrate has been shown *in vitro* to decrease pro-inflammatory cytokine mRNA expression and production in inflamed and non-inflamed colonic mucosa biopsies as well as in peripheral blood mononuclear cells [1]. All three SCFA decreased lipopolysacharide-stimulated TNF-α release from human neutrophils [2].

In human nutritional experiments we observed that whole grain products could counteract a glucose induced TNF-α and IL-6 increase [3]; this effect occurred concomitant after an increase in plasma butyrate concentrations. Consumption of intact ^{13}C-barley kernels resulted in a different ^{13}C-SCFA profile in plasma and urine than milled ^{13}C-barley .This suggests that in intact grains a combination of resistant starch and dietary fibre can produce a SCFA profile which could have an anti-inflammatory effect. To further characterize this anti-inflammatory effect we started to analyze systematically the interaction of SCFA with visceral and subcutaneous human fat tissue, macrophages, *in vitro* differentiated adipocytes and freshly isolated human adipocytes.

In human visceral adipose tissue we found that propionate in mM range concentrations could induce the adipokine leptin 2-fold, resistin was reduced while adiponectin was not affected by propionate. These effects could be inhibited by PTX, an inhibitor of Gi/o-protein coupled receptor (GPCR) signalling, suggesting that these effects are, at least partially, receptor mediated [4]. Preliminary results indicated that propionate also suppresses various inflammatory factors. Pro-inflammatory chemokines MIP-1β, IP-10 and G-CSF secretion were reduced by approximately 50% and RANTES by 70%. PTX abolished the response of MIP-1β, RANTES and G-CSF to PA treatment; whereas reduction of IP-10 was not affected by PTX.

The underlying mechanism of SCFA effects on adipose tissue is still unclear. However, the inhibition by PTX suggests involvement of GPCR, such as GPCR41 and GPCR43, which have been shown to have SCFA as ligands. The physiological relevance of these observations especially in relation to insulin resistance will be discussed.

References

1. Segain, J.P., Raingeard de la Blétière, D., Bourreille, A., Leray, V., Gervois, N., Rosales, C., Ferrier, L., Bonnet, C., Blottière, H.M. and Galmiche, J.P., 2000. Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut 47:397-403.
2. Tedelind, S., Westberg, F., Kjerrulf, M. and Vidal, A., 2007. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. World Journal of Gastroenterology 13:2826-2832.

3. Priebe, M.G., Wang, H., Weening, D., Schepers, M., Preston ,T. and Vonk, R.J., 2010. Factors related to colonic fermentation of non-digestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation, American Journal of Clinical Nutrition 9:90-97.
4. Al-Lahham, S., Roelofsen, H., Priebe, M.G., Weening, D., Dijkstra, M., Hoek, A., Rezaee, F., Venema, K. and Vonk, R.J., 2010. Regulation of adipokine production in human adipose tissue by propionic acid. European Journal of Clinical Investigation (in press).

Stable-isotope technology to gain knowledge on crosstalk between microbe and host

Albert A. de Graaf
TNO Quality of Life, the Netherlands
albert.degraaf@tno.nl

Complex metabolic networks of interacting microbes in the gastrointestinal tract of humans and other mammals yield a wide range of metabolites of which the short-chain fatty acids (SCFA, in particular butyrate, acetate, and propionate) are the most abundant. A general research objective concerning SCFA is to understand and predict how the rates of intestinal SCFA production depend on the composition of the intestinal microbiota and the given non-digestible substrate, as well as to identify and understand the regulation of the processes in the host that are directly linked to gut-derived SCFA. Stable isotopes can be highly instrumental in reaching this goal because they allow to trace the route of metabolic precursors in biochemical networks and because they enable us to quantitate the kinetics of the involved metabolic reactions.

We have studied the association between microbiota, substrate and SCFA production using the well-established TIM-2 intestinal model of the colon at TNO. Different SCFA production profiles were found in studies with [U-^{13}C]inulin, starch, lactose and glucose, leading to the hypothesis that speed of fermentation determines production profiles of SCFA. Using terminal restriction length polymorphism fingerprinting of ^{13}C-labeled 16S rRNA combined with analyses using the HITChip, different microbial species were identified that contribute to the metabolism of the different applied [U-^{13}C]substrates. Gut microbial pathways of SCFA formation were also shown to differ for the different labelled substrates as evidenced by LC-MS and NMR analysis of ^{13}C isotopomeric composition of the SCFA. The regulation of SCFA-related metabolic processes in the host *in vivo* is especially difficult to study since highly invasive techniques are required. Using a multicatheterized pig model, interorgan metabolism of ^{13}C-acetate and ^{13}C-butyrate was probed. These experiments produced a series of unexpected results that, taken together, suggested that under a certain threshold production rate, SCFA will not even cross the colon epithelium. Pilot experiments in mice showed extensive incorporation of ^{13}C label from [1-^{13}C]butyrate peritoneally injected in mice into plasma amino acids.

Together these stable isotope-derived data underline the overall metabolic importance of SCFA for the host and call for more detailed studies of SCFA metabolism *in vivo*. Integrating the knowledge obtained from stable istope experiments into computational models will be instrumental to simulate, understand and predict the behaviour of the integrated network of SCFA-related processes in gut and host.

References

1. De Graaf, A.A. and Venema, K., 2008. Gaining insight into microbial physiology in the large intestine: a special role for stable isotopes. Advances in Microbial Physiology53:73-168.
2. De Graaf, A.A., Maathuis, A., De Waard, P., Deutz, N.E.P., Dijkema, C., De Vos, W.M. and Venema, K., 2010. Profiling human gut bacterial metabolism and its kinetic using [U-^{13}C]glucose and NMR. NMR in Biomedicine 23:2-12.
3. Egert, M., De Graaf, A.A., Smidt, H., De Vos, W.M. and Venema, K., 2006. Beyond diversity: functional microbiomics of the human colon. Trends in Microbiology 14:86-91.
4. Egert, M.G.G., De Graaf, A.A., Maathuis, A., De Waard, P., Plugge, C.M., Smidt, H., Deutz, N.E.P., Dijkema, C., De Vos, W.M and Venema, K., 2007. Identification of glucose-fermenting bacteria present in an *in vitro* model of the human intestine by RNA-stable isotope probing. FEMS Microbiology Ecology 60 :126-135.

5. Kovatcheva-Datchary, P.P., Egert, M.G.G., Maathuis, A., Rajilic-Stojanovic, M., De Graaf, A.A., Smidt, H., De Vos, W.M. and Venema, K. 2009. Linking phylogenetic identities of bacteria to starch fermentation in an *in vitro* model of the large intestine by RNA-based stable isotope probing. Environmental Microbiology 11:914-926.
6. Meesters, R.J.W., Van Eijk, H.M.H., Ten Have, G.A.M., De Graaf, A.A., Venema, K., Van Rossum, B.E.J. and Deutz, N.E.P., 2007. Application of liquid chromatography-mass spectrometry to measure the concentrations and study the synthesis of short chain fatty acids following stable isotope infusions. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 854:57-62.

Gut microbes and dietary polyphenols: approaches to study bioconversion and bioavailability

Contributed paper

Doris Jacobs[1], E. van Velzen[1,4], G. Gross[1,3], S. Possemiers[3], R. Kemperman[1,2], L. Roger[1,2], A. Smilde[4], J. Doré[2], J. Westerhuis[4], T. Van de Wiele[3], J. van Duynhoven[1] and E. Vaughan[1]
[1]Unilever R&D, the Netherlands, [2]Institut National de la Recherche Agronomique (INRA), France, [3]Ghent University, Laboratory of Microbial Ecology and Technology (LabMET), Belgium and [4]University of Amsterdam, Swammerdam Institute for Life Sciences, Biosystems Data Analysis, the Netherlands
doris.jacobs@unilever.com

Dietary polyphenols are components of many foods such as tea, fruit and vegetables, and are associated with several beneficial health effects although so far largely based on epidemiological studies. Complex dietary polyphenols have limited bioavailability, with circulating levels in plasma low, and may persist to the colon whereby the human gut microbiota partakes in their degradation into potentially bioactives responsible for health effects. The unique microbiota of each individual may result in variable polyphenol bioconversion.

This complex interaction is addressed within the framework of a collaborative project (EU-GutSystem) which includes both *in vitro* gut model and human studies using metabolomics and microbiomics. The *in vitro* gut model simulator of the human intestinal microbial ecosystem (SHIME) and faecal batch fermentations were used to perform intestinal fermentation of complex tea and grape/wine polyphenol extracts. 16S ribosomal DNA-based diversity molecular tools were applied to characterize microbial community composition, and a wide range of bacterial polyphenol metabolites were determined by NMR- and GC/MS-based profiling of phenolics. The faecal batch fermentations using samples from healthy volunteers revealed considerable inter-individual variation of polyphenol bioconversion characteristics.

This inter-individual variation was confirmed *in vivo* in a placebo-controlled randomized full cross-over human intervention trial using a single oral dose of tea and grape/wine polyphenols extracts in capsules. A novel integrated approach of metabolomics, nutrikinetics and phenotyping was developed to investigate the *in vivo* kinetics of numerous polyphenol metabolites, to determine their activities in metabolic degradation pathways and to identify subpopulations such as high- and low-, slow- and fast-responders. Differences among subpopulations are likely due to individual differences in the microbial composition and/or the enzymatic activity of the colonic microbiota. Ultimately this approach will provide valuable insight into colonic metabolism of polyphenols and mechanism of action, and the infrastructure to investigate other key issues of human microbiome-metabolome interactions.

Acknowledgements
We acknowledge the financial support of the European Community under the Marie Curie Transfer of Knowledge-Industry-Academia Strategic Partnership Scheme MTKI-CT-2006-042786 (GUTSYSTEM).

Small intestinal segment perfusion test in pigs: future applications in microbe-gut crosstalk?

Contributed paper

Jan van der Meulen[1], M.M. Hulst[2], M.A. Smits[2] and T. Schuurman[1]
[1]Wageningen University and Research Center, BioMedical Research, the Netherlands and
[2]Wageningen University and Research Center, Livestock Research, the Netherlands
jan.vandermeulen@wur.nl

Both humans and pigs are true omnivores. This is reflected in the anatomy, physiology and function of the gastrointestinal system. Both species have the same type of teeth, a basal acid secretion in the stomach with a similar HCl content and pH, and a long digestive tract with identical transit times (24-26 h). Further similarities between swine and humans include the developmental program for the gastrointestinal tract (GIT), gut content (both chyme and microflora), mucosal immunity, digestion and metabolism. Some infectious diseases of the GIT of humans (traveler's diarrhoea, childhood infectious diarrhoea) and post-weaning diarrhoea in piglets share the same etiology (enterotoxigenic *Escherichia coli*: ETEC*).* Therefore pigs are a very useful (animal) model for preclinical studies on human nutrition, functional foods, microflora and metabolism.

The small intestinal segment perfusion (SISP) test has been developed to investigate the effects of food components and other test items on the net absorption of fluid (and for example electrolytes) in the healthy and infected gut. The small intestine of an anaesthetized piglet is divided into ten segments with a length of about 20 cm. Each segment is provided with a cranial inflow and a caudal outflow tube. Solutions are perfused continuously through the segments for 8 h at a rate of 8 ml/h. Non-absorbed and/or excreted materials and fluid pass through the outflow tubes into drainage bottles. Net absorption of fluid is determined from the difference between volume of inflow and volume of outflow divided by the surface area of the segment. Before the segments are perfused with test solutions they can be infected with ETEC or other (pig) gut relevant pathogenic micro organisms or toxins. Net absorption of fluid is significantly reduced in segments infected with ETEC or labile toxin (LT).

Up to now the effects of processed and non-processed dietary fibres, black tea extracts, ß-glucanes and some other prebiotics as well as *Lactobacillus plantarum* on net fluid absorption have been studied in the SISP test. Because of the availability of many 'omics' tools, such as porcine microarrays, we have begun to investigate the effects of beneficial and pathogenic microbes as well as dietary products on molecular processes of gut epithelial cells making use of the SISP technique. First results indicate that such interventions already change the gene expression profile of cells in the mucosal layer within 4-8 h. We believe that the SISP technique can successfully be applied in future experiments designed to investigate the early crosstalk between beneficial gut microbes and the host.

Life in the slime

Gunnar C. Hansson
University of Gothenburg, Department of Medical Biochemistry, Sweden
gunnar.hansson@medkem.gu.se

The normal intestinal microbiota lives in intimate contact with the mucus layers of the intestine. The organization of the intestinal mucus has only recently begun to be unravelled, first from the colon [1]. The mucus is organized in two layers; an inner to the epithelial cells firmly adherent stratified mucus layer that is about 50 µm thick and an outer non-attached mucus that can be up to 100 µm thick. These mucus layers are organized around the MUC2 mucin, an enormously large net-like highly glycosylated polymer secreted by the rapidly renewing goblet cells. The inner mucus layer is dense and blocks the bacteria from penetrating and by this keeping the epithelial cell surface free from bacteria. The inner mucus layer is converted to the outer layer, a layer that is the habitat of the commensal flora. This mucus layer has an expanded volume due to proteolytic activities provided by the host. The numerous *O*-glycans of the MUC2 mucin does not only serve as nutrients for the bacteria, but are also attachment sites. The recent observation that normal human individuals carriers a uniform MUC2 mucin glycan array may suggest a selection mechanism for these bacteria [2]. The commensal bacteria of the gut thus spend their life in the slime.

References

1. Johansson, M.E.V., Phillipson, M., Petersson, J., Holm, L., Velcich, A., Holm, L. and Hansson, G.C., 2008. The inner of the two Muc2 mucin dependent mucus layers in colon is devoid of bacteria. Proceedings of the National Academy of Sciences of the United States of America 105:15064-15069.
2. Holmén Larsson, J.M., Karlsson, H., Sjövall, H. and Hansson, G.C., 2009. A complex, but uniform *O*-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MS^n. Glycobiology 19:756-766.

The role of beneficial microbes in the gut-brain axis: reduction of pain perception

John Bienenstock
McMaster Brain-Body Institute, St. Joseph's Healthcare, Canada
bienens@mcmaster.ca

The potentially beneficial effects ascribed to commensal organisms are expanding all the time. We have been interested in whether and how these bacteria may be influencing the central and peripheral nervous systems. We initially showed that feeding 10^9 *Lactobacillus reuteri* per day for 9 days to rats strikingly inhibited the pseudo-affective autonomic responses to colorectal distension in anesthetized animals. We further showed that these effects were reproduced by gavage with the same number of heat killed bacteria and supernatants from media in which the bacteria had been grown. These observations were paralleled by recordings from single fibres from dorsal root ganglion neurons innervating the colon. Others have shown that antibiotic treatment of mice rendered them viscerally hypersensitive and this could be reversed by feeding of *L. paracasei* in spent medium. *L. acidophilus* in further experiments upregulated the number of opioid receptors expressed by intestinal epithelium. Our own experiments have determined that feeding of *L. reuteri* but not *L. salivarius* increased the excitability but decreased the opening of the calcium dependent ion channel (IKCa) specifically in AH neurons of the enteric nervous system (ENS). We have shown recently that acute perfusion of rat or mouse small intestinal lumen with Krebs buffer containing washed bacteria appears to recapitulate the findings within minutes that were previously seen after 9 days of feeding. Furthermore in a hemi-dissection 2 compartment model of intestinal tissue explants we have shown that placement of washed live but not dead bacteria on the intact epithelium results in similar effects on the local ENS IK(Ca) channels as were previously seen. Given the fact that there is now extensive literature suggesting that mast cell-nerve communication is a necessary component of visceral pain development and perception, we have examined the effects of feeding of *L. reuteri* on the IK(Ca) channel in rat mast cells.

We conclude that beneficial microbes may influence the ENS, and affect intestinal motility and pain perception. These effects may in part occur within minutes through ion channels such as IK(Ca), at least in the experimental animal. This suggests that bacterial components or products can reproduce the effects of the whole bacterium as has been shown by others with a cell wall carbohydrate reproducing the immune effects of *Bacteroides fragilis*.

Early life stress: beneficial microbes as a helper against psychiatric illnesses?

Siobhain O' Mahony
University College Cork, Alimentary Pharmabiotic Centre, Ireland
somahony@ucc.ie

Environmental signals influence the development of the central nervous system to produce an individual phenotype that determines the responsivity to stressors of the environment. Therefore, the early environment has a great impact on the development of behavioural and hormonal responses to stress. Consequently, events interrupting this development such as adverse early life stress are associated with a maladaptive stress response system and may increase the vulnerability to psychopathology in later life. Several disorders have been associated with early life stress from depression and anxiety disorders to irritable bowel syndrome.

The early postnatal period is the most dynamic stage in the establishment of the intestinal microbiota during which important commensal-host relationships are forged, including development of the neuro-humoral communication system-the brain-gut-microbiota axis. Microbial colonisation in infants is greatly associated with a range of gastrointestinal functions such as nutrient absorption and modulation, and development of the mucosal immune system. The organisation of mature microbiota into a well-balanced bacterial community also plays an important role as a barrier against colonisation by pathogenic microorganisms and the overgrowth of opportunistic microorganisms. There is also convincing evidence that exposure to the appropriate microbes at an early developmental stage is required for the hypothalamic-pituitary-adrenal (HPA) axis, the main stress axis, to become fully susceptible to inhibitory neural regulation [1]. Altering gut flora is now known to influence behaviour, for example, as seen when *Campylobacter jejuni* administration results in anxiety like behaviour as well as increased brain activation in mice [2]. Thus, it is possible that factors that interfere with the normal microbial ecology within the gut during early life, such as stress, may have deleterious effects on both end organs, the brain and gut, in adulthood and render individuals more susceptible to the development of disease.

This makes the identification of the microbiota affected by adverse experiences in early life invaluable and should lead to a greater understanding of the potential beneficial influence these microbes have on mood and behaviour.

References

1. Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., Kubo, C. and Koga, Y., 2004. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. Journal of Physiology 558:263-275.
2. Gaykema, R.P., Goehler, L.E. and Lyte, M., 2004. Brain response to cecal infection with *Campylobacter jejuni*: analysis with Fos immunohistochemistry. Brain, Bahavior and Immunity 18:238-245.

Exopolysaccharides of the probiotic *Lactobacillus rhamnosus* GG shield against innate immune factors in the intestine

Contributed paper

Sarah Lebeer[1], I.J.J. Claes[1], C. Shen[2], J.L. Ceuppens[3], J. Vanderleyden[1] and S.C.J. De Keersmaecker[1]

[1]K.U.Leuven, Centre of Microbial and Plant Genetics, Belgium, [2]K.U. Leuven, University Hospital, Division of Gastroenterology, Belgium and [3]K.U. Leuven, University Hospital, Division of Clinical Immunology, Belgium

sarah.lebeer@biw.kuleuven.be

Probiotic bacteria are considered to exert their health beneficial effects after ingestion by arriving alive in the gastro-intestinal tract (GIT) and by making close contact with the host. In this study, the role of a long galactose-rich exopolysaccharide (EPS) for the persistence of the prototypical probiotic strain *Lactobacillus rhamnosus* GG (LGG) in the murine GIT was investigated. In a competition assay with LGG wild-type, we tested the *in vivo* survival capacity of an LGG EPS mutant (CMPG5351) that was previously shown to have an enhanced adhesion capacity *in vitro.* Competitive indices for the mutant were determined by selective plating of faecal samples at several time points post administration and of tissue scrapings at distinct locations throughout the GIT. Interestingly, this EPS knock-out mutant exhibited a markedly reduced persistence capacity in the murine GIT, especially in the lower regions of the intestine. *In vitro* validation assays showed that this mutant can indeed survive the challenge with simulated gastric juice as well as LGG wild-type, but that it is more sensitive towards host defense molecules, such as the LL-37 antimicrobial peptide and complement factors. This indicates that LGG EPS form a shield that on the one hand protects against innate immune factors in the GIT, but on the other hand obstructs cell surface adhesins for contact with the host. However, EPS production seems to be dynamically regulated as to fine tune the balance between optimal adhesion and immune evasion. Culturing LGG wild-type in subinhibitory concentrations of innate defense factors such as LL-37 resulted in an upregulation of EPS expression by LGG. These data provide further insights in the mechanisms of survival and physiology of probiotic bacteria in the GIT, which is important for their application.

Identification of genetic loci in *Lactobacillus plantarum* that modulate the immune response of dendritic cells

Contributed paper

Marjolein Meijerink
Top Institute Food and Nutrition and Wageningen University, Animal Sciences, Host-Microbe Interactomics, the Netherlands
marjolein.meijerink@wur.nl

Dendritic cells (DCs) play a major role in orchestrating the responses of innate and adaptive cells to control tolerance and immunity to microbes encountered at mucosal surfaces.. *Lactobacillus* are naturally present in the human intestinal tract and several species and strains have been evaluated for their probiotic activity. Conclusive evidence for the mechanisms underlying the beneficial properties of probiotics is lacking but results obtained from *in vitro* studies and animal intervention models indicate a strong role for immunomodulation and enhancement of the epithelial barrier functions. In the small intestine DCs are known to sample microbes that gain access to the Peyer's Patches via M-cells but also directly across the epithelium by opening tight junctions and sending dendrites to the luminal side [1]. DCs are the main activators of naive T cells and their T cell polarising properties are largely governed by the nature of the microbial products encountered at mucosal sites. Recent work has shown that the ability of lactobacilli to induce a high ratio of IL-10/IL-12 production in human peripheral blood mononuclear cells correlates with their capacity protecting mice from colitis in a TNBS model [2].

We showed that the DC cytokine responses to several species of probiotics can be strikingly different and that significant variation is also seen at the strain level. This could account for the strain-dependent properties of probiotics reported in different clinical trials and animal models. Recently we identified gene loci in *Lactobacillus plantarum* WCFS1 that modulate the immune responses of DCs. The cytokine levels induced by 20 different *L. plantarum* strains were correlated with the presence or absence of genes in each strain by comparative genome hybridization using the WCFS1 genome as a reference. Eight candidate were identified and the impact of these genetic loci on the immune response to *L. plantarum* was confirmed by the construction of gene deletion mutants in the WCFS1 strain. Further insight into the possible role of these genetic loci in immunomodulation will be presented. These findings contribute to our understanding of the strain-dependent variation in immune response to probiotics and have implications for selection of probiotics with specific immunomodulatory properties.

References

1. Rescigno, M., Rotta, G., Valzasina, B. and Ricciardi-Castagnoli, P., 2001. Dendritic cells shuttle microbes across gut epithelial monolayers. Immunobiology 204:572-581.
2. Foligne, B., Nutten, S., Grangette, C., Dennin, V., Goudercourt, D., Poiret, S., Dewulf, J., Brassart, D., Mercenier, A. and Pot, B., 2007. Correlation between *in vitro* and *in vivo* immunomodulatory properties of lactic acid bacteria. World Journal of Gastroenterology 13:236-243.

Regulation of adaptive immune cell differentiation by intestinal commensal bacteria

Kenya Honda[1,2,3], K. Atarashi[2], T. Tanoue[2] and K. Itoh[4]
[1]University of Tokyo, Graduate School of Medicine, Department of Immunology, Japan, [2]Osaka University, Graduate School of Medicine, Laboratory of Immune Regulation, Japan, [3]Japan Science and Technology Agency, Precursory Research for Embryonic Science and Technology (PRESTO), Japan and [4]University of Tokyo, Department of Veterinary Public Health, Japan
kenya@m.u-tokyo.ac.jp

The intestinal mucosa has a unique and complicated immune system composed of a variety of adaptive immune cell populations, including immunoglobulin A (IgA)-producing plasma cells, γδT cells and $CD4^+$ T cells dominated by a Th1 or Th2 phenotype. In addition, we show here that $CD4^+$ T cells in the intestinal mucosa comprise significant numbers of IL-17-producing cells ('Th17 cells') and IL-10-producing regulatory T cells ('$IL\text{-}10^+$ Treg cells'). These cells are particularly abundant in the intestinal lamina propria and are present even at steady state. We also demonstrate that the Th17 and $IL\text{-}10^+$ Treg cells are both dependent on the presence of commensal bacteria for their accumulation in the gut. We will discuss how commensal bacteria provide a particular environment for the unique and well-balanced development of immune system.

Probiotic-derived lactocepin degrades the proinflammatory chemokine IP-10: impact on chronic intestinal inflammation

Gabriele Hörmannsperger, M.-A. von Schillde, C.-A. Alpert, H. Hahne, C. Bäuerl, M. Blaut and D. Haller
Technical University of Munich, Research Center for Nutrition and Food Science (ZIEL), Center for Diet and Disease (CDD), Germany
ghoerman@wzw.tum.de; haller@wzw.tum.de

Clinical studies revealed that the probiotic mixture VSL#3 is protective in the context of inflammatory bowel disease (IBD). We previously showed that VSL#3-feeding of Interleukin 10 deficient mice results in loss of epithelial interferon-inducible protein (IP-10) as well as in reduced caecal inflammation. The VSL#3-induced loss of intestinal epithelial cell (IEC)-derived IP-10 was found to be due to a post-translational mechanism and to be mediated via cell surface proteins of VSL#3-derived *Lactobacillus casei*. The aim of the present study was to identify the active probiotic structure underlying the loss of this major pro-inflammatory chemokine.

Stimulation of activated IEC (Mode-K) with *L. casei* conditioned media (CM) revealed that not only cell surface proteins, but also secreted compounds of *L. casei* mediate selective loss of IP-10. Size exclusion, heat treatment and ammonium sulphate precipitation assays showed that the active compound of CM is a protein of more than 100 kDa. Interestingly, preincubation of CM, but not preincubation of IEC, with phenylmethylsulfonylfluoride (PMSF), an irreversible serine protease inhibitor, abrogated CM-mediated loss of secreted and IEC-surface-associated IP-10.This finding suggests that extracellular IP-10 is selectively targeted by a *L. casei*-derived serine protease. Consistently, cell-free assays demonstrated rapid degradation of recombinant IP-10 via CM, which could also be inhibited by PMSF. The IP-10 degrading bacterial serine protease was then found to selectively target additional proinflammatory chemokines like interferon-inducible T cell chemoattractant (I-TAC), fractalkine and stromal cell derived factor 1α but not interleukin 8 or RANTES. Chromatographic fractionation of CM followed by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS)-analysis revealed lactocepin, a cell-wall associated as well as secreted serine protease to be the active probiotic structure of *L. casei*.

In conclusion, lactocepin was identified as a probiotic bacterial protease targeting a specific subset of proinflammatory chemokines including IP-10. This probiotic mechanism may contribute to a more structure-based evaluation of probiotic bacteria for therapeutical interventions in the context of chronic inflammation.

Probiotics and the immune system: an industrial perspective

Joost van Neerven, A. Nauta, A. Schaafsma, A.D. Siemensma and B. Smit
Royal Friesland Campina, the Netherlands
joost.vanneerven@frieslandcampina.com

The positive impact of beneficial microbes on human health already emerged in the early 20th century when Metchnikoff noted that Bulgarian peasants had a very high life expectancy, which correlated with a high intake of fermented milk (yoghurt). *Lactobacillus bulgaricus*, used in the manufacturing of yoghurt, seemed to confer this health effect. In addition to these health effects of yoghurt cultures, much work has been done in recent years to optimize these beneficial effects by adding probiotic bacteria selected for enhanced functionality. Probiotic bacteria have been included in many different types of products ranging from dairy products to candy bars. The choice of probiotic strain not only depends on functional effects, but also on many other factors as availability, price, and product characteristics. The desired functionality depends on the intended marketing strategy and target population. So far, probiotic products have been focused generally on gut health but more recent research is focusing on chronic low-grade inflammation (adults, elderly), allergy (children), and infection (all). Several intestinal functions of probiotics are well established, such as prevention of adhesion of pathogenic bacteria to intestinal epithelium, lowering of colon pH, production of enzymes (e.g. lactase) and antibacterial peptides. However, for more distant effects throughout the body (asthma, airway infections) an effect on the immune system is required. *In vitro* studies have demonstrated clear effects of probiotics on the immune system by direct activation of monocytes and dendritic cells, and modulation of intestinal epithelium. Although direct effects of probiotics on the immune system have also been demonstrated in several human studies, these effects are currently less well characterized than the effects on gut health.

From an industrial perspective two approaches are of interest to come to probiotic products. Probiotic strains with underlying dossiers can be in-licensed from probiotic suppliers, which is easy and saves time and development costs. On the other hand, in-house strain development may be more tedious and time consuming, but offers additional benefits because it enables companies to screen for specific functionalities. At FrieslandCampina we have experience with both approaches. On the one hand we have performed safety studies and have implemented *L. casei* and *Bifidobacterium lactis* in infant nutrition in Asia. *L. casei* and *B. lactis* are safe and well tolerated, and do not seem to have a major impact on colonic microbiota composition. Functionality studies in relation to infection are currently ongoing in several countries in Asia. On the other hand we have screened for probiotics with immunomodulating capacity *in vitro*, for possible applications in western societies. In this study, a panel of bacterial strains was selected from a culture collection and were screened for their immunostimulatory capacities. These strains could induce distinct cytokine production patterns in monocytes, ranging from inducing only IL-12, a combination of IL-10 and IL-12, to strains that exclusively induced the production of IL-10. We intend to study the possible effects of such novel probiotic strains on allergy and inflammation.

Another crucial industrial aspect of probiotics is the need for registration and claim substantiation. For registration of claims, clinical nutrition studies need to be performed, mostly in the countries where the products are marketed. In Europe the industry can deliver claim dossiers based on *in vitro*-, animal-, and (several) human studies to EFSA who will decide which health claims are allowed. Health claims are divided into article 13 (nutritional) and article 14 (disease prevention, infant) claims. The interpretation by EFSA of convincing evidence delivered by the dossiers allowing health claims and its wordings, may force the industry to reconsider the development of probiotics and prebiotics for non-medical uses.

Oligosaccharides synergize with Toll-Like Receptor ligands to enhance a T_H1 type immune response when applied to human intestinal epithelial cells *in vitro*

Contributed paper

Sander de Kivit[1], E. Saeland[2], A.D. Kraneveld[1], J. Garssen[1], Y. van Kooyk[2] and L.E.M. Willemsen[1]

[1]Utrecht University, Utrecht Institute Pharmaceutical Sciences (UIPS), Pharmacology and Pathophysiology, the Netherlands and [2]VU University Medical Center, Department of Molecular Cell Biology and Immunology, the Netherlands

s.dekivit@uu.nl

A mixture of short-chain galacto- and long-chain fructo-oligosaccharides (scGOS/lcFOS) stimulates T_H1 and regulatory T-cell mediated immune responses and prevents development of allergy in mice. In this study, possible mechanisms of immune modulation by scGOS/lcFOS were investigated *in vitro*. Human intestinal epithelial cells (IEC; HT-29) exposed to 0.5% scGOS/lcFOS together with Toll-like receptor (TLR) ligands were co-cultured with activated healthy donor peripheral blood mononuclear cells (PBMC) in the basolateral compartment. PBMC derived cytokines and T-cell and monocyte phenotype were analyzed.

IFN-γ secretion by PBMC upon TLR4 and TLR9-ligation of IEC was further enhanced after addition of scGOS/lcFOS ($P<0.01$). Supplementation of scGOS/lcFOS increased the percentage of T_H1 cells and decreased TLR-induced CD80 and CD83-expression ($P<0.05$) on monocytes. These effects depended on the presence of IEC. TLR9, but not TLR4, also enhanced regulatory IL-10 secretion ($P<0.01$), which was not affected by scGOS/lcFOS.

In conclusion, exposure of IEC to TLR ligands and scGOS/lcFOS enhances a T_H1 type immune response and maintains an immature phenotype of antigen presenting cells which may favour immune homeostasis.

‘Microbe-driven’ immune development in early life

Johan Garssen and L. Knippels
Danone Research - Centre for Specialised Nutrition, the Netherlands and Utrecht University, Department of Pharmaceutical Sciences, the Netherlands
johan.garssen@danone.com

The human body has an effective defense system against foreign non-self organisms or substances. This defense consists of a non-specific first line defense, a non-specific innate immunity and a specific adaptive immunity. Altogether this is called the immune system. The immune system has the ability to recognize, to remember, to destroy (non-self) cells and/or to inactivate damaging substances.

The intriguing feature of immune development is that it is a continuous process which never ends. A complex highly flexible organ-system able to react and change very fast depending on the danger signal at all ages. During pregnancy the immune system of the unborn baby is educated to inhibit the rejection between mother and child even if they are so-different. After birth the immune system should develop as fast as possible in order to recognize self from non-self and react to any other danger signal. Although there is no consensus at what age the immune system is fully developed compared to adult immune responsiveness it is generally accepted that the majority of immune functions reach adult levels during early puberty. Especially during the first few months of life the immune system changes enormously. For this reason during this period the immune system is highly susceptible to both positive as well as negative triggers affecting a healthy immune development. More and more research indicates that early events on immune development might have serious consequences on immune related diseases later at adult ages such as allergies, asthma and even autoimmunity. Several factors are playing a crucial role in immune development and as a consequence immune related disorders. Genes, epigenetic stimuli, environmental triggers, pollution, infections, and diet are recognized as example factors playing a pivotal role in immune development especially during early life.

Feeding breast milk is the best early nutrition with well described immune benefits early and later in life which might play a role in lowering the incidence of immune related disorders and of metabolic diseases such as obesity in breastfed individuals. Within this breastmilk many immunomodulating ingredients are described which can assist a healthy immune development and as a consequence less immune mediated disorders early and/or later in life. Several breastmilk ingredients can be recognized that can help the development of health promoting microbes such as several bifido species and lactobacilli. One group of ingredients that is extensively studied by many immunologists and microbiologists are the non-digestible oligosaccharides. These carbohydrate structures, or carbohydrate structures mimicking the breastmilk carbohydrates, can change the gut microbiota into a gut microbiota comparable to the microflora in breastfed babies. Additionally some of these oligo-saccharides can trigger unique receptors on immune cells and gut epithelial cells leading to serious immune effects that together with the microbiota induced immune effects can lead to an optimal immune development and as a consequence hopefully less immune related diseases.

Beneficial microbes for premature babies and children with malignancy on chemotherapy

Yuichiro Yamashiro
Juntendo University, Japan
yamasiro@juntendo.ac.jp

The process of initial colonization of bacteria in the intestine begins at the time of delivery, when the foetus leaves the germ-free intrauterine environment and enters the extra-uterine setting. It is now well accepted that the colonization of bacteria, including bifidobacteria and lactobacilli, necessary for the normal development of intestinal innate and adaptive immune defenses. Inadequate gut colonization and dysbiosis, especially low birth weight (LBW) infants and oncology patients being on chemotherapy, may lead to an increased risk of severe infection, including septicemia and gut mucosal damage. The administration of probiotics, that is 'live microbial feed supplements that beneficially affect the host by improving its microbial balance', may prove useful in subjects with such particular conditions.

We carried out several investigations of beneficial effect of *Bifidobacterium breve* in LBW infants, and oncology patients being on chemotherapy and the pertinent findings are described as follows:

- It is suggested that administration of *B. breve* within several hours of life
    - is useful in promoting the colonization of the *Bifidobacterium* and the formation of normal flora;
    - reduces the production of butylic acid, which may be helpful in protecting LBW infants from NEC (necrotizing enterocolitis); and
    - is a very effective treatment to prevent from NEC and infection, including sepsis, and as a result morbidity and mortality rates in LBW infants are decreased.
- Administration of *B. breve* also produces beneficial effects to alleviate discomfort such as frequency of febrile episode and the use of IV antibiotics.

Probiotics and allergy, a clinical perspective

Jon A. Vanderhoof
Mead Johnson Nutrition, USA
jon.vanderhoof@mjn.com

A number of probiotic organisms have been extensively studied in the prevention or treatment of various diarrheal disorders. Several benefits have been defined, and these appear to be strain specific. Despite the usefulness of probiotics in infectious diarrhoea, use of these organisms in inflammatory diseases appears to be much more exciting and potentially more useful. A number of disorders have been explored including inflammatory bowel disease, irritable bowel syndrome, necrotizing enterocolitis, and allergic enterocolitis. Most of the data regarding treatment or prevention of allergic disease have been derived from studies with *Lactobacillus* GG. This organism has been shown to be useful in both the treatment and prevention of atopic dermatitis as well as allergic enterocolitis. Studies exploring the usefulness of probiotics in other allergic diseases have been disappointing. Further data are needed. Probiotic use in allergic disease has been successfully commercialized for the dietary management of atopic dermatitis and allergic colitis through the use of therapeutic infant formulas. Further data are needed to determine if other probiotic organisms will be effective in allergic disorders and if certain prebiotics will also be useful in these conditions.

Impact of oral administration of probiotics, *Bifidobacterium breve* M-16V, in early infancy on the development of gastro-intestinal microbiota

Contributed paper

Jiro Nakayama, M. Teshima, Y. Korenori and K. Sonomoto
Kyushu University, Faculty of Agriculture, Department of Bioscience and Biotechnology, Japan
nakayama@agr.kyushu-u.ac.jp

Bifidobacterium breve M-16V has been shown to effectively aid the development of gastro-intestinal (GI) microbiota in low birth weight infants or to improve symptoms of allergic hypersensitivity to cow's milk and atopic dermatitis. In order to trace the influence of oral administration of M-16V on the developing GI tract microbiota, M-16V (10^{10} cfu) was daily administrated to seven subjects from one week (w1) to three months (m3) after the birth and faecal bacterial composition was monitored until three years old (y3) together with six placebo subjects. Pyrosequence-based 16S rDNA profiling using V6-V8 sequences was introduced to analyze the faecal bacterial composition, allowing a fine overview of bacterial community structure on any taxonomy level, from phylum to species or possibly strain. The sequence with 100% identity to M-16V 16S rRNA was detected at significantly higher frequency in probiotic group than in placebo group during the administration (*P*=0.03), suggesting the efficient colonization of M-16V. However, the relative abundance of total *Bifidobacterium* was somewhat less in probiotic group than in placebo group at m3 (83.7±9.8% vs. 95.4±2.4%, *P*=0.036), while class *Bacilli*, that is, facultatively anaerobic *Firmicutes*, was higher (5.5±2.9% vs. 1.6±1.0%, *P*=0.011). On the other hand, after the probiotics administration was over, the decrease in the *Bifidobacterium* population from m3 to y1 was less in the probiotic group than in placebo group (-31.8± 24.4% vs. -61.9± 21.0%, *P*=0.036), while the increase of class *Clostridia* was less in the probiotic group than in placebo group (17.7±19.6% vs. 49.6±23.7%, *P*=0.026). The population change from m3 to y1 was negatively correlated between with *Bifidobacterium* and *Clostridia* in each individual (R=-0.725), suggesting antagonism between these two bacterial groups in intestine. Phylotype richness based on 16S rRNA sequences in the community was significantly lower in probiotic group than in placebo group at y1 (*P*=0.04).

Taken together, it was indicated that the probiotic treatment before weaning depressed the colonization of adult-type bacteria after weaning. This could be due to an indirect effect by M-16V, maybe via a stimulation of immune development.

Beneficial microbes in the oral cavity

Jeremy Burton
BLIS Technologies, New Zealand
jeremy.burton@blis.co.nz

The contribution of the oral microbiota in the maintenance of human health has been relatively overlooked because of its relative small size compared to the intestinal tract microbiota. However, its importance as the staging post for pathogens of the mouth, throat, nasopharynx and other regions needs to be kept in mind, especially as a rich source of microorganisms capable of broadly influencing human health including various systematic diseases. A number of opportunistic pathogens reside as oral commensals such as *Streptococcus pyogenes* and *S. pneumonae* and if their contribution to the microbiota can be reduced, it may help to lessen the occurrence of disease. Microbiota modification may be achieved by the use of probiotics.

Various intestinal probiotics and a limited number oral cavity isolates have been evaluated as oral probiotics with mixed results. Indigenous bacteria from the oropharynx offer some exciting possibilities as oral probiotics, as they are specifically adapted to this region. Additionally, though the numbers of oral bacteria are low when compared to intestinal bacteria, both the commensal and potentially pathogenic bacteria that occupy the oral tissues appear to have a relatively high proportion of members that produce anti-microbial molecules such as bacteriocins that may provide functional benefits to the host. Some of these bacteria are not members of species that have been typically used as probiotics. This includes potential pathogens may produce useful molecules, that could at some stage also find application as specific therapeutic agents. This includes some relatively benign streptococcal species like *S. salivarius* are extremely common members of the oral microbiota and are good candidates for oropharynx probiotics.

The oral cavity contains of a number of different surfaces, each of which offers attachment opportunities for particular microorganisms. Bacteria from natural oral sources when used as probiotics are more capable of persistence in the oral cavity. This reflects their specific adhesive capabilities that they have adapted this particular environment. Dosing with oral probiotics can more readily achieve high proportions of colonising bacteria in the oral cavity than is possible in the intestinal tract following delivery of intestinal colonisers. The cell preservation process such as freeze-drying applied to probiotic cells can disrupt surface structures required for their attachment. In short, many of the basic attributes traditionally thought to be important for intestinal probiotics such as bile and acid resistance are irrelevant for probiotics administered for use in the oral cavity. Expellable probiotic delivery formats such as mouthwash or gum are considered cosmetic applications and may allow more diverse non-traditional probiotic species to functionally be used in this area.

Beneficial microbes in the urogenital tract

Ruben Hummelen[1, 2], J. MacKlaim[2, 3], G. Gloor[2, 3] and G. Reid[2, 4]
[1]Erasmus MC-University Medical Center Rotterdam, Department of Public Health, the Netherlands, [2]Lawson Health Research Institute, Canada, [3]University of Western Ontario, Department of Biochemistry, Canada and [4]University of Western Ontario, Department of Microbiology and Immunology, Canada
rubenhummelen@gmail.com

The microbial communities residing in the human vagina play a key role in the maintenance of health. The absence of a protective, lactobacilli-dominated, microbiota has been associated with a higher risk of a range of diseases, including sexual transmitted diseases, urinary tract infection, pre-term labour and infertility. Unfortunately a beneficial microbiota is not easily restored after antimicrobial therapy, indicated by high infection recurrence rates. Among women living with HIV, a bacterial vaginosis (BV) microbiota is most recalcitrant, and lactobacilli restoration not easy to achieve. Recently, we performed whole genome sequencing of indigenous and probiotic strains and found that the latter contained some traits potentially suitable for vaginal health. In addition, we performed a randomized, placebo-controlled study on 40 HIV positive women in Tanzania treated with metronidazole plus placebo or probiotic *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 capsules given orally. The clinical outcome showed poor eradication of BV irrespective of probiotic supplementation, a finding contrary to that in HIV-negative women where improved cure of BV resulted with probiotic addition. In order to better understand the structure and composition of the vaginal microbiota in these subjects, we analysed 273 samples using high throughput sequencing of barcoded 16S rRNA genes. Eighteen million reads were obtained, and from this, we were able to track bacterial species associated with cure of BV, restoration of a *Lactobacillus* dominated microbiota, and those in which BV persisted. This is the first such study of its kind and shows the potential for molecular techniques to provide insight into how the host microbes change with disease and interventions.

Microbes on the surface: molecular analysis of the skin microbial flora

Bart J.F. Keijser, F. Schuren and R.C. Montijn
TNO Quality of Life, Food and Biotechnology Innovations, the Netherlands
bart.keijser@tno.nl

The human body is colonized by a wide range of microbial species. With the help of recent breakthroughs such as next generation sequencing, and microarray techniques for gene expression profiling and comparative genome hybridizations, our knowledge on the interaction of the microbial consortia in relation to our health has been increased dramatically. Molecular techniques make the use of cultivation of the bacterial species obsolete, thereby providing a more complete insight in the microbial physiology and interaction with the host. Through bar-coded small ribosomal gene sequencing, detailed insight can be obtained of the species present at the ecological site, as well as their relative abundance. Through genome wide hybridization studies, the genetic potential of species of relevance van elucidated at great molecular detail, advancing further than the species level discrimination provided by the analysis of the ribosomal gene. Multivariate statistical tools can be used to identify biomarkers, either species associated with a healthy or unhealthy status of the host, of gene variants associated with pathogenicity The biomarkers hold great promise for the development of prognostic diagnostic tools and the improvement of health care.

The axillary microbiome – defining the microbial ecology of the human underarm

Contributed paper

David Taylor[1], S. Grimshaw[1], C. Quince[2] and A. Smith[3]
[1]Unilever R&D Port Sunlight, UK, [2]University of Glasgow, UK and [3]Unilever R&D Colworth, UK
david.taylor@unilever.com

In recent years the development of 16S ribosomal DNA-based approaches to characterise and identify bacteria has revolutionised the field of microbial ecology. Similarly, more recent developments in the area of next-generation sequencing, and particularly the application of Roche 454 pyrosequencing have made the analysis of complex microbial communities a tractable problem. Building the methodologies and capabilities to understand the composition of microbial communities on human substrates such as the gastro-intestinal tract and skin is a vital requirement for the future of microbial ecology and the definition of beneficial microbes. Only by understanding the situation in normal and perturbed states, at appropriate levels of detail, can approaches to rationally control microbial communities be advanced.

This paper will present results of a study carried out to characterise the human axillary microbiome. Using universal primers targeted at the V2/3 region of the 16S ribosomal gene, PCR amplicons were generated from 40 individual human axillary wash samples (20 males, 20 females). In addition, sample specific, 8-base tags were included in the amplicons to allow data to be analysed at the level of the individual sample, rather than a pool. Following amplicon sequencing, using the Roche 454 pyrosequencing platform, resulting flowgrams were processed using the PyroNoise algorithm [1], to remove inherent noise in the data which has been shown previously to drastically over-estimate true levels of α-diversity. Similarly, chimeras were removed and sequences then clustered into operational taxonomic units (OTUs) at both the 3% and 5% level. Sequences were labelled taxonomically using the ribosomal database project (RDP) classifier.

In addition, the data generated has been analysed using a number of other publically available tools including Metastats [2] and Fast UniFrac [3]. The output from these analyses, and insights generated, will be presented. To the authors' knowledge this is the most comprehensive analysis of the human axillary microbiota, at the level of individual volunteers, currently available and highlights the potential of the approach for wide application across a number of microbial ecology challenges.

References

1. Quince, C., Lanzen, A., Curtis, T.P., Davenport, R., Hall, N., Head, I.M., Read, L.F. and Sloan, W.T., 2009. Accurate determination of microbial diversity from 454 pyrosequencing data. Nature Methods 6:639-641.
2. White, J.R., Nagarajan, N. and Pop, M., 2009. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Computational Biology 5: e1000352.
3. Hamady, M., Lozupone, C. and Knight, R., 2009. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. The ISME Journal, advance online 27th August.

Dietary manipulation of rumen microbiota to reduce methane production

Diego P. Morgavi, M. Doreau, M. Eugène, C. Martin
Institut National de la Recherche Agronomique (INRA), UR 1213, Herbivores Research Unit, Research Centre of Clermont-Ferrand-Theix, France
morgavi@clermont.inra.fr

Livestock production has an important environmental impact. For ruminants in particular, the emission of enteric methane represents a large proportion of their environmental footprint and decreasing its production is a challenge facing the ruminant production sector. Methane is produced by methanogenic archaea, which together with bacteria, protozoa and fungi are ubiquitous members of the rumen microbiota. Paradoxically, it is this same microbiota that confers ruminants their capacity to digest plant structural carbohydrates, an asset that is largely responsible for their successful adaptation to diverse dietary conditions. The ability to feed on forages should not be neglected in a context of climate change and food insecurity as ruminants do not compete directly with humans for food resources.

In the rumen, production of methane is an effective microbial mechanism that improves fermentation of feeds by preventing the accumulation of dihydrogen. In order to be successful, mitigation options targeting the rumen microbiota have to maintain the proper fermentation functions of this organ and do not have to affect productivity. The reduction of methanogenesis by means of dietary components is being extensively tested with some strategies already in use in the field. The quality, type, and proportion of energy-based concentrates and forages, and the use of lipid supplements can be used to influence methane production. Specific plant components such as saponins, tannins, and essential oils modify the rumen microbiota and are also being tested as a natural approach to reduce methanogenesis. The sustainability and effect of these strategies on the total balance of greenhouse gases emitted in the production of beef or milk will be discussed.

New topics and limits related to the use of beneficial microbes in pig feeding

Paolo Bosi and P. Trevisi
University of Bologna, Department Agri-food Protection and Improvement (DIPROVAL), Italy
paolo.bosi@unibo.it

Scientific studies in *in vivo* trials highlighting the positive effects of probiotics on performance of pigs are becoming more numerous. Likewise there are numerous in vitro tests showing interesting characteristics for their use in this species. Moreover, the attention on probiotics (or probiosis) is increasing after the EU ban on the use of growth promoters, in feed antibiotics, in particular for the weaning phase . However, it is our perception that the use of probiotics in feed compounds have a slow diffusion compared to other additives, such as organic acids. This discussion will focus mainly on the most innovative aspects and problems related to the use of live gut flora stabiliser to improve the weaning and starter phases. Efforts will also be done to point out the possible problems linked with use of probiotics in pig production and on why practical results may be not constant and consistent.

A prerequisite to achieve a healthy and efficient growth of the young pig is a rapid maturation of the gut mucosa and of MALT; this is strictly related to the formation of a stable and complex commensal bacterial community in the digestive tracts. In neonatal pig, the main factors that shape the gut microbiota are the suckling and the mother environment. Then, with the weaning stress, a transient drop of some favourable bacterial strains is observed. In this phase the oral supply of microbes could contribute to re-establish the microbiota balance. Studies were conducted on strains isolated from piglets to select for the best bacteria able to settle in their intestines.

However, after weaning, the novel environment makes the piglets in contact with new and often unfavourable bacteria. On this way, the researches addressed on the identification of candidate probiotics able to contrast the enteropathogenic microorganisms, were focused to select bacteria with different properties (production of antibacterial molecules, competition on adhesion sites, stimulation of immune response, etc). It is interesting to highlight that in general, the data reported in literature, show that the oral administration of bacteria can be favourable or, at least, innocuous. However, there are some indications that the use of a probiotic when the animal is already strongly engaged in the response against an enteropathogen, can have adverse effects on the pig health. This turns to the key topic about the involvement of the different pathways related with the immune response. A more tolerogenic response can be expected when a contrasting action against pathogens is directly addressed to pathogens, while an increased activation of the immune system can be considered favourably when the probiotics are claimed to directly modulate the immune response. Data in the literature are somewhat confusing about that, may be due the different models, or the different starting microbiological environments. Surprisingly very few data are available for the pig on the stimulation of specific immune response by commensal microbes, and on how the wild microbiota interacts with orally supplied probiotics.

The microbial colonisation in the sow can be also a particular target to supply beneficial microbes, to concomitantly affect the establishment of gut microbiota in the offspring, at or immediately after birth. More knowledge about the interaction between sow genotype and dietary factors could help to design more tailored formulations of potential probiotics.

On the whole, increased comprehension about the role of commensal microbiota in the gut and its importance in the metabolism in the whole organism of pigs, could help to select the best bacterial strains and to design the best feeding strategies to improve the efficacy and the reliability of the oral use of beneficial microbes.

Applications of beneficial microbes in poultry production: an overview

John Patterson
Purdue University, Department of Animal Sciences, USA
jpatters@purdue.edu

It has long been known that fermented foods improve animal and human health and that the commensal microbiota provides colonization resistance against pathogens in the intestinal tract and is involved in development of the immune system in the neonate. During the era of growth promoting antibiotic usage the impact of commensal and probiotic microorganisms was ignored, but with pressures to replace growth promotant antibiotics, interest in alternatives, including probiotics, is increasing. Commensal, and probiotic, microorganisms are thought to provide colonization resistance by a variety of mechanisms: competition for nutrients, competition for binding sites, production of toxic compounds/environment and modulation of the immune system. However, little is known about the specific mechanism by which this is accomplished. Although scientific data in poultry is limited, probiotics have been shown to alter intestinal morphology, influence mucosal and systemic cytokines concentrations, stimulate phagocytic activity and inhibit pathogen colonization. Efficacy in commercial operations is more variable than with growth promotant antibiotics. Little is known about the conditions under which probiotics enhance animal health or improve food safety in poultry, or the relative efficacy of single vs. multiple species vs. competitive exclusion. Although current usage is relatively low, probiotic microorganisms are increasingly being used in the poultry industry as a complement to alternative to growth promotant antibiotics and alternative health promotants.

Probiotics in companion animals: a brief review

James Kyffin
Probiotics Internation, UK
james@protexin.com

The benefits of probiotics for humans have been well documented. The health of the immune system in companion animals comes mainly from a healthy gut- which can be weakened by stress, diet change, the environment or disease. Companion animals also benefit from the addition of probiotics and prebiotics to the diet, but these need to be specific to the species in order to benefit the animal. This presentation will focus on scientific details and studies of probiotics in companion animals.

Dysbiosis can occur in the gut of companion animals just as in humans. Various insults such as: gastrointestinal disease; dietary changes; dietary indiscretion; stress; life stage; and antibiotics. Feeding probiotics has shown benefit in the management of many human conditions such as eczema, antibiotics associated diarrhoea, irritable bowel syndrome and colitis. Cats and dogs can also have similar problems that may be managed with probiotics. As such there are numerous clinical applications for probiotics in these species both preventatively and therapeutically.

When considering the use of probiotics in companion animals, one needs to identify the ideal features of probiotics. Unfortunately, there is currently poor regulation of probiotics in the veterinary industry which highlights the importance of identifying a quality manufacturer for source of appropriate probiotics, capable of exerting a beneficial effect in the species for which it is dispensed.

In summary:

- Probiotics need to be safe, non-pathogenic and non-toxic microorganisms. EU strains registered for use in dogs and cats are *Enterococcus faecium* and *Lactobacillus acidophilus.*
- A product should contain sufficient numbers (cfu) to have a beneficial effect.
- Probiotics should be capable of surviving low pH of stomach and bile acids. Microencapsulation of the probiotics with cryoprotectants is recommended. Probiotic strains should also be selected based on their ability to survive these conditions.
- Probiotics should remain stable for long periods under normal storage conditions. This may be facilitated by freeze-drying processes during the final stages of manufacture.
- Quality assurance and expertise is very important. Quality products will ensure they meet label claim at the end of the shelf life of the product.

The main use of probiotics in veterinary medicine is for the treatment of acute gastroenteritis to correct the associated dysbiosis. This can be due to dietary change/indiscretion, stress (travel, shows, kennelling), antibiotic therapy and generalised gastrointestinal disease. Studies have shown that probiotic therapy can reduce the severity and duration of clinical signs associated with acute gastroenteritis in dogs. In the future there is potential for probiotics to play a pivotal role in the management of inflammatory bowel disease, atopic dermatitis and controlling infectious diseases such as feline herpes virus and giardiasis in dogs.

Establishing beneficial bacteria in the gut of chickens prior to hatching

Contributed paper

Jean E. de Oliveira[1], T.A. Scott[1], J.M.B.M. van der Vossen[2], A.M.T. Ouwens[2], E. Hangoor[1], and R.C. Montijn[2]
[1]Provimi Research and Innovation Centre, Belgium and [2]TNO Quality of Life, the Netherlands
jdeoliveira@be.provimi.com

Poultry are believed to hatch with minimal amounts of intestinal bacteria present. Gut microflora is believed to be established during or after hatch. This is considered to be a key event with consequences that affect not only the bird's health and performance, but also relates to food safety risks with establishment of food borne pathogens, particularly into the caeca. The current view is that establishment and maintenance of 'good' or probiotic microbiota can minimize or prevent overgrowth of pathogens. This study was designed to test the viability to colonize the chicken intestine with probiotic bacteria prior to hatch. Embryonized (E17) chicken eggs were *in ovo* inoculated with medium containing *Bacillus subtilis* (P1) or *Enterococcus faecium* (P2), and compared with embryos from non-inoculated eggs (control). Presence of bacteria was determined by qPCR at 48 h after inoculation in the embryo's gizzard content, and at hatch (96 h after inoculation) in the caeca content. Caeca results were also confirmed by plate culturing. P1 presence was greatly increased by inoculation when compared to the controls in both, the gizzard ($1x10^5$ vs. $3x10^2$ cells/ml, respectively) and the caeca ($4.5x10^4$ vs. $3x10^3$ cells/ml). Similar increase was found for P2 in both, the gizzard ($4x10^5$ vs. $4x10^3$ cells/ml) and in the caeca ($8x10^7$ vs. $1x10^7$ cells/ml). Culturing of caeca contents showed that P1 and P2 inoculation significantly increased the number of total bacteria colonies compared to the control (respectively, $3x10^9$, $1x10^9$ and $6x10^7$ CFU/ml). Based on these findings we conclude that the chicken intestine can be colonized with probiotic bacteria before hatching. Future research will focus in determining if chicks that hatch with gut probiotic bacteria are less susceptible to pathogenic bacteria such as *Salmonella*, *Campylobacter* and clostridia.

Pre- and probiotics for human skin

Jean Krutmann
Heinrich Heine University, Environmental Health Research Institute (IUF), Germany
krutmann@uni-duesseldorf.de

Prebiotics have been defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limiting number of, bacteria in the colon'. This concept has originally been developed for the gut, but in principle can be applied to modulate the composition of any microbial community including the skin microflora to achieve beneficial effects.

Scientific interest in the composition and function of the skin`s microflora (= the skin's microbiota) is currently experiencing a revival, and in fact, has become one of the most exciting and rapidly developing areas in cutaneous biology. A major driving force for this development has been the discovery that epidermal keratinocytes have the potential to affect the cutaneous microflora by producing antimicrobial peptides. Also, recent research efforts to understand the control of skin barrier functions unambiguously point to a close link between physical, immunological and cell biological properties of the skin and its microflora. Manipulation of the composition and / or function of the skin microflora by prebiotic strategies, which, in contrast to antibiotics, may allow selective inhibition of detrimental and at the same time preservation and /or stimulation of beneficial bacteria, is therefore of obvious interest in dermatology .

In contrast to prebiotics, probiotics are based on the use of 'living organisms which upon ingestion in certain numbers exert health beneficial effects beyond inherent general nutrition'. Probiotics have been widely used for the treatment/prevention of gastrointestinal disorders, but a growing number of clinical studies suggests that probiotic strategies induce systemic effects which extend beyond the gut and may even affect selected functions of the skin. Accordingly, modulation of the gut's microflora through probiotics appears to cause beneficial effects in healthy as well as diseased human skin.

Probiotics may help women regain their figures after pregnancy

Kirsi Laitinen and E. Isolauri
University of Turku and Turku University Hospital, Finland
kirsi.laitinen@utu.fi

Balanced maternal nutrition during pregnancy ensures growth and development of the foetus as well as the well-being of the mother. Recent evidence supports programming theory, suggesting long-lasting effects on later risk of chronic life-style-related diseases persuaded by early nutrition. Considering the mother, an excessive weight gain during pregnancy predisposes to complications in both pregnancy and labour, while also exposing the mother to a heightened risk of obesity and consequently obesity-related diseases in future years. Almost half of the female population are currently overweight, the problem often setting in after delivery of the first child.

Experimental observations indicate that gut microbiota regulate glucose metabolism and influence energy homeostasis by inducing excessive harvest and storage of nutrients. An aberrant microbiota composition has been linked with metabolic disorders including obesity and impaired glucose metabolism. On this basis an innovative hypothesis has been proposed whereby the composition of gut microbiota could be used as a target for intervention. Probiotics interact with mucosal immune system via the same pathways as commensal bacteria to influence both innate and adaptive immune responses. In consequence, the interventions by immunomodulatory diets, including certain nutrients and probiotics may be critical in coordination of the adaptive function and maintenance of systemic low-grade inflammation necessary for formation of tolerance and thus in prevention of unbalanced release of inflammatory mediators that would result in undesirable metabolic consequences.

In view of the importance of achieving a balanced nutritional environment throughout pregnancy and during postpartum period, we carried out an intervention study in which dietary counselling was combined with probiotics. Repeated dietary counselling together with administration of probiotic capsules (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis*) resulted in lower blood glucose concentrations during pregnancy and over the 12 months' postpartum period. Also a reduced risk of elevated glucose concentration was detected in women having received dietary counselling with probiotics compared to the controls (odds ratio 0.31, 95% confidence interval 0.12 to 0.78; *P*=0.013). Dietary counselling was manifested as a greater proportion of the women (44.4% in the combined intervention group) who gained weight as recommended, compared to the controls (31.4%; *P*=0.071). Central adiposity evaluated by measuring the waist circumference was lowest in the women having received dietary counselling with probiotics over the 12 months postpartum period.

Modification of gut microbiota composition by probiotics, thereby altering the intestinal immunological milieu, may be seen as a novel means of attaining regulation of glucose metabolism and adiposity.

References

1. Bäckhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F. and Gordon, J.I., 2004. The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences of the USA 101:15718-15723.
2. Godfrey, K.M. and Barker, D.J.P., 2000. Fetal nutrition and adult disease. American Journal of Clinical Nutrition 71(Suppl.):344-352.
3. Laitinen, K., Poussa, T. and Isolauri, E., 2009. Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. British Journal of Nutrition 101:1679-1687.

Can probiotics affect flu and immunity against H1N1?

Juergen Schrezenmeir
Karlsruhe, Germany
juergen.schrezenmeir@gmx.de

Probiotics were shown to enhance cellular and humoral immune response to vaccination against viral diseases such as polio (De Vrese et al., 2005) and influenza (Olivares et al., 2007; Boge et al., 2009). In a number of clinical trials effects on the severity, duration and incidence of common infectious diseases were demonstrated (Pedone et al., 1999, 2000; Agarwal et al., 2001; Hatakka et al., 2001; Turchet et al., 2003; Lodinova-Zadnikova, 2003; Tubelius et al., 2005; De Vrese et al., 2005, 2006; Weizman et al., 2005; Schrezenmeir, 2009; Leyer et al., 2009; Lin et al., 2009; Guillemard et al., 2009). A meta-analysis of 8 controlled trials (Offic, 2009) showed a decrease of the occurrence of respiratory tract diseases as defined by the number of persons having at least one episode during the observation period. In this meta-analysis the severity and duration of the episodes was not significantly affected.

In a recent double blind randomized controlled trial (DBRCT) in 1000 shift workers (Schrezenmeir, 2009) the intake of fermented milk drinks containing *Lactobacillus casei* DN-114001 twice daily reduced the occurrence if common infectious diseases during the cold season, the time until infections occurred and the duration of fever episodes. During infections the counts of leucocytes, granulocytes and Natural Killer cells were enhanced. In a multi-centre, likewise DBRCT in 1072 elderly the intake of the same drink reduced the mean duration of common infectious diseases (respiratory tract and intestinal infections) and infections of the upper respiratory tract (Guillemard et al., 2010). In a DBRCT in children aged 3-5 years *L. acidophilus* NCFM alone and together with *Bifidobacterium animalis* ssp. *lactis* Bi 07 reduced the incidence of fever, cough and sneezing, their duration, the incidence of application of antibiotics and the absence from group child care (Leyer, 2009). In 1062 children aged <5 years 2 *L. rhamnosus* strains and a mixture of 7 lactobacillus species, 3 bifidobacterium species, *Streptococcus thermophilus* and *Enterococcus faecium* were compared with no supplementation in a RCT with 4 arms. One of the *L. rhamnosus* strains reduced the incidence of respiratory infections during the cold season compared to the control (Lin et al., 2009).

In conclusion there is evidence for effects on seasonal acute respiratory diseases from a meta-analysis and from further clinical trials with large sample sizes.

References
Available from author on request.

Microbial healthcare cleaning

Contributed paper

Robin Temmerman[1], K. Van Landeghem[2] and W. Verstraete[3]
[1]Chrisal NV, Belgium, [2]Algemeen Ziekenhuis Lokeren, Belgium and [3]Ghent University, Laboratory of Microbial Ecology and Technology, Belgium
robin@chrisal.be

Because of emerging multiresistant 'bugs', governments have rightfully launched campaigns to discourage the use of antibiotics. However, equally dramatic but not yet anticipated is the increasing resistance of micro-organisms to chemical cleaning products and disinfectants used in hospitals and other healthcare facilities. Such resistance strongly undermines all hygiene protocols aiming at a safe patient's environment. The new concept of 'microbial cleaning' is to provide a sustainable hygiene solution. This concept steps away from general disinfection, but instead tries to establish a constant safe microbial environment using cleaning products containing beneficial microbes. Doing so, the biocidal pressure on pathogens will strongly decrease, resulting in less resistance among these organisms. The beneficial bacteria, upon application, penetrate into the deepest impurities of a surface, removing all stuck dirt and biofilm inside it. Because such dirt and biofilm are the perfect shelter and incubator for hospital bacteria, microbial cleaning results in microbiologically safe and stable environment.

Together with Ghent University and the hospital of Lokeren, the company Chrisal conducted a 2-year clinical trial to evaluate the efficiency of microbial cleaning compared to chemical cleaning and disinfection. With over 5,000 surface samples analysed, it was shown on average that microbial cleaning resulted in a long-term 90% reduction of hospital bacteria compared to conventional cleaning/disinfection. The first numbers on nosocomial infections indicate a 32% decrease when cleaning with beneficial microbes. The positive results of probiotic cleaning in the healthcare sector are first of all obvious at the patient level, but numerous other benefits can be listed:

- safety to the medical and nursing staff due to a lower infection pressure;
- safety to the cleaning staff because of the neutral biological composition of the products, permanently banishing all problems with skin, eye and respiratory tract irritations;
- environment friendly because of the biological degradability of the products;
- sustainability through blocking the build-up of resistant microbes against disinfectants;
- the hospital saves money through cutting down overall cleaning and disinfection costs;
- the patient no longer suffers from an elevated healthcare bill due to complications;
- the government significantly lowers its healthcare costs.

The clinical trial shows that microbial cleaning offers a sustainable solution to the enormous problems with hospital bacteria. The combination of microbial cleaning with a good hand hygiene protocol and targeted disinfection guarantees a safe healthcare facility and the lowering of human suffer and medical costs.

Systems biology strategies to uncover hidden host-microbe interactions: current insights and future applications

Peter van Baarlen[1], G. Hooiveld[2], M. Müller[2], M. Kleerebezem[3] and J. Wells[1]
[1]Wageningen University, Animal Sciences, Host-Microbe Interactomics, the Netherlands, [2]Wageningen University, Division of Human Nutrition, Nutrition, Metabolism and Genomics Group, the Netherlands and [3]Wageningen University, Laboratory of Microbiology, Bacterial Metagenomics, the Netherlands
peter.vanbaarlen@wur.nl

Understanding intestinal host-microbe interactions at the molecular level requires a level of integration that can be achieved in systems biology. We are currently undertaking detailed studies where results from transcriptomics, metabolomics and histology are combined using advanced *in silico* correlation and pathway visualisation tools. Our current main focus is: characterising the interaction between gut microbiota, their human and mouse hosts and the diet in a systems biology approach.

We make extensive use of microarray gene expression analysis, histology and immuno-histochemistry, physiological as well as chemokine and cytokine measurements and quantification of cell proliferation during interactions between microbes and human or rodent hosts. *In silico* approaches and tools include detailed annotation and enrichment of biological context of cluster-based gene sets, gene modules and gene signatures. We use gene ontology (GO) enrichment of well-defined gene clusters and evaluate the biological relevance of co-expressed genes by converting these into protein-protein interaction maps and by pathway analysis. Detailed annotation of experiments makes it possible to search for correlations between divergent datasets, and between data and the scientific literature.

We are currently developing and testing hypotheses for host-microbe interactions for two host species, human and mouse, and their microbiota. The effect of dietary interventions on the interplay between host and microbiota will be studied aimed at understanding host immune homeostasis and tolerance. To extrapolate mouse models towards human, and investigate homeostasis in light of disease, we collaborate with two Dutch university medical centres. Ultimately, we want to understand the host-microbiota-nutrition triangle such that we can begin to unravel how deviations from homeostasis lead to the progression of intestinal diseases and metabolic syndrome, and work towards personalised dietary interventions to maintain and strengthen intestinal health.

The new science of metagenomics: bioprospecting the secrets of microbial communities

L.-L. Li[1,4], S.M. McCorkle[1,4], Y.-B. Zhang[1], S.G. Tringe[2], T. Woyke[2], W.S. Adney[3,4], S.-Y. Ding[3,4], M. Himmel[3,4], S. Taghavi[1,4] and **Daniel van der Lelie[1,4]**
[1]Brookhaven National Laboratory, Biology Department, USA, [2]Joint Genome Institute, USA, [3]National Renewable Energy Laboratory, USA and [4]U.S. Department of Energy, BioEnergy Science Center, USA
vdlelied@bnl.gov

In the environment, microorganisms have evolved and accumulated remarkable physiological and functional diversity, and constitute the major reserve for genetic diversity on earth. Using metagenomics, this genetic diversity can be accessed without the need of cell cultivation. Microbial communities and their metagenomes, isolated from biotopes with high turnover rates of recalcitrant lignocellulosic plant cell wall biomass, have become a major resource for the bioprospecting and discovery of new biocatalytics (enzymes) for various industrial processes, including the production of biofuels from plant feedstocks.

Our work aims to understand the diversity and metabolic capabilities of an anaerobic microbial community actively decaying poplar biomass. Metagenomic DNA was isolated and sequenced using 454-GS-FLX Titanium pyrosequencing. Approximately 720 Mbp reads were generated which assembled into 198,375 contigs with a total size of 128 Mb, on which 653,488 putative genes were identified. 16S/18S rRNA libraries and 454-pyrotag sequencing, dinucleotide frequency analysis with agglomerative clustering (AGNES), and ensemble's G/C content analysis all suggested that the community is dominated by 5 demarcated phylogentic groups: two *Bacteroides* groups, *Firmicutes*, *Magnetospirillum* and previously uncultured *Firmicutes*. Links between phylogentic groups and functions (COG/Pfam assignments) are currently under investigation.

Approximately 4,000 glycosyl hydrolase (GHase) homologues were identified using blastx searches against CAZy database. Based on homology to GHase families/activities of interest (key enzymes for efficient decay of plant cell wall recalcitrants) and quality of sequences, candidates were selected for further investigation. One of the bottlenecks, however, is that many putative genes are incomplete or incorrectly assembled, making their full length cloning a time consuming and costly approach. Despite this limitation, full-length open reading frames of various GHases were obtained using inverse PCR and DNA walking, and subsequently cloned into an expression vector for expressing in *Escherichia coli*. Protein purification and characterization are presently in process.

How to measure health improvement in apparently healthy people?

Renger Witkamp
Wageningen University and TNO Quality of Life, the Netherlands
renger.witkamp@wur.nl

Effects of nutrition, including those of prebiotics and probiotics, are generally more subtle and act at a slower pace than those of pharmaceuticals. In addition, nutrition-based prevention and intervention strategies are targeting a population that is considered (relatively) healthy. Pharmaceuticals on the other hand are mainly intended to cure disease. This implicates that a pharmaceutical approach, based on clinical endpoints and biomarkers for disease has limited value in demonstrating health promoting effects of foods or food supplements. Instead, in nutrition it often comes down to questions whether a product can help to improve health, enhance resistance, prevent risks, delay a progressive loss of vitality etc. This requires new concepts to define and quantify health and risks. New strategies are based on early markers for deteriorating processes or intended to reflect the robustness of the homeostatic balance of the individual. In both cases these markers differ from the more classical disease biomarkers that are in use in pharma. Thanks to the developments in genomics and the integration into systems biology it is now possible to model biomarker profiles. In contrast to the classical single biomarkers, these profiles can be used to monitor subtle processes. The concept of measuring deteriorating processes or the robustness (resilience) of an individual is also coming from systems biology. It is a well-known physiological fact that any organism will try to maintain a situation of homeostasis as long as possible when its system is being disturbed, using various compensation mechanisms. Early signs of homeostatic disturbance as observed in onset of disease may be detected using a biomarker profile approach. Examples include the diagnosis of a pre-diabetic state, measuring inflammation in overweight or obesity, or analyzing metabolic fluxes. To measure the robustness of homeostasis in healthy persons, so-called challenge tests are introduced in nutrition and health research. These include variations of oral glucose and lipid tolerance tests, organ function tests, exercise- or even psychological stress challenges. Although some of these tests, for example the glucose tolerance test, are not new at all, the combination with new bio-analytical technologies and calculation power makes them particular useful to test health improving effects of nutritional products. Specifically in relation to intestinal health, challenge tests that measure resistance to infection, intestinal permeability or translocation of endotoxins show great potential.

POSTERS

P1 *Impact of maternal diet during pregnancy and breastfeeding on infant metabolic programming: a prospective randomized controlled study*
Jonna Aaltonen, T. Ojala, K. Laitinen and E. Isolauri
University of Turku, Turku University Central Hospital, Department of Pediatrics, Finland

P2 *Regulation of interferon-induced protein genes by a multi-strain commercial probiotic*
Julie Audy, O. Mathieu and T.A. Tompkins
Institut Rosell Inc., Canada

P3 *Influence of the intrinsic gut microbiota on transcriptional regulation of genes involved in the early life development of intestinal epithelial integrity*
Anders Bergström[1], M.B. Kristensen[1], H. Frøkjær[2] and T.R. Licht[1]
[1]DTU Food, National Food Institute, Division of Microbiology and Risk Assessment, Denmark and [2]University of Copenhagen, Faculty of Life Sciences, Department of Basic Sciences and Environment/Biochemistry and Natural Products Chemistry, Denmark

P4 *Monitoring of metabolic dynamics in microbial ecosystems using a combination of DNA fingerprinting and metabolome analysis based on stable isotope labelling*
Yasuhiro Date[1,2,3], Y. Nakanishi[2,3,4], S. Fukuda[3,4], T. Kato[3,4], S. Tsuneda[1], H. Ohno[3,4] and J. Kikuchi[2,3,5]
[1]Waseda University, Department of Life Science and Medical Bioscience, Japan, [2]RIKEN Plant Science Center, Japan, [3]RIKEN Research Center for Allergy and Immunology, Japan, [4]Yokohama City University, Graduate School of Nanobioscience, Japan and [5]Nagoya University, Graduate School of Bioagricultural Sciences, Japan

P5 *Comparative study of predominant faecal microbiota of a group of cystic fibrosis children and healthy siblings*
Gwen Duytschaever[1], G. Huys[1], L. Boulanger[2], K. De Boeck[2] and P. Vandamme[1]
[1]Ghent University, Laboratory of Microbiology, Department of Biochemistry and Microbiology, Belgium and [2]University Hospital of Leuven, Department of Paediatrics, Belgium

P6 *Antimicrobial peptides produced by Lactobacillus sakei 2a: cloning and heterologous expression*
K.G. De Carvalho[1], M.F. Kruger[1], M. De Souza Barbosa[1], E. Salvucci[2], F. Sesma[2] and **Bernadette D.G.M. Franco**[1]
[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental, Laboratório de Microbiologia de Alimentos, Brazil and [2]Centro de Referencia para Lactobacilos, Argentina

P7 *Potential beneficial properties of bacteriocin-producing lactic acid bacteria isolated from smoked salmon*
S.D. Todorov[1], D.N. Furtado[1], E. Chiarini[1], S.M.I. Saad[2], E. Tome[3] and **Bernadette D.G.M. Franco**[1]
[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental, Brazil, [2]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Tecnologia Bioquímico-Farmacêutica, Brazil and [3]Universidad Central de Venezuela, Escuela de Biología, Instituto de Ciencia y Tecnología de Alimentos, Venezuela

P8 *Anti-listerial properties of a bacteriocin produced by Lactobacillus plantarum ST8SH, a strain isolated from Bulgarian salami*
S.D. Todorov[1], **Bernadette D.G.M. Franco**[1] and M. Vaz-Velho[2]
[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental, Laboratório de Microbiologia de Alimentos, Brazil and [2]Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Portugal

P9 *Changes in the gut intestinal microflora influence behaviour in mice*
Mélanie G. Gareau[1], E. Wine[1], M.T. Whary[2], G. MacQueen[3] and P.M. Sherman[1]
[1]University of Toronto, Hospital for Sick Children, Research Institute, Canada, [2]Massachusetts Institute of Technology, Division of Comparative Medicine, USA and [3]University of Calgary, Department of Psychiatry, Canada

P10 *CRIB, an essential bacterium to protect us against infectious complications?*
Coline Gerritsen[1,2,3], H.M. Timmerman[4], L. Mulder[3], L.M.A. Akkermans[2], G.T. Rijkers[5] and H. Smidt[1]
[1]Wageningen University, Department of Microbiology, the Netherlands, [2]University Medical Center, Department of Surgery, the Netherlands,[3]Winclove Bio Industries B.V., Research and Development Department, the Netherlands, [4]NIZO Food Research B.V., the Netherlands and [5]University Medical Center, Department of Pediatric Immunology, the Netherlands

P11 *Treatment of infectious mastitis during lactation: antibiotics versus oral administration of lactobacilli isolated from breast milk*
Arantza Gómez de Segura, R. Arroyo, V. Martín, A. Maldonado, E. Jiménez, L. Fernández and J.M. Rodríguez
Universidad Complutense de Madrid, Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Spain

P12 *Effect of phytobiotic blends on growth performances and digestive microbiota of broiler chickens in two rearing densities*
Sarah Guardia[1], F. Recoquillay[2], H. Juin[3], M. Lessire[1], M. Leconte[1], P. Rideaud[3], C. Moreau-Vauzelle[3], C. Dupont[3], J.F. Guillot[4] and I. Gabriel[1]
[1]INRA, UR 83, URA, France, [2]Phytosynthese, France, [3]INRA, UE 1206, UEASM, France and [4]I.U.T de Tours, Microbiology laboratory, France

P13 *Antimicrobial activity of Vitreoscilla filiformis lysate: results from a randomized, double-blind, vehicle-controlled study and in vitro study*
Audrey Guéniche[1], P. Bastien[1], Y. Mahe[1], N. Billoni[1], B. Knaudt [2], E. Piche[2], T. Volz[2], M. Röcken[2], T. Biedermann[2] and L. Breton[1]
[1]L'Oreal Recherche, France and [2]University of Tuebingen, Department of Dermatology, Germany

P14 *Influence of Bacillus subtilis C3102 on microbiota in a dynamic model of the gastrointestinal tract simulating human conditions*
Misaki Hatanaka[1], Y. Nakamura[1], K. Venema[2], A.J.H. Maathuis[2], I. Murota[1] and N. Yamamoto[1]
[1]Calpis Co. Ltd.,, Functional Food and Development Laboratory, Japan and [2]TNO Quality of Life, Department of BioSciences, the Netherlands

P15 *Effects of specific carbohydrates on the intestinal microbiota*
Lene Hemmingsen[1], J. Holck[2], A.S. Meyer[2], A. Wilcks[1] and T.R. Licht[2]
[1]DTU Food, Denmark and [2]DTU Chemical Engineering, Denmark

P16 *Evaluation of viability in faeces of recombinant Lactococcus lactis following oral and rectal administration to humans*
Karolien Van Huynegem, A. van der Aa, K. Vandenbroucke, B. Coulie and L. Steidler
ActoGeniX NV, Belgium

P17 *Effect of Lactobacillus pentosus strain S-PT84 against bacterial infection in mice*
Takayuki Izumo, M. Ida, T. Maekawa, Y. Kitagawa and Y. Kiso
Suntory Wellness Limited, Institute for Health Care Science, Japan

P18 *PCR-SSCP universal primers for detection of asthma protective fungi and yeasts in farm children's environment*
Tobias Janke[1], M. Ege[2] E. von Mutius[2], M. Mayer[1] and J. Bauer[1]
[1]Technische Universität München, Institute of Animal Hygiene, Germany and [2]University of Munich, Children's Hospital, Germany

P19 *Diversity of potential probiotic lactic acid bacteria*
Hanne Jensen[1,2], L. Axelsson[1], K. Naterstad[1] and S. Grimmer[1]
[1]Nofima Mat AS, Norway and [2]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Norway

P20 *Expression of DNA damage inducible genes in Escherichia coli at the single cell and population levels*
Simona Kamenšek[1], Z. Podlesek[1], O. Gillor[2] and D. Žgur-Bertok[1]
[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia and [2]Ben-Gurion University, J. Blaustein Institutes for Desert Research, Zuckerberg Institute for Water Research, Israel

P21 *Evaluation of gut environmental dynamics based on gene expression profiling*
Tamotsu Kato[1,2,4], S. Fukuda[2], Y. Date[2,3],E. Chikayama[5], Y. Nakanishi[1,2,5], Y. Tsuboi[4,5], S. Tsuneda[3], S. Moriya[1,4], J. Kikuchi[1,5] and H. Ohno[1,2]
[1]Yokohama City University, Graduate School of Nanobioscience, Department of Supramolecular Biology, Japan, [2]RIKEN Research Center for Allergy and Immunology, Japan, [3]Waseda University, Department of Life Science and Medical BioScience, Japan, [4]RIKEN Advanced Science Institute, Japan and [5]RIKEN Plant Science Center, Japan

P22 *Detoxification of 2-amino-1-methylo-6-phenylo-1H-imidazo[4,5-b] pyridine (PhIP) by lactofementation beetroot juice in vivo in a rats model*
Elżbieta Klewicka[1], A. Nowak[1], J. Juśkiewicz[2] and Z. Zduńczyk[2]
[1]Technical University of Lodz, Institute of Fermentation Technology and Microbiology, Poland and [2]PAS, Institute of Animal Reproduction and Food Research, Division of Food Science, Poland

P23 *Use of a dynamic in vitro system to assess the ability of probiotic bacteria to survive the upper GI tract*
Marjorie Koenen, K. Venema and A. Maathuis
TNO Quality of Life, the Netherlands

P24 *Influence of D-lactate producing probiotic strains on D-lactate level in the colon*
Marjorie Koenen, K. Venema and A. Maathuis
TNO Quality of Life, Zeist, the Netherlands

P25 *Novel fluorescence-based method for real-time monitoring of viable microorganisms*
Remco Kort, A. Nocker, A. de Kat Angelino-Bart, F. Schuren and R. Montijn
TNO Quality of Life, Zeist, Microbial Genomics Group, the Netherlands

P26 *Immunomodulating effects of probiotics in the healthy and inflamed gastrointestinal tract*
Bas H.A. Kremer, R. Mariman, N. Worms, T. Lagerweij, S. Mourits, M. van Erk and L. Nagelkerken
TNO Biosciences, the Netherlands

P27 *Reduction of Enterococcus faecium in the TIM-1 dynamic gastro-intestinal model by Lactobacillus sakei subsp. sakei 2a, and evaluation of the sakacin activity*
Monika F. Kruger[1,2,5], S. van Rijn[2], K. Venema[2], R.C.R. Martinez[2,3,5], E.C.P. De Martinis[3], S.M.I. Saad[4], B.D.G.M. Franco[1], H. Smidt[5] and E.G. Zoetendal[5]
[1]Universidade de São Paulo, Departamento de Alimentos e Nutrição Experimental, Brazil, [2]TNO Quality of Life, the Netherlands, [3]Universidade de São Paulo, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Brazil, [4]Universidade de São Paulo, Departamento de Tecnologia Bioquímico-Farmacêutica, Brazil and [5]Wageningen University, Laboratory of Microbiology, the Netherlands

P28 *Nutritional intervention with NR100157 restores gut microbiota in HIV-1 infected adults not on HAART, by stimulating beneficial commensals*
A. Gori[1], K. Ben Amor[2], A. van Hees[2], **Belinda van't Land**[2,3], K. van Norren[2], N. Georgiou[2,3], D. Bray[4], G. Welling[5], C. Richter[6], P. Koopmans[7], J. Garssen[2,3], J. Knol[2], M. Clerici[8] and the COPA-COMBAT study teams
[1]University of Milano-Bicocca, 'San Gerardo' Hospital, Division of Infectious Diseases, Italy, [2]Danone Research - Centre for Specialised Nutrition, Nutricia Advanced Medical Nutrition, the Netherlands, [3]Utrecht Institute for Pharmaceutical Sciences (UIPS), the Netherlands, [4]ImmunoClin, France, [5]University Medical Center Groningen, Department of Medical Microbiology, the Netherlands, [6]Rijnstate Hospital, the Netherlands, [7]UMC St. Radboud, the Netherlands and [8]University of Milan, Italy

P29 *Interspecies cell-to-cell communication between probiotic Lactobacillus and Staphylococcus aureus inhibits production of the staphylococcal superantigen toxic shock syndrome toxin-1*
Jingru Li
University of Western Ontario, Department of Microbiology and Immunology, Canada

P30 *Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study*
Raakel Luoto, K. Laitinen, M. Nermes and E. Isolauri
Turku University Hospital and University of Turku, Finland

P31 *Digestibility and prebiotic potential of non-digestible carbohydrate fractions from novel maize-based fibres in a dynamic in vitro model of the human intestine*
Annet Maathuis[1], A. Hoffman[2], A. Evans[2], L. Sanders[2] and K. Venema[1]
[1]TNO Quality of Life, the Netherlands and [2]Tate & Lyle, USA

P32 *At the crossroads of vaginal health and disease, the genome sequence of Lactobacillus iners AB-1*
Jean M. Macklaim[1,2], G. B. Gloor[2], K.C. Anukam[1], S. Cribby[1] and G. Reid[1,3]
[1]Canadian Research & Development Centre for Probiotics, Lawson Health Research Institute, Canada, [2]The University of Western Ontario, Department of Biochemistry, Canada and [3]The University of Western Ontario, Departments of Microbiology and Immunology and Surgery, Canada

P33 *Fructansucrase encoding gene from Weissella confusa MBF-CNC2(1) isolated from Indonesian Cincau reveals high similarity to inulosucrase from Lactobacillus reuteri*
Amarila Malik[1], S. Ishikawa[2] and N. Ogasawara[2]
[1]Universitas Indonesia, Faculty of Mathematics and Natural Sciences, Department of Pharmacy, Laboratory of Microbiology and Biotechnology, Indonesia and [2]Institute of Science and Technology (NAIST), Graduate School of Biological Sciences, Division of Bioinformatics and Genomics, Japan

P34 *Effect of galacto-oligossaccharide, and milk on the survival of Lactobacillus sobrius 16698 in a computer-controlled model of the stomach and the small intestine*
Rafael C.R. Martinez[1,2], A.-E. Aynaou[3], E.C. Pereira De Martinis[1], E. Zoetendal[2], K. Venema[3], S.M.I. Saad[4] and H. Smidt[2]
[1]University of São Paulo, Faculty of Pharmaceutical Sciences of Ribeirão Preto, Department of Clinical, Toxicological and Bromatological Analysis, Brazil, [2]Wageningen University, Laboratory of Microbiology, the Netherlands, [3]TNO Quality of Life, the Netherlands and [4]University of São Paulo, Faculty of harmaceutical Sciences, Department of Biochemical-Pharmaceutical Technology, Brazil

P35 *Arabinoxylans and metabolic homeostasis: effect on prebiotic properties, glycemic control, weight management and immune modulation*
Massimo Marzorati[1], P. Van den Abbeele[1], A. Neyrinck[2], P. Cani[2], N. Delzenne[2] and W. Verstraete[1], S. Possemiers[1]
[1]Ghent University, Laboratory of Microbial Ecology and Technology (LabMET), Belgium and [2]Université Catholique de Louvain, Unit PMNT, Belgium

P36 *Molecular-epidemiological screening on allergy- and asthma- preventive bacteria in farming environment*
Melanie Mayer[1], M.J. Ege[2], K. Schwaiger[1], E. von Mutius[2] and J. Bauer[1]
[1]Technische Universität München, Chair of Animal Hygiene, Germany and [2]University of Munich, Dr. von Hauner Children's Hospital, Germany

P37 *Characterization of potential allergy- and asthma- prophylactive or probiotic bacteria isolated from farming environment*
Melanie Mayer, K. Schwaiger, C. Hölzel and J. Bauer
Technische Universität München, Chair of Animal Hygiene, Germany

P38 *The effect of a six-week exercise and weight control intervention on the intestinal microbiota of the overweight/obese Finnish women*
Eveliina Munukka[1], A. Lyytikäinen[1], E. Völgyi[1], L. Xu[1], P. Wiklund[1], J. Vaahtovuo[2,3] and S. Cheng[1]
[1]University of Jyväskylä, Department of Health Sciences, Finland, [2]CyFlo Ltd., Finland and [3]Paimion terveyskeskus, Finland

P39 *Does kefir intake interact with the pig microbial gut? Preliminary study on pig faeces*
Véronique Ninane, J. Wavreille, V. Planchon and G. Berben
Walloon Agricultural Research Centre (CRA-W), Belgium

P40 *Cyto- and genotoxicity of faecal water after incubation of 2-amino-3-methyl-3H-imidazo[4.5-ƒ]quinoline (IQ) with faecal microorganisms and Lactobacillus casei DN 114 001, with usage of Caco-2 cell line*
Adriana Nowak[1], S. Malgorzata[2] and Z. Libudzisz[1]
[1]Technical University of Lodz, Institute of Fermentation Technology and Microbiology, Poland and [2]University of Lodz, Department of Molecular Genetics, Poland

P41 *Automated extraction of microbial DNA from faeces for 16S rRNA microarray analysis*
Lotta Nylund[1,2], H.G.H.J. Heilig[3], S. Salminen[1], W.M. de Vos[2, 3] and R. Satokari[1]
[1]University of Turku, Functional Foods Forum, Finland, [2]University of Helsinki, Division of Microbiology and Epidemiology, Department of Basic Veterinary Sciences, Finland and [3]Wageningen University, Laboratory of Microbiology, the Netherlands

P42 *Food, fibre and satiety – how does fibre make you feel full?*
Shanthi Parkar[1], T. Trower[1], D. Stevenson[1], D. Sun-Waterhouse[2], J. Monro[3] and M. Skinner[2]
[1]The New Zealand Institute for Plant & Food Research Limited, Ruakura Research Centre, New Zealand, [2]The New Zealand Institute for Plant & Food Research Limited, Mount Albert Research Centre, New Zealand and [3]The New Zealand Institute for Plant & Food Research Limited, New Zealand

P43 *Colicinogenic Escherichia coli isolates from healthy individuals exhibit a similar extra-intestinal virulence profile as non-colicinogenic strains*
Živa Petkovšek[1], M. Čitar[1], D. Žgur Bertok[1], M. Starčič Erjavec[1]
[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia;

P44 *Microbial Toll-signalling at the intestinal epithelial surface stimulates apical secretion of CXCL8 and autocrine signalling in villus epithelial cells*
Oriana Rossi[1], J. Karczewski[1], M. Meijerink[1], P. van Baarlen[1], J. van Neerven[1], S. van IJzendoorn[2] and J. Wells[1]
[1]University of Wageningen, ASG, Host-Microbe Interactomics Group, the Netherlands and [2]University of Groningen, Faculty Medical Sciences, Cell Biology Group, the Netherlands

P45 *Modulation of the gut derived microbiota by dietary fibres in an in vitro fermentation system*
Ida Rud[1], I.M. Aasen[2], B. Moen[1] and S.H. Knutsen[1]
[1]Nofima Mat, Norwegian Institute of Food, Fisheries and Aquaculture Research, Norway and [2]SINTEF Materials and Chemistry, Department of Biotechnology, Norway

P46 *Bifidobacterium animalis Bb-12 survival in probiotic and synbiotic margarine and its resistance*
under in vitro simulated gastro-intestinal conditions
C.H.B. de Souza, R.C.S. Estrotra, L.A..Gioielli and **Susana M.I. Saad**
University of São Paulo, Faculty of Pharmaceutical Sciences, Department of Biochemical-Pharmaceutical Technology, Brazil

P47 *Viability of probiotic strains in a non-dairy rice-based synbiotic dessert*
D.M. Kakinoki and **Susana M.I. Saad**
University of São Paulo, Faculty of Pharmaceutical Sciences, Department of Biochemical-Pharmaceutical Technology, Brazil

P48 *Probiotic Lactococcus lactis (NCC2287) reduces allergic symptoms in sensitized mice*
A. Zuercher, S. Holvoet, M. Weiss, **Anurag Singh**, S. Nutten, G. Prioult and A. Mercenier
Nestlé Research Center, Nutrition and Health Department, Allergy Group, Switzerland

P49 *The human ileal microbiota and its function: development of a dynamic in vitro model for the human ileum*
Maria Stolaki[1,2,3], E.G. Zoetendal[1,2], E.J. Smid[1,4], M. Minekus[1,3], K. Venema[1,3] and M. Kleerebezem[1,2]
[1]Top Institute Food and Nutrition, the Netherlands, [2]Wageningen University, Laboratory of Microbiology, the Netherlands, [3]TNO Quality of Life, the Netherlands and [4]NIZO food research, the Netherlands

P50 *Resident microbiota and Streptococcus thermophilus modulate the amount of cell-cycle related proteins of colonic epithelium*
C. Cherbuy, F. Rul, L. Wzrosek, L. Ben-Yahia, N. Bouznad, E. Honvo-Houeto, M.L. Noordine, C. Mayeur, P. Langella and **Muriel Thomas**
INRA, Micalis UMR 1319, France

P51 *The use of stable isotope labelled substrates to study fermentation in the gut*
Koen Venema, A. Maathuis and A.A. de Graaf
TNO Quality of Life, Department of BioSciences, the Netherlands

P52 *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*
Koen Venema[1], A. Maathuis[1], I. Giesbrecht[2], P. Bohnhorst[2] and A. Christ[2]
[1]TNO Quality of Life, Department of BioSciences, the Netherlands and [2]Merck Selbstmedikation GmbH, Department Research & Development, Germany

P53 *Faecal bacterial communities in healthy controls and ulcerative colitis patients*
Louise K. Vigsnæs[1], A. Wilcks[1], J. Brynskov[2] and T.R. Licht[1]
[1]DTU Food, Gut Ecology, Denmark and [2]Herlev University Hospital, Department of Medical Gastroenterology, Denmark

P54 *Lactobacillus plantarum enhances human intestinal barrier function via a TLR2 and PKCδ-mediated mechanism*
J. Karczewski[1,2], P. van Baarlen[1,2], J. Dekker[2] and **Jerry Wells**[1,2]
[1]University of Wageningen, Host-Microbe Interactomics, the Netherlands and [2]Top Institute Food and Nutrition, the Netherlands

P55 *Dietary non-digestible carbohydrates induce CD25$^+$ regulatory T-cells that protect mice from developing casein allergy*
B. Schouten[1], B.C.A.M. van Esch[1,2], G.A. Hofman[1], S. de Kivit[1], L. Boon[3], L.M.J. Knippels[3], J. Garssen[1,2] and **Linette E.M.Willemsen**[1]
[1]Utrecht University, Utrecht Institute for Pharmaceutical Sciences, Pharmacology and Pathophysiology, the Netherlands, [2]Danone Research, Centre for Specialised Nutrition, the Netherlands and [3]Bioceros, the Netherlands

P56 *Cow's milk allergy symptoms are reduced in mice fed dietary synbiotics during oral sensitization with whey*
B. Schouten[1], B.C.A.M. van Esch[1,2], G.A. Hofman[1], S.A.C.M. van Doorn[2], J. Knol[2], A.J. Nauta[2], J. Garssen[1,2], **Linette E.M.Willemsen**[1] and L.M.J. Knippels[1,2]
[1]Utrecht University, Utrecht Institute for Pharmaceutical Sciences, Pharmacology and Pathophysiology, the Netherlands and [2]Danone Research - Centre for Specialised Nutrition, the Netherlands

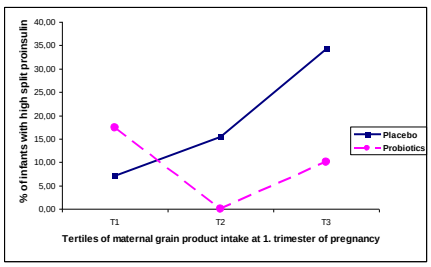
P57 *Lipoteichoic acid from Gram-positive bacteria shows positivity in the Limulus amebocyte lysate assay and binds to polymyxin B*
Zdenek Zídek and E. Kmoníčková
Academy of Sciences, Institute of Experimental Medicine, Czech Republic

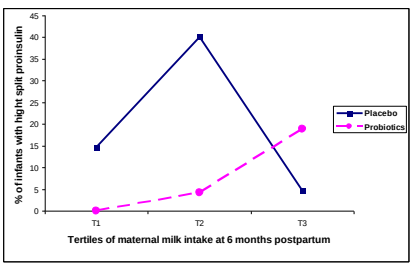
P1

Impact of maternal diet during pregnancy and breastfeeding on infant metabolic programming: a prospective randomized controlled study

Jonna Aaltonen, T. Ojala, K. Laitinen and E. Isolauri
University of Turku, Turku University Central Hospital, Department of Pediatrics, Finland;
jmaalt@utu.fi

The Western societies are faced with an epidemic of metabolic disorders, and the velocity of propagation is particularly outstanding in the paediatric population. The gut microbiota composition is linked not only to microbiological programming but also to the immunological and metabolic development of the infant. Evidence that metabolic diseases are programmed by early nutrition points to the foetal and early postnatal life as critical periods and intervention targets. To evaluate the impact of maternal dietary intake with and without probiotics during pregnancy and breastfeeding on infant's metabolic programming, specifically on glucose metabolism measured by high serum 32-33 split proinsulin at six months of age. At the first trimester of pregnancy 256 women were randomized into a control group (control/placebo) and two intensive dietary intervention groups with double-blind randomization to probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis*) or placebo (diet/probiotics and diet/placebo). The pregnant women in the control group received dietary counselling in well-women clinics, while the intervention groups received in addition detailed dietary counselling by nutritionist targeting excessive saturated fat and low fibre consumption. Maternal diet was evaluated repeatedly during pregnancy and postpartum by means of three days' food diaries. Serum 32-33 split proinsulin and intact proinsulin concentrations were measured from 196 infants at 6 months of age as markers of glucose-insulin metabolism. The 32-33 split proinsulin concentrations above the 85th percentile cut-off point were taken to depict unfavourable metabolic programming in these healthy infants. The group comparisons in dichotomized 32-33 split proinsulin concentrations were assessed by univariate logistic regression analysis and the associations between dietary intakes, evaluated in tertiles (T1=lowest, T2=middle and T3=highest intake), and high split proinsulin concentration by Chi-squared test. The effect of probiotic intervention on the association between dietary components and infant's high split proinsulin was analyzed by the method of Mantel-Haenszel and the Beslow-Day test. Maternal dietary intervention, with or without probiotics, reduced the infants' risk of high 32-33 split proinsulin concentrations compared with that in controls, OR 0.30 (95% CI 0.13 to 0.66), p=0.003. The independent effect remained in multivariable analysis. The consumption of most dietary factors was non-linearly associated with the infants' risk of high split proinsulin concentration. The probiotic intake contributed to the pattern of association between maternal grain product and milk intake and infants' high split proinsulin (p=0.017 and 0.002, respectively) as shown in the figures. Specifically, the probiotics reduced the detrimental effect of maternal high grain product intake at 1st trimester of pregnancy and middle milk consumption six months post partum. Our results indicate that favourable programming of infants' glucose metabolism can be achieved by balanced nutrient intake during pregnancy, and the concomitant probiotic intake of the mother may enhance the association between maternal dietary intake and infants' high 32-33 split proinsulin.





P2

Regulation of interferon-induced protein genes by a multi-strain commercial probiotic

Julie Audy, O. Mathieu and T.A. Tompkins
Institut Rosell Inc., Canada; jaudy@lallemand.com

The objective of the study was to evaluate the immune response of human intestinal epithelial cells (IEC) to a live or heat-killed multi-strain probiotic product. Previous studies have shown live and dead probiotics can modulate immune associated genes. IEC (HT-29) were exposed 3 h to ProbioKid (PK) which is an industrially-prepared blend of strains (*Lactobacillus helveticus* R0052, *Bifidobacterium infantis* R0033 and *B. bifidum* R0071, 60:20:20) and to heat-inactivated PK (HK-PK). Gene modulation was evaluated with an Immune Array, custom-designed with 1354 oligos (70-mers) covering pathways related to innate and adaptive immunity. Assessment of slide quality, normalization and statistical analysis were conducted with the Limma Package from BioConductor in R software on at least four biological replicates. IEC responded differently to PK and HK-PK; 29 and 25 unique genes were respectively modulated. More specifically, only PK induced STAT-1 gene involved in IFN-γ–Jak1,2–STAT-1 signalling transduction cascade, an essential innate immune response required to combat microbial infection. Previous findings had shown the ability of R0052 to maintain IFN-γ–Jak1,2–STAT-1 activation following *Escherichia coli* O157:H7 infection. Further studies are need, but two potential activators for the pathway were identified: 1. PK induced interferon cascades in HT-29 cells, at least 13 interferon induced protein genes were up-regulated; and 2. PK up-regulated the LIF gene a member of IL-6 cytokine family. We concluded that viable bacteria in the commercial probiotic product were required to induce STAT-1 gene expression.

P3

ınfluence of the intrinsic gut microbiota on transcriptional regulation of genes involved in the early life development of intestinal epithelial integrity

Anders Bergström[1], M.B. Kristensen[1], H. Frøkjær[2] and T.R. Licht[1]
[1]DTU Food, National Food Institute, Division of Microbiology and Risk Assessment, Denmark and [2]University of Copenhagen, Faculty of Life Sciences, Department of Basic Sciences and Environment/Biochemistry and Natural Products Chemistry, Denmark; adbe@food.dtu.dk

The interplay between the gut microbiota and the integrity of the intestinal mucus layer is important both in the maintenance of the epithelial barrier as part of the innate immune defense, and in the conservation of gut homeostasis. Interesting parameters are the mucins, which protect the mucosal surfaces of all epithelial linings by physical or specific hindrance of pathogenic species e.g. virus and bacteria. Moreover, the proteins constituting the tight junctions in the apical membrane of the epithelial cells are important as they take part in controlling, which substances can penetrate the barrier from the gut lumen to the blood circulation. Previously, it has been shown that the early life mucus layer in germ-free mice has a distinctly different composition than in conventionally colonized animals. In this study, four groups of differently colonized mice were used to analyze mRNA expression by real-time quantitative PCR of relevant mucin (Muc1-4) and tight junction genes (JAM-A, E-Cad, Tjp-1) on RNA purified from isolated ileum samples (n=8 in each group). The groups were: (i) germ free (GF);(ii) specific pathogen free (SPF), i.e. 'conventional microbiota'; (iii) NCFM (GF monocolonized with *Lactobacillus* NCFM); and (iv) *Escherichia coli* (GF monocolonized with *E. coli*). Ileal samples were taken on day 1 and day 6 after birth in order to analyze early life developmental parameters. On the day 6 samples, mucin-related mRNAs showed significantly higher expression levels in the GF animals compared to the SPF animals, possibly as part of protective mechanism. Monocolonization with *Lactobacillus* NCFM and *E. coli* seemed to decrease levels towards levels observed in the SPF animals (except for Muc-3 in E*. coli*). Two of the tight junction genes (JAM-A, E-Cad) showed similar tendencies, whereas Tjp-1 showed high levels in both GF and SPF. Comelli et al. (2008) have shown very similar results on the mucin genes, when colonizing with human adult or baby 'full' microbiota. This is the first study with monocolonization, however. Finally, we observed inverse correlation between Muc-1 and *Lactobacillus* 16S rRNA expression. The analysis of the day 1 samples is ongoing and results will be presented at the meeting.

P4

Monitoring of metabolic dynamics in microbial ecosystems using a combination of DNA fingerprinting and metabolome analysis based on stable isotope labelling

Yasuhiro Date[1,2,3], Y. Nakanishi[2,3,4], S. Fukuda[3,4], T. Kato[3,4], S. Tsuneda[1], H. Ohno[3,4] and J. Kikuchi[2,3,5]
[1]Waseda University, Department of Life Science and Medical Bioscience, Japan, [2]RIKEN Plant Science Center, Japan, [3]RIKEN Research Center for Allergy and Immunology, Japan, [4]Yokohama City University, Graduate School of Nanobioscience, Japan and [5]Nagoya University, Graduate School of Bioagricultural Sciences, Japan;
ceryle-lugubris@suou.waseda.jp

Microorganisms inhabit in various environments such as soils, oceans and symbiotic ecosystems, and are responsible for driving the biogeochemical cycling of elements on earth. In addition, the animal gastrointestinal tract, including that of humans, is colonized by a multitude of bacteria that play roles in the maintenance of health or conversely in the pathogenesis of infection due to crosstalk between commensal and pathogenic bacteria. Hence, the bacterial crosstalk in the gut environment impacts the host health condition. Despite their importance and diversity, the vast majority of bacteria continue to resist cultivation in the laboratory, and even when cultivation can be achieved, the traits expressed by a particular bacteria in culture may not be equivalent to those expressed when the organism is present in its natural environment. Therefore, one of the biggest challenges that microbial ecologists face is to identify which microorganisms are carrying out a specific set of metabolic processes in the natural environment. In this study, we have developed a new approach for monitoring the metabolic dynamics in microbial ecosystems using a combination of DNA fingerprinting and metabolome analysis based on stable isotope labelling. For the development and validation of our monitoring approach, faecal microbiota were analyzed with stable-isotope-labelled glucose used as the sole carbon source. In order to link the metabolic information and the microbial variability, we performed metabolic-microbial correlation analysis based on nuclear magnetic resonance (NMR) profiles and denaturing gradient gel electrophoresis (DGGE) fingerprints, which successfully identified the glucose-utilizing bacteria and their related extracellular metabolites. Moreover, our approach revealed information regarding the carbon flux, in that the ‘first’ wave of extracellular metabolites secreted by the glucose-utilizing bacteria were incorporated into the ‘secondary’ group of substrate-utilizing bacteria, and that this ‘secondary’ group further produced their own secondary metabolized substrates. Thus, this approach is a powerful tool for monitoring the metabolic dynamics in microbial ecosystems and allows for the tracking of the carbon flux within a microbial community or within food webs. By applying our approach to other organic substrates such as oligosaccharides, amino acids, and aromatic compounds, we will make it possible to unlock the secrets of complex metabolic dynamics starting from the incorporation of the substrates into microbial communities in a variety of microbial ecosystems.

P5

Comparative study of predominant faecal microbiota of a group of cystic fibrosis children and healthy siblings

Gwen Duytschaever[1], G. Huys[1], L. Boulanger[2], K. De Boeck[2] and P. Vandamme[1]
[1]Ghent University, Laboratory of Microbiology, Department of Biochemistry and Microbiology, Belgium and [2]University Hospital of Leuven, Department of Paediatrics, Belgium;
gwen.duytschaever@ugent.be

The human gastrointestinal tract harbours a highly dynamic and complex microbial community which plays a key role in health and disease. Previous population fingerprinting research has indicated that the healthy microbiome is host-specific and temporally stable. However, external factors such as antibiotic treatment may lead to dysbiosis and subsequently contribute to several disorders. Cystic fibrosis (CF) patients are frequently treated with multiple antimicrobial agents to conquer lung infections. Although these antibiotic therapies are necessary to prevent further lung function decline, they may also have some side effects such as triggering a state of dysbiosis in the gut. An improved knowledge of the bacterial diversity and the population dynamics of the CF-microbiome could enhance the development of alternative or supplementary therapies based on pro- and/or prebiotics. This study aims to compare the predominant faecal microbiota in a group of CF infants with these of healthy siblings through a combination of culture-dependent and culture-independent techniques. One general medium for colon bacteria and six selective media were selected to enumerate the predominant members of the faecal microbiota. Denaturing Gradient Gel Electrophoresis (DGGE) of 16S rDNA amplicons was applied to evaluate the community structure and to monitor the population dynamics. Paired t-test was performed using SPSS software version 17.0. In total, 21 families each consisting of one CF-patient and one to two healthy siblings (age ranging from 10 months to 15 years old) were investigated. When comparing one faecal sample per subject, it was found that the counts on the general medium and the counts of lactic acid bacteria, bifidobacteria, *Veillonella* sp., *Bacteroides*/*Prevotella* sp. and clostridia were consistently higher (ranging from 0.035 to 3.36 log cfu/g difference) for sibling samples compared to CF samples whereas the opposite was found for enterobacterial counts. None of these trends were statistically significant (95% CI). Between the two subject groups, the average number of bands in the DGGE fingerprints of faecal samples was comparable (CF: 9.71 and sibling: 10). However, fingerprints obtained from cultured fractions were less complex than those from total DNA extracts and both types of profiles contained common as well as unique bands within a given sample. Profile clustering analysis of 8 samples per subject collected over a two-year period indicated a generally less stable predominant faecal microbiota in CF-patients compared to the healthy siblings.

P6

Antimicrobial peptides produced by *Lactobacillus sakei* 2a: cloning and heterologous expression

K.G. De Carvalho[1], M.F. Kruger[1], M. De Souza Barbosa[1], E. Salvucci[2], F. Sesma[2] and **Bernadette D.G.M. Franco[1]**
[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental, Laboratório de Microbiologia de Alimentos, Brazil and [2]Centro de Referencia para Lactobacilos, Argentina; bfranco@usp.br

Strain *Lactobacillus sakei* 2a, isolated from a Brazilian pork product, is capable of producing several antimicrobial peptides, active against *Listeria monocytogenes in vitro, in situ* and *in vivo*. Activity of *L. sakei* 2a in foods as biopreservative is limited, so the use of partially purified peptides as food additives may result in better inhibition. Three peptides are constantly produced: one is the classical sakacin P, the second is identical to the 30S ribosomal protein S21 of *L. sakei* subsp. *sakei* 23K, and the third is identical to a histone-like DNA-binding protein HV produced by *L. sakei* subsp. *sakei* 23K. Their molecular masses are 4.4, 6.8 and 9.5 kDa, respectively. Purification of these peptides is cumbersome, and cloning of the genes responsible for their production and heterologous expression in *Escherichia coli* may be an alternative for enhanced production of these peptides. In this study, total genomic DNA was extracted and used as target DNA for PCR amplification of the genes *sak*, *lis* and *his* involved in the synthesis of the three antimicrobial peptides. The fragments were cloned in pET28b expression vector and the resulting plasmids transformed in *E. coli* KRX competent cells. Supernatants of cultures of these cells obtained after their rupture were active against *L. monocytogenes* Scott A. The molecular masses of the cloned peptides were confirmed by mass spectrometry. Despite preliminary, these results indicate that the activity of the classical sakacin P produced by *L. sakei* 2a can be complemented by other antimicrobial peptides, and their enhanced production can be successfully achieved by cloning and heterologous expression in transformed *E. coli*.

Acknowledgements
FAPESP and CNPq.

P7

Potential beneficial properties of bacteriocin-producing lactic acid bacteria isolated from smoked salmon

S.D. Todorov[1], D.N. Furtado[1], E. Chiarini[1], S.M.I. Saad[2], E. Tome[3] and **Bernadette D.G.M. Franco[1]**
[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental, Brazil, [2]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Tecnologia Bioquímico-Farmacêutica, Brazil and [3]Universidad Central de Venezuela, Escuela de Biología, Instituto de Ciencia y Tecnología de Alimentos, Venezuela; bfranco@usp.br

Lactic acid bacteria (LAB) tested in this study (*Lactobacillus curvatus* ET06, ET30 and ET31, *L. fermentum* ET35, *L. delbrueckii* ET32, *Pediococcus acidilactici* ET34 and *Enterococcus faecium* ET05, ET12 and ET88) survived conditions simulating the GI-tract and produced bacteriocins active against a number of *L. monocytogenes* strains and presented a very low activity against some other LAB. All of cell free supernatant containing bacteriocins ET05, ET06, ET12, ET30, ET31, ET32, ET34, ET35 and ET88, added to 3 hours old cultures of *L. monocytogenes* 603 serotype 1/2b (OD_{600nm} ≈ 0.11), suppressed cell growth over 12 h. Auto-aggregation showed to be strain-specific, and values ranged from 7.2% for strain *L. fermentum* ET35 to 12.1% for *E. faecium* ET05. Various degrees of co-aggregation were observed with *L. monocytogenes* ScottA, *L. sakei* ATCC15521 and *E. faecium* ATCC19443. Adherence of the bacteriocinogenig LAB to Caco-2 cells was within the range reported for *L. rhamnosus* GG, a well-known probiotic. Hydrophobicity values of 12.6%-14.7% were recorded for strains *E. faecium* ET05, ET12 and ET88. The highest levels of hydrophobicity were recorded for *L. curvatus* (61.9%-64.6%), *L. fermentum* (78.9%), *L. delbrueckii* (43.7%) and *P. acidilactici* (51.3%). These values were higher than the one recorded for *L. rhamnosus* GG (53.3%) indicating good potential probiotic properties. According to susceptibility profile these strains are highly sensitive to several antibiotics. In addition, the effect of several drugs from different generic group was tested in order to determine if these potential probiotics strains may be applied in combination with tested medical products. This effect of tested drugs was strain dependent. Minimal inhibition concentration for the drugs inhibiting the growth of tested LAB was determined. Although these properties are all characteristic of good probiotics, in-depth *in vivo* studies will have to be performed to determine the performance in the human GI-tract.

Acknowledgements
FAPESP, CAPES and CNPq.

P8

Anti-listerial properties of a bacteriocin produced by *Lactobacillus plantarum* ST8SH, a strain isolated from Bulgarian salami

S.D. Todorov[1], **Bernadette D.G.M. Franco[1]** and M. Vaz-Velho[2]
[1]Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Alimentos e Nutrição Experimental, Laboratório de Microbiologia de Alimentos, Brazil and [2]Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Portugal; bfranco@usp.br

Strain ST8SH, isolated from Bulgarian salami, produces a 3.0kDa class IIa bacteriocin, active against *Streptococcus caprinus*, *Streptococcus* spp., *Listeria monocytogenes*, *L. innocua*, *Lactobacillus casei*, *L. curvatus*, *L. salivarius, L. pentosus*, *L. lactis* subsp. *lactis*, *Enterococcus mundtii* and *E. faecalis*. Strain ST8SH was identified as *Lactobacillus plantarum* based on biochemical tests, sugar fermentation reactions (API50CHL), PCR with species-specific primers and 16S rDNA sequencing. The peptide is inactivated by proteolytic enzymes, but resistant to α-amylase, SDS, Triton X-100, Triton X-114, SDS, Tween-20, Tween-80, urea and EDTA. No change in activity was recorded after 2 h at pH values between 2.0 and 12.0, and after treatment at 100°C for 120 min or 121^{o}C for 20 min. The mode of activity against *L. casei*, *L. sakei, L. innocua, L. monocytogenes* and *E. faecalis* is bactericidal, resulting in cell lyses and enzyme- and DNA-leakage, confirmed by atomic force microscopy. The highest level of activity (25600 AU/ml) was recorded when cells were grown at 37^{o}C or 30^{o}C in MRS broth at pH 6.5. Peptide ST8SH adsorbs at low levels (400 AU/ml) to the producer cells. High cell numbers of *L. plantarum* and *L. innocua* were recorded at beginning when co-cultured. However, the cell numbers of *L. innocua* decreased from $1.6x10^4$ cfu/ml to $2.5x10^2$ cfu/ml in 12 h and to undetectable levels in 24 h. Bacteriocin ST8SH production was stimulated by the presence of *L. innocua* (102400 AU/ml). Similar results were obtained with addition of 10% autoclaved overnight culture of *L. innocua* to MRS growth media. Based on the genetic approach, strain ST8SH harbours associated genetic determinants for production of a variation of the well known plantaricin 423. A future purification of the produced bacteriocin are required to determined if *L. plantarum* ST8SH produces a plantaricin 423 like bacteriocin or harbours more than one bacteriocin operon.

Acknowledgements
CAPES and CNPq.

P9

Changes in the gut intestinal microflora influence behaviour in mice

Mélanie G. Gareau[1], E. Wine[1], M.T. Whary[2], G. MacQueen[3] and P.M. Sherman[1]
[1]University of Toronto, Hospital for Sick Children, Research Institute, Canada,
[2]Massachusetts Institute of Technology, Division of Comparative Medicine, USA and
[3]University of Calgary, Department of Psychiatry, Canada; melanie.gareau@utoronto.ca

The brain-gut axis is a key regulator of normal intestinal physiology and psychological stress is linked to altered gut barrier function, the development of food allergies, and changes in behaviour. Whether intestinal events, such as intercurrent enteric bacterial infection and bacterial colonization, exert an effect on stress-associated behaviour has not been determined. The objective of this study was to determine the effect of acute enteric infection, probiotics, or the absence of a gut microflora on animal behaviour, including anxiety and non-spatial memory formation. The impact of psychological stress (1h water avoidance stress; WAS) on behaviour was assessed following acute infection with the non-invasive enteric pathogen, *Citrobacter rodentium*, and in germ-free mice. Mice were infected on day 1 and behaviour assessed both during acute infection (day 10) and post-infection (day 30). Whether daily treatment with probiotics (starting one week prior to infection) would normalize behaviour was then determined. Potential mechanisms through which behavioural alterations are mediated was determined by immunohistochemistry to assess levels of brain-derived neurotropic factor (BDNF) and c-Fos. No behavioural abnormalities were observed either at the height of infection (10d) or following bacterial clearance (30d) in *C. rodentium*-infected mice. When infected mice were exposed to WAS, however, memory dysfunction was apparent during acute infection and still present after clearance of the pathogen. This defect was in part mediated by reduced levels of BDNF and c-Fos in the hippocampus. Memory dysfunction was prevented by daily treatment of infected mice with probiotics, which was in part mediated by normalization of hippocampal c-Fos levels. Non-spatial memory formation was also absent in germ-free mice, with or without exposure to stress, in contrast to conventionally reared, control animals with an intact intestinal microbiota. The intestinal microflora can influence the capacity to form non-spatial memory. The results presented herein emphasize that alterations in the composition of the intestinal microbiota exert a measurable impact on certain aspects of behaviour, and may be relevant in the development of post-infectious irritable bowel syndrome or major depressive disorders.

P10

CRIB, an essential bacterium to protect us against infectious complications?

Coline Gerritsen[1,2,3], H.M. Timmerman[4], L. Mulder[3], L.M.A. Akkermans[2], G.T. Rijkers[5] and H. Smidt[1]
[1]Wageningen University, Department of Microbiology, the Netherlands, [2]University Medical Center, Department of Surgery, the Netherlands,[3]Winclove Bio Industries B.V., Research and Development Department, the Netherlands, [4]NIZO Food Research B.V., the Netherlands and [5]University Medical Center, Department of Pediatric Immunology, the Netherlands;
coline.gerritsen@winclove.nl

In the last years, the scientific as well as the public attention for probiotics is increasing and evidence for the beneficial effects of these products to the health of animals and humans is accumulation. We have demonstrated that prophylactic administration of a multispecies probiotics to rats in an experimental model of acute pancreatitis shows beneficially effects to the health status of the animals. A reduction of bacterial translocation to the pancreas, lymph nodes and spleen was seen, and clinical severity and late-phase mortality was reduced. It was demonstrated that the presence of an until then unidentified bacterium was significantly upregulated. This bacterium was named Commensal Rat Ileum Bacterium (CRIB) and we have succeeded in isolation, identification and cultivation of this bacterium. Increased presence of CRIB was positively and significantly correlated with improved pancreas pathology, reduced bacterial counts in multiple organs, and reduced plasma levels of pro-inflammatory cytokines. In the future, CRIB will be characterized and the mutualistic relationship between CRIB, probiotics and the host will be studied. These studies will demonstrate whether CRIB is an essential bacterium that can protect us against infectious complications.

P11

Treatment of infectious mastitis during lactation: antibiotics versus oral administration of lactobacilli isolated from breast milk

Arantza Gómez de Segura, R. Arroyo, V. Martín, A. Maldonado, E. Jiménez, L. Fernández and J.M. Rodríguez
Universidad Complutense de Madrid, Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Spain; arantza.gomezdesegura@gmail.com

Mastitis is a common infectious disease during lactation, involving staphylococci, streptococci and/or corynebacteria. The efficacy of oral administration of two lactobacilli strains isolated from breast milk, *Lactobacillus fermentum* CECT5716 or *L. salivarius* CECT5713, for treating staphylococcal lactational mastitis was evaluated and compared to antibiotic therapy. In this study, 352 women with infectious mastitis were randomly divided in three groups. Those in groups A (*n*=124) and B (*n*=127) daily ingested 9 $\log_{10}$ cfu of *L. fermentum* CECT5716 or *L. salivarius* CECT5713, respectively, for 3 weeks while those in group C (n=101) were submitted to antibiotherapy prescribed in their respective Primary Care Centres. On day 0, the mean bacterial counts in the samples of the three groups were similar (4.35–4.47 $\log_{10}$ cfu/ml) but lactobacilli could not be detected. On day 21, the mean bacterial counts in the probiotic groups (2.61 and 2.33 $\log_{10}$ cfu/ml) were lower than that of the control group (3.28 $\log_{10}$ cfu/ml). During the treatments, *L. fermentum* CECT5716 and *L. salivarius* CECT5713 were isolated from the milk samples of women of the probiotic groups A and B, respectively. Women ascribed to any of the probiotic groups improved more and had lower recurrences than those ascribed to the antibiotic group. *L. fermentum* CECT5716 or *L. salivarius* CECT5713 appear to be an efficient alternative to commonly prescribed antibiotics for the treatment of lactational infectious mastitis during lactation.

P12

Effect of phytobiotic blends on growth performances and digestive microbiota of broiler chickens in two rearing densities

Sarah Guardia[1], F. Recoquillay[2], H. Juin[3], M. Lessire[1], M. Leconte[1], P. Rideaud[3], C. Moreau-Vauzelle[3], C. Dupont[3], J.F. Guillot[4] and I. Gabriel[1]
[1]INRA, UR 83, URA, France, [2]Phytosynthese, France, [3]INRA, UE 1206, UEASM, France and [4]I.U.T de Tours, Microbiology laboratory, France ; sarah.guardia@tours.inra.fr

In poultry, phytobiotics are used to improve growth performances. However, the effect of these additives are variable, which may be partly explained by difference in poultry breeding conditions. Several mechanisms for growth enhancement have been proposed for phytobiotics. Due to their *in vitro* activities on bacteria, currently, digestive microbiota is thought to be the most likely target. In this study, the effects of phytobiotic blends on the performances and digestive microbiota of chicken were studied according to stocking densities (normal and high density: 12 and 17 birds/m²). Three experimental dietary treatments were performed: a control diet , a Exp1 diet with a phytobiotic blend from d22 with an *in vitro* antimicrobial effect, and a Exp2 diet with a second phytobiotic blend with antioxidant and immunomodulator properties until d10, followed by the first phytobiotic blend. Body weights were recorded until d39. Digestive microbiota was studied in digestive content (crop, ileum, caeca) at 3 and 6 weeks by a fingerprint method. Analysis of similarity (ANOSIM) between the profiles were performed on the Pearson distance matrix (differences were taken into account for $p<0.05$ and $R>0.5$). At normal density, Exp1 and Exp2 diets improved weight gain from d24 to d32 (9.9% and 11.1% respectively) and from d32 to d39 (8.1% and 10.8% respectively). At high density weight gain was improved with Exp2 diet from d24 to d32 (+8.0%). At normal density Exp1 diet led to different fingerprints of caecal microbiota at 6 weeks than control (R=0.68), and Exp2 diet led to different fingerprints of microbiota in crop (R=0.69) and in caeca (R=0.75). At high density, the effects of experimental diets compared to control diet were more pronounced. With Exp1 diet, changes were observed at 6 weeks in ileum (R=0.55) and caeca (R=0.92), and with Exp2 diet, changes were observed as soon as 3 weeks in caeca (R=0.53) and later at 6 weeks in ileum (R=1) and caeca (R=0.90). Thus the modifications of caecal microbiota as well as with Exp1 and Exp2 diets may be responsible for the beneficial effect observed on the growth performance at normal density. In the same manner, the higher performances observed with Exp2 diet at high density may be due in part to the high modifications of digestive microbiota. However, the lack of effect of Exp1 diet on growth performance at high density, whereas microbiota was also highly modified, showed that microbiota is not the one and only factor involved in the growth promoting effect of these phytobiotics.

P13

Antimicrobial activity of *Vitreoscilla filiformis* lysate: results from a randomized, double-blind, vehicle-controlled study and *in vitro* study

Audrey Guéniche[1], P. Bastien[1], Y. Mahe[1], N. Billoni[1], B. Knaudt [2], E. Piche[2], T. Volz[2], M. Röcken[2], T. Biedermann[2] and L. Breton[1]
[1]L'Oreal Recherche, France and [2]University of Tuebingen, Department of Dermatology, Germany; agueniche@rd.loreal.com

Atopic dermatitis(AD) is a chronic and relapsing inflammatory skin disease that is treated with immunosuppressants in acute phases and with emollients to stabilize the skin barrier thereafter. Novel treatments for AD focusing on establishing the physical and immunological barrier are needed. Directly applying non-pathogenic bacterial extracts to the skin of AD patients could be such a new therapeutic rationale to not only treat, but also stabilize AD skin. Therefore, we designed a study to investigate the effects of non pathogenic bacterial lysate *Vitreoscilla filiformis* strain at 5% v/v on atopic probands with mild AD. As demonstrated by the study's data, Vf is highly effective in AD significantly improving not only SCORAD but also reducing 'pruritus' and consequently 'loss of sleep'. There is a better clearance of pathogenic bacteria in the group using the verum. After finishing treatment on day 29, *Staphylococcus aureus* was reduced on skin of 29.6% (n=8) volunteers, when treated with bacterial lysate. These differences between the two groups may be related to the stimulation of antimicrobial defense proteins seen by in vitro approach using proteomic and genomic in air exposed skin (Mahe, 2007). One interpretation could be that *V. filiformis* lysates improves AD skin by immunomodulation and anti-inflammatory effects. Changes of bacterial colonization such as decrease of *S. aureus* colonization could be a direct effect of *V. filiformis* bacterial extract and an indirect effect secondary due to the anti-inflammatory properties resulting in less auto-inoculation of bacteria due to reduced scratching and changing the basic conditions for bacterial growth by restoring the skin barrier. The direct application of non-pathogenic bacteria to atopic dermatitis skin offers a totally new concept of product, effective through local innate immunomodulatory mechanisms. Further steps should include clinical studies with *V. filiformis* application to AD skin that include the study of biomarkers in the skin.

P14

Influence of *Bacillus subtilis* C3102 on microbiota in a dynamic model of the gastrointestinal tract simulating human conditions

Misaki Hatanaka[1], Y. Nakamura[1], K. Venema[2], A.J.H. Maathuis[2], I. Murota[1] and N. Yamamoto[1]
[1]Calpis Co. Ltd.,, Functional Food and Development Laboratory, Japan and [2]TNO Quality of Life, Department of BioSciences, the Netherlands; misaki.hatanaka@calpis.co.jp

Bacillus subtilis C3102 is used as probiotics for animal consumption. In this study, C3102 was evaluated for its potential use in humans using in vitro dynamic models developed by TNO. The aim was to investigate germination and survival of the C3102 strain in the stomach and small intestine (TIM-1 model) and the impact of C3102 germination on complex human microbiota in the model of large intestine (TIM-2 model). The spore formed C3102 was introduced into the TIM-1 model under fed conditions. Gastric emptying, intestinal residence time and the gastric and duodenal pH-curve mimics the situation found in humans after consuming a light meal. Every 60 minutes the intestinal content was collected from the end of the ileum compartment. The difference in the amount of cfu counted on an agar plate, with or without heating, was the germination count. The germination percentage after 6 h was 8.0% in the TIM-1 model. Total recovery of the cells was 98.8%, which meant that no bacteria were killed during passage through the TIM-1 model. In the TIM-2 model study, a standardized microbiota from human healthy adults was inoculated. After adaptation of human microbiota for 16 h, C3102 with or without TIM-1 treatment was added to the TIM-2 model at 0, 24, and 48 h. Samples at the start (0 h) and at the end of the experiment (72 h) were used to evaluate the changes of the microbiota. To determine whether C3102 had an effect on the microbiota, analytical methods using the TNO I-Chip and real time-PCR were studied. Both analytical data showed changes in some members of microbiota after feeding of the TIM-1 sample. These results suggest that *Bacillus subtilis* C3102 influences human microbiota and may contribute to human health: the germination of C3102 during passage through the gastro-intestinal tract may be relevant to its effect.

P15

Effects of specific carbohydrates on the intestinal microbiota

Lene Hemmingsen[1], J. Holck[2], A.S. Meyer[2], A. Wilcks[1] and T.R. Licht[2]
[1]DTU Food, Denmark and [2]DTU Chemical Engineering, Denmark; lehem@food.dtu.dk

The current screening study aimed at testing a set of well-characterized carbohydrates derived from pectic oligosaccharides (POS) from sugar beet for their specific effect on intestinal microbiota derived from healthy people and from patients suffering from the inflammatory bowel disease designated ulcerative colitis (UC). Two such oligosaccharides having different degrees of polymerization, in the following designated S1 and S2, respectively, were tested. Small scale anaerobic fermentation studies were performed to test the effect of S1 and S2 on the composition of the intestinal microbiota. Changes in the microbial composition were addressed by denaturing gradient gel electrophoresis (DGGE) using fructo-oligosaccharides (FOS, a golden standard prebiotic) and glucose as reference substrates. Comparison between the DGGE profiles obtained by fermentations of S1, S2 and FOS showed that S2 produced a DGGE profile different from fermentations of S1 and the control substrate FOS in a Pearson correlation cluster analysis, indicating that the degree of polymerization (DP) was decisive for which bacteria were stimulated by the oligosaccharides. Additionally, DGGE results of this screening study showed that there were no significant differences between the numbers of bands in the fermentations of all four substrates, indicating that S1, S2 and FOS had similar degrees of selectivity.

P16

Evaluation of viability in faeces of recombinant *Lactococcus lactis* following oral and rectal administration to humans

Karolien Van Huynegem, A. van der Aa, K. Vandenbroucke, B. Coulie and L. Steidler
ActoGeniX NV, Belgium; karolien.vanhuynegem@actogenix.com

ActoGeniX has recently finalized MDUC-201, a phase 2A, double blind, placebo controlled dose escalation clinical trial in mild to moderate UC patients. The active component was a formulation of live, freeze dried *Lactococcus lactis* sAGX0037, administered as enteric coated capsules and enema. The strain produces interleukin-10 from a unique, synthetic IL-10 gene embedded in the bacterial chromosome. Groups of active patients received $4.8x10^{10}$ cfu (#10, low dose), $4.8x10^{11}$ cfu (#10, mid dose) or $1.9x10^{12}$ cfu (#20, high dose) sAGX0037 daily. One of the primary endpoints of the study was the evaluation of viability, i.e. the live/ total ratio of sAGX0037 in faeces, before (d0), during (d7) and 7 days post treatment (D7PT). Total (live + dead) bacteria were quantified by QPCR on the synthetic IL-10 DNA. The method allows determination in the range of 10^{12} to 10^{5} sAGX0037 per gram of faeces. The determination of the number of live sAGX0037 is more challenging. We have evaluated molecular methods for the determination of 16s rRNA and IL-10 mRNA but found them unreliable. We determined a broad antibiogram of sAGX0037 and found that the strain, as well as its ancestor *L. lactis* MG1363, is resistant to nalidixic (N) acid, metronidazole (M) and (Tr) trimetoprim. Faecal samples spiked with sAGX0037 showed that plating on NMTr agar provides a very reliable and extremely sensitive method which can be used in the range of 10^{12} to 50 CFU per gram of faeces. Discrimination of sAGX0037 from food-derived *L. lactis* was made by the inclusion of X-Gal in the plates. Analysis of stool samples from patients enrolled in the MDUC-201 clinical study (40 active, 20 placebo) showed no traces of sAGX0037 at D0 nor in any of the placebo treated patients. Total bacteria at D7 reflect the dosage groups, while live bacteria were recovered in a very broad viability range. At D8PT total sAGX0037 could be found in 1 patient in the low dose, 2 patients in the middle dose and 10 patients in the high dose. At D7PT, only one patient's faecal sample (high dose) showed a very low number of live sAGX0037 (266 cfu/g). In conclusion we have established a reliable method for the determination in faces of live and total numbers of a recombinant *L. lactis* for clinical use. The evaluation of viability shows that, shortly after end of treatment, sAGX0037 disappears from the faeces.

P17

Effect of *Lactobacillus pentosus* strain S-PT84 against bacterial infection in mice

Takayuki Izumo, M. Ida, T. Maekawa, Y. Kitagawa and Y. Kiso
Suntory Wellness Limited, Institute for Health Care Science, Japan;
takayki_izumo@suntory.co.jp

Lactobacillus pentosus S-PT84, a plant origin lactic acid bacteria, has been found to enhance immunological functions. We have reported that *L. pentosus* S-PT84 enhances NK activity, and exhibits anti-allergic effects by modulating the Th1/Th2 balance and inducing regulatory T cells. In addition, *L. pentosus* S-PT84 stimulated Th1 cytokine production through interaction between dendritic cells and NK cells. In the present study, we elucidated the effects of *L. pentosus* S-PT84 against bacterial infection in mice. BALB/c mice were administered *L. pentosus* S-PT84 from 7 days before bacterial infection or toxin administration. In three models – (i), caecal ligation and puncture model (CLP model); (ii) *Salmonella typhimurium* infection model; and (iii) revelation model of the cholera toxin (CT) – we examined the effects of *L. pentosus* S-PT84. Experiment 1: In the CLP model, survival rate of control group was 28.6%, but the mortality rate was improved by *L. pentosus* S-PT84 intake. Dexamethasone and gentamycin had no effect on the mortality rate. Experiment 2: In the *S. typhimurium* infection model, body weight, food intake and water intake were decreased for 7 days after infection in control group. *L. pentosus* S-PT84 could improve these phenomena and survival rate. *Salmonella*-specific IgA production in *L. pentosus* S-PT84 administered group increased compared to that in control group. The numbers of *Salmonella* in faeces, liver, and spleen decreased significantly. Experiment 3: In the revelation model of the CT, CT-specific IgA production in faeces were induced by CT administration. CT-specific antibody production was significantly enhanced by *L. pentosus* S-PT84 intake. These results indicated that *L. pentosus* S-PT84 can promote the bacteria specific antibody production. It is suggested that the improvement of the mortality rate for bacterial infection (intestinal bacteria or food poisoning bacteria) was caused by specific antibody production. Therefore, *L. pentosus* S-PT84 intake may be beneficial for prevention against bacterial infection diseases.

P18

PCR-SSCP universal primers for detection of asthma protective fungi and yeasts in farm children's environment

Tobias Janke[1], M. Ege[2] E. von Mutius[2], M. Mayer[1] and J. Bauer[1]
[1]Technische Universität München, Institute of Animal Hygiene, Germany and [2]University of Munich, Children's Hospital, Germany; tobias.janke@wzw.tum.de

Several epidemiological studies have shown that growing up on a farm and consumption of unprocessed farm milk may protect from the development of atopic diseases such as asthma. Due to the close contact with animals, plants and soil during farming activities farm children are exposed to high numbers and great diversity of microorganisms which may affect the maturation of the immune system. Furthermore, it has been shown that the exposure to mould components as determined by measurement of extracellular polysaccharide and beta-1,3-glucan levels in mattress dust was inversely related to the prevalence of asthma. The objective of the study is to establish PCR-SSCP as a screening method for potentially asthma protective fungi. In this pilot study we used single fungal cultures from the institutes stocks as well as DSMZ strains. DNA was extracted by three different DNA methods. For PCR amplification six universal fungal primers for the 18S and 28S rDNA as well as ITS regions were compared. The primer pairs were assessed with respect to detection on a species level, the amplification rate of several fungi and yeast species and a consistent PCR product size. The different PCR products of the respective primers were evaluated for separation and performance in SSCP gel analysis. Furthermore, the method evaluated for fungal in DNA derived from raw milk samples. DNA extraction from fungal spores using microbead beating led to significant higher DNA yields in comparison to non-bead beating extraction. By testing 28 different fungal strains the primer pairs ITS1 / ITS4 and 0817 / 1536 were found to be most suitable for detecting fungal species, differentiation in SSCP gels, and amplification. Additionally, PCR-SSCP could be applied to the detection of fungal or yeast DNA in dairy products. This newly established method of PCR-SSCP analysis for fungi and yeasts may be useful for the identification of novel candidates with asthma protective attributes in environmental specimens and raw milk samples.

P19

Diversity of potential probiotic lactic acid bacteria

Hanne Jensen[1,2], L. Axelsson[1], K. Naterstad[1] and S. Grimmer[1]
[1]Nofima Mat AS, Norway and [2]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Norway; hanne.jensen@nofima.no

Probiotics are most commonly defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Several mechanisms by which probiotics mediate their health benefits on the host have been suggested, but the relative importance of these remains unknown. Furthermore, different strains are most likely to have different mechanisms of action and different health benefits on the host. The objective of this study is to investigate the diversity of selected potential probiotic bacteria in various *in vitro* models. Five strains of *Lactobacillus plantarum*, four *L. sakei*, four *L. reuteri*, one *Pediococcus pentosaceus,* one *L. farciminis,* one *L. gasseri,* one *L. rhamnosus,* and one *L. pentosus* were screened in various *in vitro* models. Adhesion capacity to the human colon adenocarsinoma cell line Caco-2 was examined. Changes in epithelial barrier function were investigated with transepithelial electrical resistance (TER) measurements of polarized monolayers of Caco-2. Transit tolerance in the upper human gastrointestinal tract was assessed in an *in vitro* model simulating gastric and pancreatic juice. One *L. plantarum* and three *L. reuteri* strains had a significant higher adhesion capacity compared to the other strains investigated. Data from the TER measurements indicate that *L. reuteri* possibly increase the epithelial barrier over time. *In vitro* transit tolerance in the upper gastrointestinal tract revealed significant differences between the strains investigated. The eighteen strains investigated showed various results in the *in vitro* models applied. The strains are likely to have different mechanisms of action in the host. In the future we aim to pursue the *in vitro* screening of the eighteen strains selected, and finally study selected mechanisms of action in the most heterogenic probiotic bacteria investigated.

P20

Expression of DNA damage inducible genes in *Escherichia coli* at the single cell and population levels

Simona Kamenšek[1], Z. Podlesek[1], O. Gillor[2] and D. Žgur-Bertok[1]
[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia and [2]Ben-Gurion University, J. Blaustein Institutes for Desert Research, Zuckerberg Institute for Water Research, Israel; simona.kamensek@bf.uni-lj.si

Escherichia coli strains are part of the colon microbiota however, particular strains are also the causative agents of intestinal and extra-intestinal infections. A number of studies have revealed that about forty percent of *E. coli* strains sampled from natural populations produce a class of anti-microbial compounds known as colicins. Colicins are antibacterial cytotoxins active against strains of the same or related species. They are released into the environment to reduce competition from other bacterial strains. Colicinogenic strains can be induced, under stress, to produce high levels of toxin. Studies focused on colicins help to reveal more about their biology and their potential use as probiotics, antibiotic alternatives and even in cancer treatment, etc. *E. coli* has evolved a coordinated cellular response to DNA damage named the SOS response with the LexA protein as a key regulator. In addition to genes involved in DNA repair, LexA also regulates colicin encoding genes. LexA binds as a dimer to a 20 nucleotide long operator sequence and thus represses expression. Among isogenic bacterial cells phenotypic variability is observed. Heterogeneity within bacterial cell populations enables a small part of the population to be prepared for unfavourable environmental conditions or alternatively, the sacrifice of a subpopulation enhances the likelihood of survival of clonal siblings. In our study we examined the kinetics of expression of a few SOS and colicin genes at the population level fluorimetrically, using promoter fusions with the promoterless *gfp* gene. In addition, to observe heterogeneity in gene expression single cell analysis by fluorescence microscopy was performed. Expression was analysed in a wild type and isogenic *recA* defective strain that cannot elicit a SOS response. At the single cell level our results revealed heterogeneity in expression of the SOS genes *recA, lexA*, *polB*, *uvrA* and also in expression of colicin encoding genes. The *umuD* gene is completely repressed under normal conditions. Heterogeneity in expression of SOS genes among genetically identical cells is possible due to a spontaneous SOS response induced by endogenous events and stochastic events such as, random distribution of cell content and the LexA repressor among daughter cells and fluctuations in transcription and translation of LexA. Expression also depends upon the LexA binding sequence, its localisation within the promoter region; the number of LexA binding sequences and their position as well as promoter strength.

P21

Evaluation of gut environmental dynamics based on gene expression profiling

Tamotsu Kato[1,2,4], S. Fukuda[2], Y. Date[2,3],E. Chikayama[5], Y. Nakanishi[1,2,5], Y. Tsuboi[4,5], S. Tsuneda[3], S. Moriya[1,4], J. Kikuchi[1,5] and H. Ohno[1,2]
[1]Yokohama City University, Graduate School of Nanobioscience, Department of Supramolecular Biology, Japan, [2]RIKEN Research Center for Allergy and Immunology, Japan, [3]Waseda University, Department of Life Science and Medical BioScience, Japan, [4]RIKEN Advanced Science Institute, Japan and [5]RIKEN Plant Science Center, Japan;
tamo2@tsurumi.yokohama-cu.ac.jp

Our gastrointestinal tract provides residence to a wide variety of commensal microbiota containing both beneficial and potentially pathogenic bacteria. It has been postulated that imbalance in the composition of microbiota could result in human disorders including inflammatory bowel disease, metabolic syndrome, allergy and cancer. Control of the intestinal microbiota to adequate composition should therefore be important. However, only limited information in the gut environment is currently available, which hampers evidence-based intervention of gut microenvironment for improvement of our health. To understand the molecular basis for the gut environmental dynamics including host-bacterial crosstalk, we developed a meta-analysis-based profiling system. In our previous experiments, we performed a genome-metabolome correlation analysis, or DGGE (Denaturing Gradient Gel Electrophoresis)-NMR analysis, using faeces from BALB/c mice to assess gut environmental dynamics. In this work, we focused on the gene expression profiles of intestinal microbiota. Bacterial gene expression profiles from BALB/c mice fed with food containing plant-origin fibres under the SPF condition were analyzed with a novel cDNA library method in that cDNA library was constructed by reverse transcription after rRNA removal from total RNA. DNA sequence data were classified according to MG-RAST (Meta Genome Rapid Annotation using subsystem Technology: http://metagenomics.nmpdr.org/), and sequences related to the gut environmental dynamics were identified. To combine these expression data and the results gained by the DGGE-NMR correlation analysis, we achieved the prediction of metabolic pathways of major microbial symbionts affected by the fibre diet intakes. Combined DGGE-cDNA-NMR analysis is considered to be a useful method to assess the gut environmental dynamics.

P22

Detoxification of 2-amino-1-methylo-6-phenylo-1H-imidazo[4,5-*b*] pyridine (PhIP) by lactofementation beetroot juice *in vivo* in a rats model

Elżbieta Klewicka[1], A. Nowak[1], J. Juśkiewicz[2] and Z. Zduńczyk[2]
[1]Technical University of Lodz, Institute of Fermentation Technology and Microbiology, Poland and [2]PAS, Institute of Animal Reproduction and Food Research, Division of Food Science, Poland; klewicka@p.lodz.pl

The aim of this study was to assess the reduction of toxic activity of 2-amino-1-methyl-6phenylo-1H-imidazo [4,5-b] pyridine (PhIP) by using into the diet of experimental animals fermented juice of beetroot. Beetroot juice was fermented with two strains of probiotic bacteria of *Lactobacillus brevis* 0944 and *L. paracasei* 0920. The juice after fermentation contained 4.5×10^9 of a live cells per ml. Heterocyclic amine PhIP was administered to experimental animals (rats) at a dose of 10 µg per day for 8 weeks. The animals were divided into four groups of 8 animals each (male). Group B - basic diet; BJ - basic diet and 3 ml/day of fermented juice; P - basic diet; and PJ - basic diet and PhIP and 3 ml/day of fermented juice. In assessing the toxicity of PhIP in rats faecal water the genotoxicity test (the comet assay) on cell line Caco2 and the MTT cytotoxicity assay were used. Faecal water genotoxicity, expressed as percentage of DNA damage in the control groups B and BJ were 7.07% (SEM 0,706) and 8.52% (SEM 0.544). For the group of animals that received the heterocyclic amine PhIP, DNA damage percentage was 10.35% (SEM 0.717). Faecal water genotoxicity of the group of animals PJ (amine and beetroot juice) was 3.9% (SEM 0.254) of DNA damage. The reduction in DNA damage about 7% was observed in a group of animals, which in addition to amine consumed also fermented beetroot juice and the results are statistically significant. The MTT cell viability test line Caco2 in the control group (P - only PhIP) was 52.22 (23.4)%. In the group of animals PJ (PhIP and fermented juice of beetroot) cell viability was developed at the level of 50.35 (25.5)%. Usage in diet of experimental animals of fermented beetroot juice containing alive *Lactobacillus* bacteria significantly lowers the toxicity of PhIP, which can promote tumour processes in the gut.

Acknowledgements
The work was supported by Polish State Committee for Scientific Research Project N N312 356934.

P23

Use of a dynamic *in vitro* system to assess the ability of probiotic bacteria to survive the upper GI tract

Marjorie Koenen, K. Venema and A. Maathuis
TNO Quality of Life, the Netherlands; marjorie.koenen@tno.nl

The objective of the study was to use a dynamic, *in vitro* model to assess the ability of different probiotic strains, with or without a prebiotic, to survive transit thru the upper GI tract. The ESPGHAN Committee on Nutrition has established a recommendation that the dose of a probiotic be justified for use in infant formula (Journal of Pediatric Gastroenterology and Nutrition 38 (2004) 365-374). We chose to use a validated, dynamic *in vitro* model of the stomach and small intestine (TIM-1), simulating GI tract conditions of 2-4 wk old infants, to assess survival in the upper GI tract of probiotic strains, with and without prebiotics. TIM-1 was given two 200 ml meals at 3 hr intervals. A meal consisted of infant formula containing approx. 10^6 cfu per ml of each probiotic strain; some variables also included a prebiotic. Ileum effluent was sampled hourly during a 6 hours experiment, and cumulative survival of the probiotics in time was determined. Variables were tested in duplicate. Survival increased for *Lactobacillus* and *Bifidobacterium* strains when tested in combination versus separately (*L. acidophilus* (LA), 17.5%; *B. lactis* (BL), 35.9%; and LA + BL, 31.5% and 53.6%, respectively). For combinations of LA and BL from different suppliers, cumulative survival of probiotic strains could be high (LA 31.5% + BL 53.6%) or low (LA 4.5% + BL 31.5%). Supplier production practices had more influence on survival of probiotic combinations than did strain differences: BL from two suppliers in combination with a *Lactobacillus* varied in survival from 31.5% to 90.0%. For *Lactobacillus* strains, survival in the presence of a *Bifidobacterium* ranged from 4.5% to 38.1%. A prebiotic impacted survival of probiotic strains during passage thru the upper GI tract. FOS added to an infant formula that contained a combination of LA and BL reduced survival of the probiotic strains, but this was dependent on the dose of prebiotics added. Only for BL with 2% FOS added, cumulative survival (77.8%) was still higher than without FOS (53.6%). It is concluded that a validated, dynamic *in vitro* model can be used to study survival of probiotic strains in the upper GI tract and help establish the dose for use in infant formula. A combination of strains might provide more benefit to the infant than an individual strain. Supplier practices, a combination of strains, and presence *and* dose of a prebiotic, can impact survival and need to be taken into account when recommending a probiotic feeding dose.

Acknowledgements
Sponsored by Discovery Technology, Abbott Nutrition, Chicago, IL, USA.

P24

Influence of D-lactate producing probiotic strains on D-lactate level in the colon

Marjorie Koenen, K. Venema and A. Maathuis
TNO Quality of Life, Zeist, the Netherlands; marjorie.koenen@tno.nl

The objective of this study was to understand the impact of D-lactate producing probiotic strains on the amount of D-lactate present after a 72-hr dynamic, *in vitro* colonic fermentation. Two D-lactate producing *Lactobacillus acidophilus* strains and two non-D lactate producing *Bifidobacterium lactis* probiotics, alone or in combination and with or without prebiotics (short or long chain inulin), were evaluated for the influence on D-lactate production over a 72 h fermentation period in a dynamic *in vitro* model of the colon (TIM-2). A sample of predigested milk-based starter infant formula was combined with one of the test variables and introduced into the TIM-2 chamber containing a microbiota originating from faeces of 2 to 4 months old breastfed infants. Multiple doses of pre- and probiotics were evaluated to determine if dose impacted D-lactate level. A total of 14 variables were tested. Measurement of total cumulative lactate and of each isomeric form were made at the end of fermentation and compared to experiments with the predigested milk only (control without pre- or probiotics). The control produced a total cumulative ratio of L- to D-lactate of 1:1.16 (76 mmol: 88 mmol) after 72 h fermentation. Of the 14 variables, 8 variables produced less total cumulative lactate than the control (<164 mmol). Seven variables produced a greater amount of cumulative D-lactate than the control, with only 1 having a decreased ratio of L:D (<1:1.16). Individual strains of D-lactate-producing lactobacilli, even at 10^9 cfu/ml, did not impact the cumulative total amount of lactate or proportion of D-lactate produced. The most significant impact on both the total amount of lactate produced and the proportion of L:D, was found with a combination of *L. acidophilus* + *B. lactis* at either a low (10^4 cfu/ml) or higher (10^7 cfu/ml) dose of each strain, together with 0.5 g/L oligofructose (lower level probiotic - 205 mmol total lactate, 114 mmol D; higher level probiotic - 198 mmol total, 111 mmol D). It can be concluded that the presence of D-lactate-producing probiotic strains did not influence the proportion of D-lactate produced in the colon to the same extent as did a combination of D- and L-lactate producing strains. The largest increase in both proportion and amount of D-lactate was seen with the prebiotic-containing variables. These data suggest that some D-lactate producing strains of probiotics may not significantly impact the safety of healthy infants fed formula containing these strains.

Acknowledgements
Sponsored by Discovery Technology, Abbott Nutrition, Chicago, IL, USA.

P25

Novel fluorescence-based method for real-time monitoring of viable microorganisms

Remco Kort, A. Nocker, A. de Kat Angelino-Bart, F. Schuren and R. Montijn
TNO Quality of Life, Zeist, Microbial Genomics Group, the Netherlands; remco.kort@tno.nl

Ever since the pioneering work by Louis Pasteur and Robert Koch at the end of the nineteenth century, the detection of viable microorganisms has been carried out by cultivation and enumeration of colony forming units (cfus). Almost all judgments on hygiene, food safety, drinking water quality, infections of pathogens, and microbial content of probiotic formulas are based on growth on solid agar medium followed by cfu counts. However, the assessment of the number of viable cells on agar plates is laborious, requires at least an overnight incubation, and is limited to microorganisms that are readily culturable under laboratory conditions. These difficulties to directly measure the number of viable cells renders increasing importance to methods that measure indirect parameters (viability indicators) that live cells should possess. Such assays have been developed for the assessment of a variety of viability indicators, including membrane integrity, membrane potential, redox activity, ATP content, enzymatic activity, release of intracellular components and presence of specific gene transcripts. Here we present a novel and generic principle for the real-time viable (RTV) cell detection that exclusively occurs in live cells exposed to a specific probe. The innovative method allows for rapid fluorescence-based detection of viable cells. The principle is based on the loss of a functional membrane in dead cells. As a result, the intracellular environment of dead cells reflects to some extent that of the surrounding environment. This is completely different in case of live cells that actively maintain a distinct intracellular environment. The novel method includes the addition of a fluorescent cell-permeable probe to a cell suspension. The conditions have been selected such that in the surrounding medium and in dead cells the fluorescence of the probe is quenched, whereas the probe brightly fluoresces in the intracellular environment of live cells. The signal is quantitatively monitored and correlates to the number of live cells present in the sample. The method in addition monitors the ability of the live cells to maintain a controlled intracellular environment under biocidal conditions, which can serve as a fitness parameter. The novel method reported here can be used as a diagnostic tool for all applications where knowledge about microbial viability is important. The broad range of applications includes both process optimization and assessment of product quality. Relatively large number of samples can be rapidly screened. The technology in its current stage is especially well-suited for assessment of the number of live cells in spray- or freeze dried probiotics and starter cultures. The method is compatible with sample filtration and is not affected by organic matter in the cell suspension. However, as the method is dependent on fluorescence signals, dilution or clearance of light scattering samples such as dairy products is required.

References

1. Kort, R., Schuren, F. and Montijn, R., 2008. Real-time method for the detection of viable microorganisms. TNO Patent. PCT/NL2008/050828.

P26

Immunomodulating effects of probiotics in the healthy and inflamed gastrointestinal tract

Bas H.A. Kremer, R. Mariman, N. Worms, T. Lagerweij, S. Mourits, M. van Erk and L. Nagelkerken
TNO Biosciences, the Netherlands; bas.kremer@tno.nl

Probiotics are known to have immunomodulatory effects. We are currently exploring the effects of long-term probiotic administration on intestinal homeostasis and on recurrent chemically induced colitis in BALB/c mice. Colitis was induced by weekly intrarectal installation of low dosages TNBS, following initial skin sensitization with TNBS. In addition to tissue damage, as assessed by histopathological analysis, TNBS-induced inflammation was associated with increased numbers of CD4+, CD8+ and CD11b+ cells in intestinal mucosal tissue. Furthermore, multiplex analysis revealed elevated serum levels of pro-inflammatory cytokines, including IL-17, IFN-γ, IL-1β, and MIP-1α, implicating the involvement of the Th1/Th17 axis of the immune response, in line with the processes suggested to be involved in Crohn's Disease. As expected, oral administration of either *Lactobacillus plantarum* or the VSL#3 probiotic mixture did not affect the macroscopical and microscopical appearance of the small intestine and colon. However, initial analysis of gene expression suggests putative effects of long-term probiotic administration on the immune status in both parts of the gastrointestinal tract. Probiotic administration did ameliorate histopathological changes induced by repeated TNBS challenge. These effects were associated with reduced mucosal infiltration of CD4+ and CD11b+ cells and suppression of serum levels of multiple cytokines to concentrations similar to those detected in healthy mice. Preliminary transcriptomics data also show that probiotics suppress transcriptional changes of mucosal immune response associated genes induced by TNBS, in line with reduced colon inflammation. Follow-up experiments to establish whether the transcriptomics data indeed reflect modulation of the mucosal immune system by probiotics are ongoing. In conclusion, our data suggest that prolonged administration of probiotics may have immunomodulating effects both in the healthy and inflamed gastrointestinal tract.

P27

Reduction of *Enterococcus faecium* in the TIM-1 dynamic gastro-intestinal model by *Lactobacillus sakei* subsp. *sakei* 2a, and evaluation of the sakacin activity

Monika F. Kruger[1,2,5], S. van Rijn[2], K. Venema[2], R.C.R. Martinez[2,3,5], E.C.P. De Martinis[3], S.M.I. Saad[4], B.D.G.M. Franco[1], H. Smidt[5] and E.G. Zoetendal[5]
[1]Universidade de São Paulo, Departamento de Alimentos e Nutrição Experimental, Brazil, [2]TNO Quality of Life, the Netherlands, [3]Universidade de São Paulo, Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Brazil, [4]Universidade de São Paulo, Departamento de Tecnologia Bioquímico-Farmacêutica, Brazil and [5]Wageningen University, Laboratory of Microbiology, the Netherlands; monika.kruger@ig.com.br

Lactic acid bacteria are naturally present in the gut and have been intensively investigated as a dietary adjunct for gastro-intestinal disorders in animals and humans. A major concern in studies of LAB as probiotic bacteria is the assessment of their persistence in the gastro-intestinal tract (GIT) and also their ability to inhibit pathogenic bacteria in the intestinal environment. Probiotic cultures must adapt to different stresses during food processing to provide adequate amounts of active, living cells to withstand harsh conditions in the stomach and small intestine before reaching the colon. Considering that the viability of these cultures in the GIT is affected mainly by acid conditions in the stomach and bile salts in the duodenum, we evaluated the survival of *Lactobacillus sakei* subsp. *sakei* 2a in the human small intestine and its impact on the survival of *Enterococcus faecium,* using the TNO model (TIM-1). *L. sakei* 2a and *E. faecium* were cultivated overnight in MRS at 30°C and BHI at 37°C, respectively, washed twice, resuspended in PFZ (peptone physiological salt solution) or in commercial cow's milk and introduced in the TIM-1 model at 6-7 log cfu/ml. Samples were taken and analyzed from time 0 until 6 hours of running in the TIM-1 model. The viability of the microorganisms was assessed by the pour plate method in MRS or BHI agar. The results showed that *L. sakei* 2a was able to persist in the small intestine until the sixth hour of experiment, but CFUs were drastically reduced (5 log). In contrast, *E. faecium* presented high ability to colonize the GIT, reaching a population of 8 log cfu/ml at the second hour, persisting until the sixth hour of analysis. When co-inoculated, *L. sakei* 2a was able to persist with approx. 10^4 cfu/ml. Remarkably, its presence caused a substantive decrease (6 log) in the number of *E. faecium*. This effect was confirmed when both strains were co-inoculated in milk, although milk presented a protective effect on both strains. Since *L. sakei* 2a produces sakacin, the activity of the bacteriocin was evaluated during the transit in the TIM-1, but no bacteriocin activity was detected. We concluded that, due to the survival rate and the ability to inhibit *E. faecium* in the simulated small intestinal conditions, *L. sakei* 2a becomes an interesting strain for future studies as a potential probiotic culture. We are currently investigating the mechanisms underlying the inhibitory effect by *L. sakei* 2a.

P28

Nutritional intervention with NR100157 restores gut microbiota in HIV-1 infected adults not on HAART, by stimulating beneficial commensals

A. Gori[1], K. Ben Amor[2], A. van Hees[2], **Belinda van't Land[2,3]**, K. van Norren[2], N. Georgiou[2,3], D. Bray[4], G. Welling[5], C. Richter[6], P. Koopmans[7], J. Garssen[2,3], J. Knol[2], M. Clerici[8] and the COPA-COMBAT study teams
[1]University of Milano-Bicocca, 'San Gerardo' Hospital, Division of Infectious Diseases, Italy, [2]Danone Research - Centre for Specialised Nutrition, Nutricia Advanced Medical Nutrition, the Netherlands, [3]Utrecht Institute for Pharmaceutical Sciences (UIPS), the Netherlands, [4]ImmunoClin, France, [5]University Medical Center Groningen, Department of Medical Microbiology, the Netherlands, [6]Rijnstate Hospital, the Netherlands, [7]UMC St. Radboud, the Netherlands and [8]University of Milan, Italy; belinda.vantland@danone.com

Alterations in the gastrointestinal tract may be a key factor in the pathogenesis of human immunodeficiency virus (HIV). Previously, we have shown that HIV-1 infected adults often exhibit elevated levels of intestinal inflammatory parameters and have clear alterations in gut commensal microbiota with the outgrowth of pathogenic species. We hypothesized that specific nutritional components may restore the disbalance in the gut microbiota composition in HIV-1 infected adults. The nutritional concept NR100157 has been developed containing specific oligosaccharides, long-chain polyunsaturated fatty acids, colostrum, N-acetyl cysteine, and a vitamin/mineral mix. The effects of oligosaccharides, colostrum, and the complete nutritional concept (NR100157) on gut microbiota composition were assessed in HAART-naïve HIV-1 infected adults in separate clinical studies. Stool samples were collected at baseline and after nutritional intervention with either the investigational product or a control product. Faecal microbiota was analyzed by fluorescent in situ hybridization, quantitative real-time PCR, and/or the semi-quantitative denaturating gradient gel electrophoresis (DGGE) method, after DNA extraction from fixed faecal cells. Intake of the oligosaccharides alone, but not colostrum alone, resulted in the increase of bifidobacteria levels, whereas no effects on lactobacilli were observed. In addition bifidobacteria significantly increased after 6 weeks intake of NR100157 (median (range): 1.11 (0-30.01) to 2.75 (0-33.08)%, *P*=0.016), decreased after discontinuation of product intake for 2 months, and increased again during a second 6-week intervention with NR100157 in the same subjects (2.26 (0.01-18.98) to 4.97 (0.33-19.65)%, *P*=0.080). Levels of the *Clostridium coccoides-Eubacterium rectale* cluster and the *Clostridium histolyticum/Clostridium lituseburense* cluster were significantly decreased after nutritional intervention, especially with the oligosaccharide mixture, whereas levels of the *Atopobium* cluster significantly increased after intervention with NR100157. DGGE analysis showed that the bacterial profile of the HIV-infected adults clustered separately from a healthy control group at baseline (similarity index of 24%), whereas similarity increased to 52% after 6-week intervention with NR100157. In conclusion, intervention with the complete nutritional concept NR100157 and the oligosaccharide mixture alone restored gut microbiota towards levels seen in healthy individuals, mainly by stimulating bifidobacteria and decreasing clostridia-related species. Continuous intake of NR100157 was necessary to maintain these beneficial effects. Restoration of the microbiota balance may contribute in the reduction of disease progression in HIV-1 infected individuals.

P29

Interspecies cell-to-cell communication between probiotic *Lactobacillus* and *Staphylococcus aureus* inhibits production of the staphylococcal superantigen toxic shock syndrome toxin-1

Jingru Li
University of Western Ontario, Department of Microbiology and Immunology, Canada;
jli494@uwo.ca

Staphylococcus aureus produces multiple extracellular toxins that significantly contribute to its ability to cause disease. Particularly, the production of the staphylococcal superantigen toxic shock syndrome toxin-1 (TSST-1) has been associated with essentially all cases of menstruation-associated toxic shock syndrome. *Lactobacillus reuteri* RC-14, a well-defined probiotic isolated from the human vagina, was previously demonstrated to produce small signalling molecules that are able to interfere with the staphylococcal quorum-sensing system *agr*, and independently repress the expression of a potential virulence factor termed staphylococcal superantigen-like protein 11 (SSL11) in *S. aureus* Newman. In this work, the effect of *L. reuteri* RC-14 on TSST-1 production in *S. aureus* MN8, a prototype of menstrual TSS *S. aureus* strains, was evaluated. Quantitative RT-PCR (qPCR) data clearly showed that the transcription of the TSST-1 promoter (P_{tst}) in MN8 was greatly decreased in response to growth with *L. reuteri* RC-14 cultural supernatant. Alterations in the transcriptional levels of the P2 and P3 promoters from the *agr* system were also observed, indicating a potential overall influence of RC-14 signals on the production of other secreted virulence factors. In addition, several luciferase-based reporter plasmids were constructed, in which the expression of the *luxABCDE* operon was driven by different promoters including P_{tst}, P2, or P3. The effect of *L. reuteri* RC-14 on promoter activities was confirmed by the repression of luciferase activity upon the addition of RC-14 methanol extracts, with no impact on the growth of *S. aureus*. Currently, these promoter-reporter constructs are being used to screen fractions of *L. reuteri* RC-14 supernatant for the ability to repress TSST-1 production and/or the *agr* system, with the goal of identifying and characterizing the putative signalling molecules responsible for the antagonistic activity against Staphylococcal virulence factors. The results from this work will contribute to a better understanding of interspecies cell-to-cell communication between *Lactobacillus* and *Staphylococcus*, and moreover, may lead to the development of novel antimicrobial therapy for *S. aureus* infection.

P30

Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study*

Raakel Luoto, K. Laitinen, M. Nermes and E. Isolauri
Turku University Hospital and University of Turku, Finland; raakel.luoto@utu.fi

The foetal and neonatal periods critically guide the development, growth and functional maturation of the organ systems. Specifically, the perinatal nutritional environment impacts upon the health and well-being of child and also mother long-term. The objective of this study is to determine the safety and efficacy of the combined probiotic intervention and dietary counselling on pregnancy outcome and foetal and infant growth during the 24 months' follow-up. Altogether 256 women were randomised at their first trimester of pregnancy into a control and a dietary intervention group. The intervention group received intensive dietary counselling, complying with current recommendations, provided by a nutritionist and were further randomised, double-blind, to receive probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12; diet/probiotics) or placebo (diet/placebo). Study visits were scheduled three times during pregnancy and after delivery at the infant ages of 1, 6, 12 and 12 months. Firstly, probiotic intervention reduced the frequency of gestational diabetes mellitus (GDM); 36% (diet/placebo) and 34% (control) vs. 13% (diet/probiotics); P = 0.003. Secondly, the safety of this approach was attested by normal duration of pregnancies with no adverse events in mothers or children. No significant differences in prenatal or postnatal growth rates among the study groups were detected. Thirdly, significant interaction of the two interventions was detected; probiotic intervention reduced the risk of GDM and dietary intervention diminished the risk of larger birth size in affected cases; P = .035 for birth weight and .028 for birth length. The results of this study can be interpreted to indicate, that long-term health benefits for mothers and children may be conferred by balanced maternal nutrition during pregnancy and lactation and by promoting the healthy gut microbiota, in the mother and the child. In view of the fact that birth size is a risk marker for later obesity, the present results are of significance for public health in demonstrating that this risk is modifiable. Probiotic-supplemented perinatal dietary counselling could be a safe and cost-effective tool in addressing the obesity epidemic.

*Authors have no conflict of interest related to this study. This abstract is based on an article which is accepted, but not yet published, for publication in British Journal of Nutrition.

P31

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

Annet Maathuis[1], A. Hoffman[2], A. Evans[2], L. Sanders[2] and K. Venema[1]
[1]TNO Quality of Life, the Netherlands and [2]Tate & Lyle, USA; annet.maathuis@tno.nl

A well balanced intestinal microbiota can offer numerous potential health benefits to the host. Some non-digestible dietary fibres act as prebiotics by stimulating the growth of beneficial – or minimizing the growth of undesirable – microbes. In this study, the prebiotic potential of novel fibres were evaluated using an in vitro model of the human intestine (TIM). Resistant starch (RS), resistant maltodextrin (RM), soluble corn fibre (SCF), soluble fibre dextrin (SFD) and biogum (BG) were pre-digested in the model of the stomach and small intestine (TIM-1) 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 fibre carbohydrate mixture, all novel fibres 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 fibres show different yet favourable fermentation profiles and prebiotic potential in this dynamic in vitro system of the human intestine.

Acknowledgements
Research supported by Tate & Lyle.

P32

At the crossroads of vaginal health and disease, the genome sequence of *Lactobacillus iners* AB-1

Jean M. Macklaim[1,2], G. B. Gloor[2], K.C. Anukam[1], S. Cribby[1] and G. Reid[1,3]
[1]Canadian Research & Development Centre for Probiotics, Lawson Health Research Institute, Canada, [2]The University of Western Ontario, Department of Biochemistry, Canada and [3]The University of Western Ontario, Departments of Microbiology and Immunology and Surgery, Canada; mmacklai@uwo.ca

Lactobacilli have long been regarded as important constituents of the healthy human vagina. *Lactobacillus iners* is the most frequently detected species, but little is known about its characteristics. We report the first description of the whole genome sequence of *L. iners* AB-1, a vaginal strain, along with comparative analysis of published genomes of gut and cheese strains of lactobacilli. The genome is the smallest *Lactobacillus* reported to date with a 1.28Mbp single chromosome, and appears to be rapidly evolving through both gene loss and conversely through horizontal acquisition of a number of genes for survival in the vagina. It appears to have specialized vaginal environmental adaptations such as an iron-sulphur cluster assembly system, and several unique sigma factors to regulate gene transcription in this fluctuating environment. A potentially highly expressed cholesterol-binding lysine, the first reported in lactobacilli, may also contribute to host-cell adhesion, or potentially act as a defense mechanism against other microbes. Notably there is a lack of apparent adhesion proteins despite evidence of adherence and pathogen displacement, but several novel cell-anchor proteins are likely related to host cell adhesion and retention. In women who have a urogenital infection, their recovery not only requires eradication or depletion of the pathogens and alleviation of any inflammatory process, but also restoration of the indigenous lactobacilli. The fact that *L. iners* is present in healthy females and those suffering from bacterial vaginosis (BV) or who have undergone antimicrobial therapy, suggests it may be a critical species for recovery to health.

P33

Fructansucrase encoding gene from *Weissella confusa* MBF-CNC2(1) isolated from Indonesian Cincau reveals high similarity to inulosucrase from *Lactobacillus reuteri*

Amarila Malik[1], S. Ishikawa[2] and N. Ogasawara[2]
[1]Universitas Indonesia, Faculty of Mathematics and Natural Sciences, Department of Pharmacy, Laboratory of Microbiology and Biotechnology, Indonesia and [2]Institute of Science and Technology (NAIST), Graduate School of Biological Sciences, Division of Bioinformatics and Genomics, Japan; amarila.malik@ui.ac.id

Fructan type of exopolysaccharide (EPS) such as inulin and its oligos (fructooligosaccharide/ FOS), in addition to levan, has been well known to be used and developed in food and pharmaceutical industries. Inulin has been used widely but it is mostly produced from plant, e.g. chicory root. EPS producing-lactic acid bacteria (LAB) have been reported to produce EPS fructan, besides glucan, with characteristic as inulin as well as levan. A collection of EPS-producing LAB, isolated from various sources in Indonesia, e.g. local foods and beverages, were screened for fructansucrase (FS) gene using degenerated primers designed with a tag for specific cloning system in *Escherichia coli*. A *Weissella confusa* strain MBFCNC-2(1), which was isolated from Cincau previously, was shown to be potential to harbour FS gene coding for inulosucrase, an enzyme that synthesize EPS inulin. A full length DNA fragment for FTF gene was successfully cloned as the result by several rounds of inversed-PCR (iPCR) technique and DNA sequencing, simultaneously. By using tblastx and GeneWorks® the DNA sequences analysis was obtained. Result revealed that this fragment showed an open reading frame of FS-coding gene which has the closest DNA sequence similarity to a putative inulosucrase-coding gene of *Lactobacillus reuteri*. Cloning is still in the process to obtain *E. coli* recombinant harbouring this inulosucrase type of FS gene using Gateway® system for overexpression and to study the enzyme characterization.

P34

Effect of galacto-oligossaccharide, and milk on the survival of *Lactobacillus sobrius* 16698 in a computer-controlled model of the stomach and the small intestine

Rafael C.R. Martinez[1,2], A.-E. Aynaou[3], E.C. Pereira De Martinis[1], E. Zoetendal[2], K. Venema[3], S.M.I. Saad[4] and H. Smidt[2]
[1]University of São Paulo, Faculty of Pharmaceutical Sciences of Ribeirão Preto, Department of Clinical, Toxicological and Bromatological Analysis, Brazil, [2]Wageningen University, Laboratory of Microbiology, the Netherlands, [3]TNO Quality of Life, the Netherlands and [4]University of São Paulo, Faculty of Pharmaceutical Sciences, Department of Biochemical-Pharmaceutical Technology, Brazil; rafacrmartinez@yahoo.com.br

Survival rates of probiotic microorganisms in the gastrointestinal tract (GIT) can be influenced by the co-administration with prebiotics and/or different food matrixes. The use of TIM-1, a validated *in vitro* model to simulate the physiological conditions found in the stomach and small intestine is useful to address such studies, since the experiments are relatively easy to perform under controlled conditions, display reproducible results and are not limited by ethical constraints. The survival of the probiotic microorganism *Lactobacillus sobrius* 16698 was determined in TIM-1: (i) alone (control); (ii) in the presence of the prebiotic galacto-oligossaccharide (GOS); and (iii) in the presence of partially skimmed milk (PSM). *L. sobrius* 16698 was grown in MRS broth overnight in anaerobic jars at 37°C. Subsequently, the culture was re-cultivated in fresh MRS broth, centrifuged, re-suspended in sterile PBS (phosphate buffer solution), and finally introduced into the TIM-1 system together with other components (including artificial salivary juice). For condition (ii) instead, *L. sobrius* was cultivated in MRS broth, modified by the substitution of glucose (2%) for Vivinal GOS (1%), as the main carbohydrate source; besides that, 5 g of Vivinal GOS were added to the model. Samples were collected from the initial meal (t=0 h) and from the ileum efflux of TIM-1 for six hours with one-hour time intervals and subsequently used to enumerate *L. sobrius* populations on MRS agar plates. For the confirmation of their identities, colonies were tested by the randomly amplified polymorphic DNA (RAPD) method. Results indicated that *L. sobrius* alone displayed ascending survival rates (determined as cumulative delivery) varying from approx.15% (t=1 h) to 30% (t=6 h). On the other hand, when *L. sobrius* was co-inoculated with GOS or PSM the survival rates of the probiotic microorganism were statistically higher (P<0.05) than those observed for the control from time-points ≥ t=3 h, reaching ca. 109% and 115% for conditions (ii) and (iii) assessed at t=6 h. We can conclude that the addition of GOS and PSM in probiotic preparations containing *L. sobrius* may be useful for improving the ability of the microorganism to survive and even multiply during the passage through the GIT and therefore optimize its probiotic property.

P35

Arabinoxylans and metabolic homeostasis: effect on prebiotic properties, glycemic control, weight management and immune modulation

Massimo Marzorati[1], P. Van den Abbeele[1], A. Neyrinck[2], P. Cani[2], N. Delzenne[2] and W. Verstraete[1], S. Possemiers[1]
[1]Ghent University, Laboratory of Microbial Ecology and Technology (LabMET), Belgium and
[2]Université Catholique de Louvain, Unit PMNT, Belgium; massimo.marzorati@ugent.be

Arabinoxylan (AX), is a dietary fibre constituent from cereals, consisting of β-(1,4)-linked D-xylopyranosyl residues with α-L-arabinofuranose side chains and potential substitution with ferulic acid. Indications of potent health effects of AX with different structure and chain length have been reported in several studies. In this work, we will focus on long-chain water-extractable AX, which were studied using *in vitro*, animal and human intervention studies. Endpoints of the investigation was the complex system of metabolic homeostasis. This was studied by investigating the effect on glucose and insulin control, prebiotic properties in the intestine and at the gut mucosa, effects on weight gain after AX consumption in combination with high fat intake and modulation of the immune system. The glycemic control was investigated in a human intervention study with pre-diabetic volunteers. A glucose challenge after the interventions showed significant improvement of the glucose and insulin response and a lowering of fasted blood triglyceride concentrations. Prebiotic properties were investigated using the dynamic *in vitro* SHIME (Simulator of the Human Intestinal Microbial Ecosystem) and in gnotobiotic rats. Whereas inulin increased butyrate production, AX significantly increased the production of propionate, known to beneficially regulate cholesterol and fatty acid synthesis in the liver. Both carbohydrates support growth of bifidobacteria, yet different species were selectively enhanced. Overall selective effects towards positive commensalistic bacteria were summarized by calculating a Prebiotic Index (PI) for the luminal and an Adhesion Related Prebiotic Index (AR-PI) for the mucosal community. This confirmed that both products exert prebiotic properties at both the gut lumen and gut mucosa, in which a much more potent activity was observed for AX compared to inulin in the SHIME study. In the mice study, high fat administration (4 wks) induced a significantly increased fasted glycaemia, plasma total cholesterol and more than doubled the animals' body weight gain. In case of AX co-administration, significantly lower total cholesterol, adiposity and body weight gain were observed, although kcal consumption was unaffected. In addition, AX administration led to a significantly lower inflammatory tone. With respect to the microbial community, AX co-administration neutralized the negative effects of high fat intake and increased bacterial fermentation. These data indicate that the AX concentrate has a unique biological activity by affecting multiple targets related to metabolic homeostasis, both in the gut and in the rest of the body, which may lead to promising applications related to diabetes, weight management and immune health.

P36

Molecular-epidemiological screening on allergy- and asthma-preventive bacteria in farming environment

Melanie Mayer[1], M.J. Ege[2], K. Schwaiger[1], E. von Mutius[2] and J. Bauer[1]
[1]Technische Universität München, Chair of Animal Hygiene, Germany and [2]University of Munich, Dr. von Hauner Children's Hospital, Germany; melanie.korthals@wzw.tum.de

An increasing number of epidemiological studies show that exposure to farming environment during early childhood inversely influences the development of allergic reactions later in life ('hygiene hypothesis'). The cause for this association is still unknown but preliminary studies indicate that exposure to farm environment bacteria may mediate this effect [1-3]. However, it remains unclear whether this association is related to the amount or diversity of exposure or to specific strains. Diversity of bacterial communities from mattress dust samples of 489 school age children from rural and suburban regions in Germany were assessed by PCR-SSCP using the 16S rRNA gene. The individual band patterns were tested for associations with asthma or atopy in quantitative and qualitative multivariable analyses. Bands that were significantly associated with asthma, atopic sensitization or hay fever were isolated from the gels and sequenced. They were identified by comparison to the public database. A specific PCR assay was designed for the bacterium showing the strongest effect on asthma in order to confirm the SSCP results. Specifity of PCR was tested by comparison with its closely relatives and other bacteria occurring in farming environment. In total 7 SSCP-bands were found to be inversely related to childhood asthma and allergy. The sequences of the bands represented well known candidates for asthma protection such as *Acinetobacter lwoffii* but also novel candidates. Most of them are apathogenic. The candidate specific PCR assay confirmed the SSCP results. Prevention of asthma and atopy might be achieved by exposure to certain bacterial species whereas the diversity of the exposure might be irrelevant. Currently, these candidates are tested by *in vivo* mouse models in order to investigate their allergy- and asthma-preventive properties.

References

1. Riedler, J., Braun-Fahrländer, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D. and Von Mutius, E., 2001. Exposure to farming in early life and development of asthma and allergy: a cross sectional survey. The Lancet 358:1129-33.
2. Debarry, J., Garn, H., Hanuszkiewicz, A., Dickgreber, N., Blümer, N., Von Mutius, E., Bufe, A., Gatermann, S., Renz, H., Holst, O. and Heine, H., 2007. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. Journal of Allergy and Clinical Immunology 119:1514-1521.
3. Vogel, K., Blümer, N., Korthals, M., Mittelstädt, J., Garn, H., Ege, M., Von Mutius, E., Gatermann, S., Bufe, A., Goldmann, D., Schwaiger, K., Renz, H., Brandau, S., Bauer, J., Heine, H. and Holst, O., 2008. Animal shed *Bacillus licheniformis* spores possess allergy-protective as well as inflammatory properties. Journal of Allergy and Clinical Immunology 122:307-312.

P37

Characterization of potential allergy- and asthma- prophylactive or probiotic bacteria isolated from farming environment

Melanie Mayer, K. Schwaiger, C. Hölzel and J. Bauer
Technische Universität München, Chair of Animal Hygiene, Germany;
melanie.korthals@wzw.tum.de

Several epidemiological studies have shown an allergy protective effect of farm life in early childhood. Children who grow up in this environment reveal a significantly reduced occurrence of atopic sensitization, hay fever and asthmatic symptoms in comparison to children living in the same area. Preliminary investigations provided good evidence that this is due to microbial exposure and consumption of raw milk [1-3]. We previously found an inverse association between exposure to specific bacterial strains ('candidates') and the occurrence of childhood asthma and allergy by molecular-epidemiological screening using PCR-SSCP and statistical evaluation. A selection of allergy- and asthma-preventive candidate bacteria such as the already known species *Lactococcus lactis G121* and *Acinetobacter lwoffii F78* and novel candidates were further characterized in order to screen for suitable applications such as prophylactive vaccine or probiotic. Therefore novel candidates were cultured from farming environments including shed dusts, raw milk and silage. They were characterized concerning pH-stability, antibiotic resistance and lack of cytotoxicity (MTT test using Vero-cells). Several candidates were successfully cultured from different farming environments. The characterization concerning pH-stability of these strains revealed that most of them do not tolerate low pH down to pH3. The evaluation of antibiotic resistances showed that candidates were mostly intrinsic resistant. Some strains not belonging to the lactic acid bacteria group are very susceptible to antibiotic treatment. Investigations concerning cytotoxicity revealed that down to a 1:20 dilution of supernatants the strains are not cytotoxic in most of the cases. In conclusion, since most of the candidates do not tolerate low pH, application by the gastrointestinal route is not suitable for these strains. In these cases, implementation as prophylactive vaccine using the nasal exposure seems to be more comprising. This is currently tested by *in vivo* asthma mouse models.

References

1. Riedler, J., Braun-Fahrländer, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D. and Von Mutius, E., 2001. Exposure to farming in early life and development of asthma and allergy: a cross sectional survey. The Lancet 358:1129-33.
2. Debarry, J., Garn, H., Hanuszkiewicz, A., Dickgreber, N., Blümer, N., Von Mutius, E., Bufe, A., Gatermann, S., Renz, H., Holst, O. and Heine, H., 2007. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. Journal of Allergy and Clinical Immunology 119:1514-1521.
3. Vogel, K., Blümer, N., Korthals, M., Mittelstädt, J., Garn, H., Ege, M., Von Mutius, E., Gatermann, S., Bufe, A., Goldmann, D., Schwaiger, K., Renz, H., Brandau, S., Bauer, J., Heine, H. and Holst, O., 2008. Animal shed *Bacillus licheniformis* spores possess allergy-protective as well as inflammatory properties. Journal of Allergy and Clinical Immunology 122:307-312.

P38

The effect of a six-week exercise and weight control intervention on the intestinal microbiota of the overweight/obese Finnish women

Eveliina Munukka[1], A. Lyytikäinen[1], E. Völgyi[1], L. Xu[1], P. Wiklund[1], J. Vaahtovuo[2,3] and S. Cheng[1]
[1]University of Jyväskylä, Department of Health Sciences, Finland, [2]CyFlo Ltd., Finland and [3]Paimion terveyskeskus, Finland; eveliina.munukka@jyu.fi

The objective was to study the effect of exercise and weight control intervention on the composition of the faecal microbiota, and physiological parameters of the overweight and obese Finnish women. One hundred previously physically inactive (participating regular leisure time aerobic exercise less than two times per week, less than 45 min per session), overweight and obese women (body mass index > 25 kg/m^2) 20-50 years of age were randomly assigned into exercise (EX) or weight control (WC) groups. The EX group underwent a progressive, supervised, exercise programme (Nordic walking) according to the American College of Sport guidelines of exercise for health promotion. The WC group received a dietary instruction from a clinical nutritionist according to the guidelines of Finnish nutrition recommendation targeting to weight loss during the six week period. Body weight and body composition were measured (InBody 720) before and after the intervention. Total energy and energy yield nutrient intakes were collected via food dairy (one day before and six randomly selected days during the intervention). Faecal samples were collected at the baseline and follow-up. Bacterial compositions of the faecal samples were analyzed with a method based on flow cytometry (FCM), 16S rRNA hybridization and DNA-staining. Set of seven 5'-end labelled oligonucleotide probes hybridizing bacteria belonging to common genera and groups in the faecal microbiota of humans was used. Genera and groups associated with obesity, energy metabolism and weight loss were included in the panel of probes. The counts of the total bacteria and relative amounts of the hybridized bacteria were determined. We found no significant change of the body weight in the EX group (mean baseline 78.5 kg (SD ± 8.0) vs. mean follow-up 78.3 kg (SD±8.0), *P*=0.23), while the WC group loss their weight significantly (mean baseline 85.9 kg (SD±11.1) vs. mean follow-up 84.7 kg (SD±10.8), *P*<0.001) after the 6-week intervention. Furthermore FCM analyses revealed that the relative amount of bacteria belonging to the *Eubacterium rectale-Clostridium coccoides* group was reduced in the EX group 33.1% (*P*=0.003) and 22.9% in the WC group (*P*=0.04). Similar changes were found (34.1% reduction in EX group (*P*=0.003) and 9.3% in WC group (*P*=0.40)) for the ratio calculated from the relative amounts of *Eubacterium rectale-Clostridium coccoides* group and *Bacteroides-Porphyromonas-Prevotella* group bacteria. We also found no significant change of the total energy intake both in the EX group (Mean baseline 1,767 kcal (SD ± 520) vs. Mean intervention 1,689 kcal (SD ±418), *P*=0.22) and the WC group (Mean baseline 1,779 kcal (SD ± 465) vs. Mean intervention 1656 kcal (SD ±314), *P*=0.10). In conclusion, the six-week exercise or weight control program altered the relative amounts of the bacterial groups that have been previously known to associate with energy metabolism and weight loss. However the intervention may not be long enough to have significant effect on the overall composition of faecal microbiota as it is acknowledged that without any dramatic changes in diet or life habits the composition of adult's intestinal microbiota remains stable on the time scale of months.

P39

Does kefir intake interact with the pig microbial gut? Preliminary study on pig faeces

Véronique Ninane, J. Wavreille, V. Planchon and G. Berben
Walloon Agricultural Research Centre (CRA-W), Belgium; ninane@cra.wallonie.be

Kefir is fermented milk with healthy reputation that includes lactic acid bacteria and yeasts [1]. Some *Lactobacillus* strains isolated from kefir show, *in vitro,* interesting properties for probiotics application in the traditional way of preventing or treating gut microflora disorders [2-4]. The aim of this work was to determine *in vivo*, by microbial enumeration of pig faeces, the potentialities of kefir to interact with the gut microflora. Traditional kefir, prepared by incubation of milk with kefir KJ grains (10% w/v), was given daily to 2 months old pigs fed with a standard fattening diet. The amount of kefir given daily per pig, 500 ml, provided an extra microbial intake of about 10^{10} lactobacilli, 10^{11} lactic acid streptococci and 10^{9} yeasts in term of colony forming unit (cfu). Faeces of 4 pigs were collected after 2 and 3 weeks of kefir feeding and after 4 and 8 weeks of kefir starvation. Faeces were compared for total anaerobes, lactobacilli, lactic acid streptococci, coliforms and yeasts content determined by plate counts. As no difference in microbial counts were observed between faeces collected after 2 and 3 weeks of kefir feeding and between faeces collected after 4 and 8 weeks of kefir starvation, it was assumed that microbial flora was stabilised within those two feeding periods (with and without kefir). Values within those feeding periods were therefore pooled and their geometric means were compared. The number of total anaerobes and this of lactobacilli were unchanged under the two feeding periods: they stay around 10^{9} CFU per gram of faeces. In contrast, the number of lactic acid streptococci, the number of coliforms and this of yeasts were significantly modified (P<0.05): they turned respectively from $1.3x10^{7}$, $4.1x10^{6}$ and $5.3x10^{3}$ cfu/g with kefir to $4.6x10^{5}$, $4.8x10^{5}$ and $2.6x10^{4}$ cfu/g without kefir. The enhancement of lactic acid streptococci and of coliforms observed under kefir feeding is expected to be the expression of a modification in the microbial balance of the gut flora. It however could also be the expression of a lactose adaptation of some commensal bacteria (i.e. *Enterococcus faecalis*), resulting in a better growth on these culture media for which the carbohydrate source is lactose. Nevertheless, kefir had an indisputable effect on the microbial balance by lowering the charge of yeasts.

References

1. Ninane V., 2008. Ph.D. Thesis FUSAGx, Belgium
2. Santos, A., Mauro, M.S., Sanchez, A., et al, 2003. The antimicrobial properties of different strains of *Lactobacillus* spp. isolated from kefir. Systematic and Applied Microbiology 26:434-437.
3. Golowczyc, M.A., Mobili, P., Garrote, G.L., Abraham, A.G. and De Antoni, G.L., 2007. Protective action of *Lactobacillus kefir* carrying S-layer protein against *Salmonella enterica* serovar *enteritidis*. International Journal of Food Microbiology 118:264-273.
4. Golowczyc, M.A., Gugliada, M.J., Hollmann, A., Delfederico, L., Garrote, G.L., Abraham, A.G., Semorile, L. and De Antoni, G., 2008. Characterization of homofermentative lactobacilli isolated from kefir grains: potential use as probiotic. Journal of Dairy Research 75:211-217.

P40

Cyto- and genotoxicity of faecal water after incubation of 2-amino-3-methyl-3H-imidazo[4.5-*f*]quinoline (IQ) with faecal microorganisms and *Lactobacillus casei* DN 114 001, with usage of Caco-2 cell line

Adriana Nowak[1], S. Malgorzata[2] and Z. Libudzisz[1]
[1]Technical University of Lodz, Institute of Fermentation Technology and Microbiology, Poland and [2]University of Lodz, Department of Molecular Genetics, Poland;
adriana.nowak@p.lodz.pl

The aim of the study was to evaluate cyto- and genotoxicity of faecal water (FW) after incubation of heterocyclic amine (IQ) with faecal microorganisms and/or probiotic *Lactobacillus casei* DN 114 001. The stool samples derived from 15 healthy non-smoking persons (5 of each group: 0-24 months of life; 30-40 and 75-85 years old), on basic diet, without the history of gastrointestinal tract diseases, who did not take antibiotics and probiotics for at least 3 months. To 1 g of the faeces were added respectively: IQ (50 µg/ml); probiotic strain (10^{10} cfu/ml); IQ (50 µg/ml) and probiotic strain (10^{10} cfu/ml). The control sample was faeces without appendix. The samples were incubated for 72 h at 37^{o}C in anaerobic conditions and then centrifuged (10.700 x g, 30 min). The supernatants were frozen (–20^{o}C) and stored till analysis. Cytotoxicity of faecal water was evaluated with MTT and NRU tests, genotoxicity with comet assay, with usage of Caco-2 cell line. In the comet assay the most genotoxic (10.9%) was FW of persons between 75 and 85 years old and the least of children (4.2%). *L. casei* DN 114001 lowered genotoxicity of FW after incubation with IQ the most effectively (from 15.5 to 5.6%) in case of adults (30-40 years old). In case of group of children genotoxicity after incubation of FW with IQ and *L. casei* DN 114001 increased from 4.2 to 9.7%. There was no effect in case of the group of the old persons, in which the genotoxicity did not change (and it was at about 12%). In MTT test after incubation of FW with IQ and *L. casei* DN 114001 the increase in cytotoxicity was observed for the group of persons between 30 and 40 years old (from 32.3 to 41.7%). In case of the group of children the cytotoxicity was decreased from 54.4 to 33.5% after incubation with probiotic. There was no influence in the group of persons between 75 and 85 years old. In NRU test the most cytotoxic was FW of persons between 75 and 85 years old (63.2%) and the least of children (28.3%). The addition of *L. casei* DN 114001 did not influenced on the cytotoxicity of FW after incubation with IQ. It was observed that the genototoxicity and cytotoxicity of FW depended on the person's age and its individual intestinal microbiota.

P41

Automated extraction of microbial DNA from faeces for 16S rRNA microarray analysis

Lotta Nylund[1,2], H.G.H.J. Heilig[3], S. Salminen[1], W.M. de Vos[2, 3] and R. Satokari[1]
[1]University of Turku, Functional Foods Forum, Finland, [2]University of Helsinki, Division of Microbiology and Epidemiology, Department of Basic Veterinary Sciences, Finland and [3]Wageningen University, Laboratory of Microbiology, the Netherlands; lotta.nylund@utu.fi

The human gastrointestinal tract (GI-tract) harbours a complex microbial ecosystem, largely composed of so far unculturable species, which can be detected only by using culture-independent techniques such as PCR and by different hybridization techniques including phylogenetic microarrays. Manual DNA extraction from faeces is laborious and is one of the bottlenecks holding up the application of microarray and other DNA-based techniques in large cohort studies. We compared two (semi)automated DNA extraction methods, KingFisher with InviMag Stool DNA kit (KF) and NucliSENS easyMAG (NeM), to the manual extraction method currently considered the most suited method for faecal DNA extraction by analyzing the three parallel DNA extracts with qPCR and the phylogenetic microarray HITChip. The KF and manual method gave comparable yields of 16S rDNA as assessed by qPCR, whereas NeM showed a significantly lower yield. However, all three methods showed highly similar microbiota profiles in HITChip. Both KF and NeM were found to be good methods for (semi)automated DNA extraction from faecal samples after the mechanical disruption of microbial cells by bead-beating.

P42

Food, fibre and satiety – how does fibre make you feel full?

Shanthi Parkar[1], T. Trower[1], D. Stevenson[1], D. Sun-Waterhouse[2], J. Monro[3] and M. Skinner[2]
[1]The New Zealand Institute for Plant & Food Research Limited, Ruakura Research Centre, New Zealand, [2]The New Zealand Institute for Plant & Food Research Limited, Mount Albert Research Centre, New Zealand and [3]The New Zealand Institute for Plant & Food Research Limited, New Zealand; shanthi.parkar@plantandfood.co.nz

With obesity reaching epidemic proportions, there is a need to influence food intake by enhancing or prolonging the feeling of satiety after a meal. One of the goals of our research is to develop a food matrix that can influence satiety. Apolipoprotein A-IV (apoA-IV) is rapidly secreted by cells of the small intestine following a lipid-based meal, and then acts as a satiety signal to limit food intake. While dietary fibre is widely acknowledged to play a positive role in satiety, the mechanism of its action in inducing satiety at the intestinal level is not understood. Our objective was to investigate the role of dietary fibre in regulating the expression of apolipoprotein A-IV gene in intestinal differentiated Caco-2 cells, which share many characteristics with a mature intestinal epithelium such as apolipoprotein synthesis. Dietary fibre prepared from apple, broccoli and tomato were subjected to *in vitro* fermentation with faecal microflora. Fibre at 10 mg/ml (equivalent to a dietary intake of 4 g/day) was incubated with a fresh faecal sample (0.4 % v/v) for up to 48 h in an anaerobic chamber at 37ºC. Quantitative real-time PCR (qPCR) was used to demonstrate that the fibre samples significantly increased total bacteria as compared with a control (the growth medium inoculated with the faecal microflora alone). The fermenta had increased metabolites such as short chain fatty acids consistent with increased biotransformation. For example, with apple fibre butyric acid increased from 0 to 16 µmol/ml of fermenta. The fermenta obtained at different time points (0, 6, 12, 24 and 48 h) were then incubated with Caco-2 cells to examine the expression of apolipoprotein A-IV by qPCR. Apple fibre increased expression of apoA-IV only at 48 h, while tomato fibre was effective at the end of 6 h and 12 h. Broccoli fibre was the least effective in increasing apo-IV expression. Preliminary results reveal that this regulation of apoA-IV expression by fibres may at least partially correlate with their fermentative ability. We conclude that regular consumption of apple and tomato fibre may influence intestinal apoA-IV synthesis and secretion, thereby influencing satiety. Thus continued and optimised intake of modulators such as dietary fibre may regularly enhance satiety, resulting in long-term dietary change – moderate food consumption, and therefore a better lifestyle.

P43

Colicinogenic *Escherichia coli* isolates from healthy individuals exhibit a similar extra-intestinal virulence profile as non-colicinogenic strains

Živa Petkovšek[1], M. Čitar[1], D. Žgur Bertok[1], M. Starčič Erjavec[1]
[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia;
ziva.petkovsek@bf.uni-lj.si

Colicins are toxic proteins frequently produced by natural isolates of *Escherichia coli* and are lethal for strains of the same or related species. This fact has made them an object of extensive research studying their potential use as antimicrobial agents. As it is well recognised that laboratory strains cannot persist in the natural environment, natural colicinogenic strains should be applied to destroy pathogenic strains by producing effective colicins. However, cases of tight linkage of colicin (ColV) and virulence factor encoding genes have been previously reported. The threat of potential virulence is especially great in strains where colicins are encoded on large conjugative plasmids that can also encode virulence determinants. Since it is known that the bowel flora is a reservoir of pathogenic *E. coli* strains causing extra-intestinal infections (ExPEC), each colicinogenic commensal *E. coli* strain that might be used as a probiotic strain has to be examined for its virulence potential. In order to find potentially useful colicinogenic commensal strains a collection of 90 *E. coli* isolates from healthy individuals (commensal strains) was screened for colicin production and virulence factors. Our results showed that 39 (43%) of the tested strains were colicinogenic. The tested virulence factors (VFs) were quite evenly distributed between colicinogenic and non-colicinogenic strains. A single statistically significant difference was found namely, the association of the aerobactin iron uptake system and colicinogeny (P<0,01), while the prevalence of *iro*, although also more common among colicinogenic strains (41% vs. 20%), was not statistically significant. Iron uptake systems are well recognised for being encoded on large, type II pCol plasmids (pColBM, pColV) therefore, this outcome is not surprising. Other tested VFs were quite evenly distributed among the two subgroups. Of the two toxin genes screened for, *cnf1* and *hlyA*, only *hlyA* was found to be in a single strain associated with colicinogeny. Of 39 colicinogenic strains, 6 were found not to encode any of the 10 tested VFs and could be, in view of virulence hazard, safely used for application with beneficial intentions. Our findings imply that colicinogenic strains are not more virulent than non-colicinogenic strains, however each colicinogenic strain intended for prevention or treatment of disease, should be preliminary tested for possession of virulence determinants, even if isolated from healthy individuals.

P44

Microbial Toll-signalling at the intestinal epithelial surface stimulates apical secretion of CXCL8 and autocrine signalling in villus epithelial cells

Oriana Rossi[1], J. Karczewski[1], M. Meijerink[1], P. van Baarlen[1], J. van Neerven[1], S. van IJzendoorn[2] and J. Wells[1]
[1]University of Wageningen, ASG, Host-Microbe Interactomics Group, the Netherlands and [2]University of Groningen, Faculty Medical Sciences, Cell Biology Group, the Netherlands; oriana.rossi@wur.nl

The commensal microbiota of the intestinal tract is largely symbiotic in nature and over eons of co-evolution multiple adaptations appear to have developed to help maintain this peaceful co-existence. Here we propose a new homeostatic mechanism involving a polarised host response to indigenous microbial TLR ligands. Stimulation of Caco2 monolayers with specific ligands for TLR1/2, TLR2/6, TLR5 and NOD-2 or cytokines TNFα and IL-1β induced the secretion of CXCL8 (also known as Interleukin-8). The polarity of the CXCL8 secretion correlated strongly with the location of the stimulus. Basolaterally secreted CXCL8 is a known inflammatory initiator of leukocyte migration and activator of neutrophils. To investigate the possible effects of apically secreted CXCL8 we used immunofluorescence to localize CXCR1, the major receptor for CXCL8. This receptor was exclusively located on the apical surface of polarized Caco2 cells and the differentiated villus enterocytes in human small intestinal tissue suggesting that apical CXCL8 may have an autocrine function. CXCL8 has previously been shown to induce migration in epithelial cells suggesting that it may initiate pathways involved in epithelial repair. To investigate CXCL8 signalling in epithelial cells a transcriptome analysis was performed on CXCL8 treated Caco2 cells and the results will be presented. Our findings demonstrate that the polarity of intestinal epithelial cells has a major effect on the host response to microbial TLR ligands and the directionality of IL-8 secretion. This homeostatic mechanism implicates a role for microbial ligands and CXCL8 in the process of epithelial restitution and repair.

P45

Modulation of the gut derived microbiota by dietary fibres in an *in vitro* fermentation system

Ida Rud[1], I.M. Aasen[2], B. Moen[1] and S.H. Knutsen[1]
[1]Nofima Mat, Norwegian Institute of Food, Fisheries and Aquaculture Research, Norway and
[2]SINTEF Materials and Chemistry, Department of Biotechnology, Norway;
ida.rud@nofima.no

Dietary fibres may act as prebiotics, i.e. stimulation of growth of health-promoting bacteria such as bifidobacteria and lactobacilli. Fermentation of fibres by the gut microbiota generates short-chain fatty acids (SCFA) and there are indications that SCFA profiles are directly linked to the fibre substrate. Evidently this may include selection of certain bacteria. A range of fibres have been screened by in vitro batch fermentations with faeces from human infants (3-7 months age) and adults. The fibres included polysaccharides or selected fractions, such as β-glucans, arabinoxylans and poly-uronic acids (pectin, alginate). The commercial prebiotic inulin was included for comparison. Changes in the overall composition of the microbiota after growth on the fibres were characterized by establishment and analyses of 16S rRNA clone libraries. SCFA were monitored by HPLC analyses. Distinct differences in the development of the microbiota during the fermentations with infant faeces were observed. For instance, β-glucan and barley fibres highly stimulated *Bacteroides*, while pectin from cabbage stimulated growth of *Lachnospiraceae*. Inulin promoted an increase in *Bifidobacterium*, a well-known effect of this prebiotic. The highest amount of SCFA was produced from inulin followed by pectin, with acetate as the dominating acid. Inulin and barley fibres produced the highest amounts of propionic and butyric acids. Further comparison of the microbiota composition after fermentation with adult faeces will be included, which might reveal different results due to the higher microbial diversity in adult guts compared to infants.

P46

Bifidobacterium animalis Bb-12 survival in probiotic and synbiotic margarine and its resistance under *in vitro* simulated gastro-intestinal conditions

C.H.B. de Souza, R.C.S. Estrotra, L.A..Gioielli and **Susana M.I. Saad**
University of São Paulo, Faculty of Pharmaceutical Sciences, Department of Biochemical-Pharmaceutical Technology, Brazil; susaad@usp.br

Prebiotic ingredients like inulin are frequently added to probiotic foods, in order to improve probiotic populations. Also, whey protein might help probiotic survival. The effect of inulin (I), whey protein concentrate (WPC), and caseinomacropeptide (CMP) on probiotic *Bifidobacterium animalis* Bb-12 viability in margarine supplemented with the probiotic microorganism was investigated. In addition, the *in vitro* resistance of Bb-12 incorporated in margarine was checked. Seven margarine-making trials (60% of fat) were produced and supplemented with Bb-12 - M1: I; M2: WPC; M3: CMP; M4: I+WPC; M5: I+CMP; M6: WPC+CMP, and M7: I+WPC+CMP. Probiotic viability was determined after 1, 7, 14, 21, 28, and 35 days of storage at 5±1ºC. The *in vitro* gastrointestinal simulated resistance of Bb-12, at 37 ºC, covered the pH range from 1.65 to 6.77, with pepsin (3 g/l), pancreatin (1.6 g/l) and bile (5g/L), and was monitored after 7, 14, 21, and 28 days of storage. Samples were analyzed after 2 h (gastric phase), 4 h and 6 h (enteric phase). Margarines supplemented with inulin presented suitable Bb-12 populations throughout the whole storage period, reaching up to 8 log cfu/g by the end of storage (M1). Also, M3 and M6, revealed Bb-12 populations of 6.87 log cfu/g and of 7.27 log cfu/g (day 35), respectively. Even though whey protein is largely employed in probiotic foods, margarine supplementation with WPC without I or CMP did not lead to Bb-12 satisfactory populations, decreasing from 7.82 (day 1) to 4.64 log cfu/g (M2, day 35). During the whole *in vitro* assays, Bb-12 survived significantly better ($p<0.05$) in M1 and revealed populations above 6 log cfu/g after 6 h even after 28 days of storage. M2 populations decreased drastically during the *in vitro* assays for all storage period tested (reduction of 5 log cfu/g after 2 h of *in vitro* assays on day 7 and populations of 2.8 log cfu/g after 6h). For the other formulations, Bb-12 populations decreased 2 log cfu/g after 2 h of the *in vitro* assays on day 7. After 7 and 14 days of storage, all formulations, except M2, presented Bb-12 counts above 6 log cfu/g after 6h of the *in vitro* assays. The supplementation of margarine with inulin and CMP guaranteed appropriate Bb-12 populations during storage for at least 28 days, and also contributed for its survival throughout the *in vitro* assays. Therefore, margarine might be considered an appropriate food matrix for Bb-12 survival, mainly when inulin is also added.

P47

Viability of probiotic strains in a non-dairy rice-based synbiotic dessert

D.M. Kakinoki and **Susana M.I. Saad**
University of São Paulo, Faculty of Pharmaceutical Sciences, Department of Biochemical-Pharmaceutical Technology, Brazil; susaad@usp.br

Functional foods containing probiotic strains and/or prebiotic ingredients are available in different forms, and most of them are dairy products. Nevertheless, the milk content in these products may restrict their intake by a considerable proportion (approximately 70%) of the worldwide population with lactose intolerance. Rice (*Oryza sativa*) is an alternative crop with low residual taste, which is extremely abundant, being the basic food for more than half of the world´s population. This study aimed to evaluate the inclusion or not of a fermentation stage during the production of a potentially non-dairy rice-based synbiotic dessert, prepared with the addition of an ABT culture, containing *Bifidobacterium animalis* subsp. *lactis* Bb-12, *Lactobacillus acidophilus* La-5 and *Streptococcus thermophilus*. The non-dairy dessert was prepared with the addition of inulin and fructo-oligosaccharide (FOS), enriched with whey protein isolate, and stored for up to 21 days at 4-5 ^{o}C. Two different trials were prepared (in triplicates): T1, without the previous fermentation stage after the addition of the ABT culture, and directly cooled for storage; and T2, desserts were previously fermented, at 37 °C for 2 h, after the addition of the culture. Viability of *B. animalis* Bb-12, *L. acidophilus* La-5 and *S. thermophilus* were monitored during the storage period (days 0, 7, 14 and 21 of storage) for each trial. Viability of Bb-12 was higher than La-5 in both trials. Nevertheless, the fermentation stage had implications on growth in different ways for each microorganism. When compared to T2, viability of La-5 was significantly higher (P<0.05) in T1, decreasing from 8.2 log cfu/g (day 1 - pH 5.1) to 7.0 log cfu/g (day 21 - pH 4.9), whereas viability of Bb-12 was just slightly higher in T2, decreasing from 8.8 log cfu/g (day 1 - pH 4.7) to 8.1 log cfu/g (day 21 - pH 4.5). Trial T2 revealed La-5 populations below 5.0 log cfu/g, after 21 days of storage. *S. thermophilus* maintained population above 8.0 log cfu/g after 21 days of storage in all trials. This study suggests each strain must be evaluated independently for a specific product and condition. Moreover, the inclusion of a fermentation stage during production may affect the viability of probiotic strains in different ways.

Acknowledgments
FAPESP, CAPES, CNPq.

P48

Probiotic *Lactococcus lactis* (NCC2287) reduces allergic symptoms in sensitized mice

A. Zuercher, S. Holvoet, M. Weiss, **Anurag Singh**, S. Nutten, G. Prioult and A. Mercenier
Nestlé Research Center, Nutrition and Health Department, Allergy Group, Switzerland;
anurag.singh@rdls.nestle.com

Lactococci are traditional dairy starter lactic acid bacteria and are also evaluated for their capacity to provide a health benefit to the host. Utilizing both an *in vitro* assay and a food allergy mouse model, we investigated the anti-allergic potential of *Lactococcus lactis* strain NCC2287. In an initial step we developed a cell culture model of Th2-skewed peripheral blood mononuclear cells (PBMC) using interleukin 4 (IL-4) and anti-CD40 antibody. After screening of a series of lactic acid bacterial strains we selected 2 *Lactococcus* strains for further evaluation based on their cytokine profile. NCC2287 strongly induced secretion of IFN and IL-10, concomitant with inhibition of IL-5. Based on this data we further evaluated the effect of this strain in an *in vivo* food allergy model. BALB/c mice were sensitized at weekly intervals with ovalbumin (OVA) + cholera toxin (CT) by the oral route for 7 weeks. In this model, oral challenge with a high dose of OVA (100 mg) at the end of the sensitization period leads to clinical symptoms such as diarrhoea, scratching episodes, bristled fur, and reduced mobility. *L. lactis* NCC2287 ($5x10^8$ cfu/ml; *ad libitum*) was given to mice via the drinking water during the sensitization phase from day 1 to 43 (prevention) or during the last week of the experiment (day 43-50; symptom reduction). While no preventive effect with the strain was observed, NCC2287 administration to sensitized mice strikingly reduced allergic manifestations upon challenge when compared to control mice. Future studies are needed to better elucidate the mechanisms of action via which NCC2287 exerts its immunomodulatory effect.

P49

The human ileal microbiota and its function: development of a dynamic *in vitro* model for the human ileum

Maria Stolaki[1,2,3], E.G. Zoetendal[1,2], E.J. Smid[1,4], M. Minekus[1,3], K. Venema[1,3] and M. Kleerebezem[1,2]
[1]Top Institute Food and Nutrition, the Netherlands, [2]Wageningen University, Laboratory of Microbiology, the Netherlands, [3]TNO Quality of Life, the Netherlands and [4]NIZO food research, the Netherlands; maria.stolaki@tno.nl

The responses of the small intestinal microbiota to food intake at the level of species composition and metabolic activity are assumed to be highly dynamic. However, the composition of the microbes in the small intestine and their function remain largely unexplored due to the poor accessibility of the small intestine in healthy individuals. A preliminary study that monitored the population dynamics in the small intestine by using a phylogenetic microarray indicated that the microbiota in ileostomy effluent is fluctuating in time, even within a day [1]. To obtain insight in dominant factors that determine the dynamics in phylogenetic and functional composition of the ileum microbiota, we aim to develop, validate and apply a dynamic *in vitro* model simulating the human distal small intestine (ileum) for long term experiments, including complex fermentation. The model prototype has currently been developed based on knowledge obtained in TNO Research Institute with the *in vitro* intestinal models (TIM models) simulating the small and the large intestine. To determine if a stable and complex microbiota can be obtained in this *in vitro* ileum model-system, a faecal sample was used as inoculum and the microbiota composition was followed in time using HITChip phylogenetic microarray technique. The microbial profiles obtained with HITChip analyses indicate that the microbial population diversity and complexity within the *in vitro* ileum could be maintained for a long time-period. However, even after 15 days no steady state of the microbiota had been achieved, which may be due to lack of sufficient control of the physico-chemical conditions within the system. Nevertheless streptococci and actinobacteria populations increased during the experiment, from which the first one is known to be dominantly present in ileostoma effluent [1]. However, the other prominent small intestinal groups, such as *Veillonella* appeared to be absent in the model system, which may be caused by the use of a faecal inoculum. Further experiments are underway to reach a stable microbial community in the ileum model, using ileostoma effluent as inoculum.

References

1. Booijink, C.C.G.M., 2009 Ph.D. Thesis, Wageningen University, the Netherlands.

P50

Resident microbiota and *Streptococcus thermophilus* modulate the amount of cell-cycle related proteins of colonic epithelium

C. Cherbuy, F. Rul, L. Wzrosek, L. Ben-Yahia, N. Bouznad, E. Honvo-Houeto, M.L. Noordine, C. Mayeur, P. Langella and **Muriel Thomas**
INRA, Micalis UMR 1319, France; muriel.thomas@jouy.inra.fr

Convergent data suggest that intestinal microbiota modulates the proliferation and differentiation processes of the intestinal epithelium, which is one of the most dynamic tissues in the whole organism. We recently observed in gnotobiotic rat colon a coordinated induction of cell cycle related proteins in response to bacterial colonization. Two days after the transfer of a complex microbiota in germfree (GF) rats, we observed a rapid recruitment of proliferative cells, stained with PCNA and Ki67. This process is counterbalanced by the induction of $p21^{cip1}$ and $p27^{Kip1}$, two proteins restraining proliferation. These mechanisms has been associated with the increase of colonic crypt depth observed during the colonization process [1]. In this context, the objectives of the present work were (i) to explore the effects of a complex microbiota colonization on cell cycle-related proteins playing a key role in proliferation control, and (ii) to determine whether transiting lactic acid bacteria may modulate gut proliferation/differentiation by using *Streptococcus thermophilus*, which is daily largely consumed through dairy products (yoghurt in particular). All experiments were conducted in GF rats either inoculated with a faecal conventional microbiota or with *S. thermophilus*. The cell-cycle related proteins in colonic epithelial cells were studied by immunohistochemistry and Western blot. We also profiled the protein expression of *S. thermophilus* after its adaptation in the gut thanks to the facilities of the PAPSSO proteomic platform (http:// www.jouy.inra.fr/unites/proteines/papsso/). In conclusion, $p27^{Kip1}$ was induced in colon by a complex microbiota but also by *S. thermophilus in vivo.* Since the glycolytic pathway was preferentially activated by *S. thermophilus* in gut*,* we propose that lactate may serve as a biological signal to communicate with host epithelium by increasing $p27^{Kip1}$.

References

1. Cherbuy, C., Honvo-Houeto, E., Noordine, M.L., Mayeur, C., Bruneau, A., Bridonneau, C., Langella, P., Duée, P.-H. and Thomas, M. Intestinal microbiota matures the colonic epithelium through a well synchronized induction of cell-cycle related proteins. (under review in American Journal of Physiology).

P51

The use of stable isotope labelled substrates to study fermentation in the gut

Koen Venema, A. Maathuis and A.A. de Graaf
TNO Quality of Life, Department of BioSciences, the Netherlands; koen.venema@tno.nl

The gastro-intestinal tract accommodates trillions of living bacteria (collectively called the gut microbiota) that have a symbiotic relationship with the host. The composition of the gut microbiota is different in every individual. However, the activity displayed by the collective microbiota is markedly similar between different individuals, likely because there is enormous functional overlap between the different species in terms of fermentation capacity. Short-chain fatty acids (SCFA; primarily acetate, propionate and butyrate) are the major microbial metabolites produced within the intestinal lumen by bacterial fermentation of undigested dietary carbohydrates. These SCFA are beneficial to the host [1]. The influence on the microbiota and the subsequent health effects of functional food components should be investigated in order to proof possible health claims of functional foods. We have used a dynamic, validate computer-controlled *in vitro* laboratory model of the colon (nick-named TIM-2) in the development of new potential prebiotic substrates, for which it is imperative to know exactly what is going on in the complex microbiota. Currently, it is unknown which micro-organisms are involved in direct fermentation of added substrates, and exactly which metabolites are formed from the substrates. Therefore, we have developed a technology using stable isotopes (primarily ^{13}C) to investigate this is detail. The technologies that have been developed using ^{13}C-labeled substrates are stable-isotope probing of 16S rRNA (16S rRNA SIP; [2]) and specific analytical methods to measure the different isotopomers of the SCFA produced from the labelled substrate [3,4]. Using model substrates, the members of the microbiota that play a role in fermentation of the model substrates have been identified. In addition, the metabolites that have been produced from the substrates have been characterized using LC-MS and NMR. In addition, tools have been developed to carry these technologies from *in vitro* models to the human clinical application, including a lab-on-a-chip that takes samples from the human gastro-intestinal tract, and a nasal-tube to deliver (^{13}C-labeled) substrates to the terminal ileum/caecum. Several clinical studies have been performed in human volunteers to study the role and effect of butyrate on gut health. This presentation will high-light the latest results obtained in this exiting, fast moving field of science, relating microbiota activity to health and disease. With the developed techniques it is possible to trace the label to both microbial metabolites as well as microbial biomass, and this allows for (i) the precise determination of which microbes are involved in fermentation of the prebiotic, and (ii) characterization of the metabolites that are produced from it. This is crucial information in both claim support of the health benefits of prebiotics, as well as for understanding mechanistically what happens in such a complex microbiota. The understanding of these processes will allow for tailor-made new prebiotics to be developed, or for different applications of existing compounds.

References

1. Hamer, H., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. and Brummer, R.-J., 2008. Review article: the role of butyrate on colonic function. Alimentary Pharmacology and Therapeutics 27:104-119.
2. Egert, M., De Graaf, A.A., Smidt, H., De Vos, W.M. and Venema, K., 2006. Beyond diversity: functional microbiomics of the human colon. Trends in Microbiology 14:86-91.
3. Meesters, R.J.W., Van Eijk, H.M.H., Ten Have, G.A.M., De Graaf, A.A., Venema, K., Van Rossum, B.E.J. and Deutz, N.E.P., 2007. Application of liquid chromatography-mass spectrometry to measure the concentrations and study the synthesis of short chain fatty acids following stable isotope infusions. Journal of Chromatography B 854:57-62.
4. De Graaf, A.A. and Venema, K., 2007. Gaining insight in microbial physiology in the large intestine: a special role for stable isotopes. Advances in Microbial Physiology 53:73-168.

P52

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

Koen Venema[1], A. Maathuis[1], I. Giesbrecht[2], P. Bohnhorst[2] and A. Christ[2]
[1]TNO Quality of Life, Department of BioSciences, the Netherlands and [2]Merck Selbstmedikation GmbH, Department Research & Development, Germany;
koen.venema@tno.nl

The aim of this study was to investigate the 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, 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 hour 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 hour after the meal, survival was 0.6% and 0.8%, respectively. In conclusion, use of the 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.

Acknowledgements
Research support by Merck Selbstmedikation GmbH.

P53

Faecal bacterial communities in healthy controls and ulcerative colitis patients

Louise K. Vigsnæs[1], A. Wilcks[1], J. Brynskov[2] and T.R. Licht[1]
[1]DTU Food, Gut Ecology, Denmark and [2]Herlev University Hospital, Department of Medical Gastroenterology, Denmark; lokv@food.dtu.dk

Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease (IBD) that is characterized by chronic inflammation of the colonic mucosa. The aetiology of IBD is not well understood, however the commensal intestinal microbiota is thought to play an important pathogenetic role. Hence, a detailed knowledge about the composition of the intestinal microbiota may be critical to unravel the pathogenesis of IBD. The aim of this study was to examine if the faecal microbiota of patients with UC differs from that of healthy subjects. Faecal samples were collected from healthy subjects and from UC patients with either clinically inactive or active disease. To analyse the composition of the faecal microbiota, we performed quantitative PCR (qPCR) using species and group-specific primers targeting *Bifidobacterium* spp., *Lactobacillus* spp., *Firmicutes*, *Bacteroidetes* and *Faecalibacterium prausnitzii*. Denaturing gradient gel electrophoresis (DGGE) analysis using a universal primer targeting bacterial 16S rRNA genes were carried out in order to identify differences in species composition. The results obtained from the qPCR showed that the UC patients, irrespective of the stage of disease activity had a significantly lower amount of *Bacteroidetes* compared to the healthy controls ($P<0.001$). In addition, UC patients with inactive disease had a significantly higher amount of *Firmicutes* compared to the other two groups (healthy controls ($P<0.05$); active group ($P<0.001$)). However, there was no significant difference between groups in the amount of *Bifidobacterium* spp., *Lactobacillus* spp. and *F. prausnitzii*. The DGGE profiles demonstrated large individual variation. However, principal component analysis (PCA) revealed that the composition of faecal bacteria from active UC patients differed compared to the other two groups. Sequencing of bands, which are either abundant or absent in the profiles of the patients with active UC will be carried out to reveal which bacterial species the bands represent. In conclusion, we have shown that the faecal microbiota in UC patients is associated with loss of dominant bacterial phyla such as *Bacteroidetes*. Furthermore, the composition of bacteria in the faecal microbiota in patients with active UC differs from that in inactive UC and healthy controls. These findings may help to improve understanding the role of intestinal microbiota in UC.

P54

Lactobacillus plantarum enhances human intestinal barrier function via a TLR2 and PKCδ-mediated mechanism

J. Karczewski[1,2], P. van Baarlen[1,2], J. Dekker[2] and **Jerry Wells[1,2]**
[1]University of Wageningen, Host-Microbe Interactomics, the Netherlands and [2]Top Institute Food and Nutrition, the Netherlands; jerry.wells@wur.nl

Lactobacillus plantarum (LP), a commensal bacterium of humans, is able to modify the intestinal barrier in of human volunteers and intestinal cell-lines via a Toll-like receptor (TLR) 2 pathway. To study the mechanism by which TLR signalling can modify tight junction proteins a genomic study was performed. Caco-2 BBE cells were treated for 2 and 6 h with LP, and TLR2 ligands PAM_3CSK and lipoteichoic acid isolated from LP. 538 Genes were differentially regulated after 6 h exposure to LP ($P \leq 0.01$). No unregulated expression of tight junction proteins or tight junction associated proteins was observed but network analysis indicated that epithelial cells remodel after TLR2 stimulation. Affected networks include: cellular development, lipid metabolism, molecular transport, cellular assembly and organization, post-translational modification and lipid metabolism. Using confocal laser scanning microscopy, phosphorylation of ERK1/2 and p38 was observed in LP, LTA and PAM_3CSK -treated Caco-2 cells. Furthermore, we observed TLR2 induced translocation of cytosolic PKCδ towards the apical part of the cell and to the tight junctions. Analysis of human biopsies of the duodenum also showed a very strong apical and junctional staining of PKCδ in villus cells. Surprisingly, no PKCδ was observed in crypt cells of the duodenal biopsy. This suggests that PKCδ is associated with differentiation of crypt cells into villus epithelial cells. TLR2 signalling can enhance the activation of PKCδ which may play a role in the modification of tight junctions. We are now investigating the specific targets of PKCδ in TLR2 triggered intestinal epithelial cells.

P55

Dietary non-digestible carbohydrates induce CD25$^+$ regulatory T-cells that protect mice from developing casein allergy

B. Schouten[1], B.C.A.M. van Esch[1,2], G.A. Hofman[1], S. de Kivit[1], L. Boon[3], L.M.J. Knippels[3], J. Garssen[1,2] and **Linette E.M. Willemsen[1]**
[1]Utrecht University, Utrecht Institute for Pharmaceutical Sciences, Pharmacology and Pathophysiology, the Netherlands, [2]Danone Research - Centre for Specialised Nutrition, the Netherlands and [3]Bioceros, the Netherlands; l.e.m.willemsen@uu.nl

Specific dietary prebiotic non-digestible carbohydrates have been shown to reduce the incidence of atopic dermatitis in infants at risk when introduced early in life provided via infant milk formula. They may exert their effect via selective stimulation of health promoting bifidobacteria and lactobacilli or they may directly interact with the (mucosal) immune system. These non-digestible carbohydrates were also found to partially prevent the development of cow's milk allergy in mice. In this study the contribution of CD25$^+$ regulatory T-cells (Treg) to the protective effect of specific dietary prebiotics was investigated using *in vivo* CD25$^+$ depletion and adoptive transfer studies. Mice were sensitized with cow's milk protein casein and fed a diet containing 2% short-chain galacto-, long-chain fructo-, and acidic-oligosaccharides (GFA) or control diet. *In vivo* depletion of CD25$^+$ Treg was performed using anti-CD25 (PC61). In addition, donor splenocytes of mice sensitized with casein and fed the GFA or control diet were adoptively transferred to naïve recipient mice in presence or absence of *ex vivo* depleted CD25$^+$ Treg. Recipient mice were sham or casein sensitized and fed the control diet. The acute allergic skin reaction upon i.d. casein challenge, casein-specific immunoglobulins (Ig) and T_H1 and T_H2 counts were determined. The GFA diet enhanced T_H1 and tended to reduce the percentage of T_H2 cells in mesenteric lymph nodes. The acute allergic skin reaction was reduced by GFA and *in vivo* anti-CD25 treatment abrogated this. Splenocytes from casein-sensitized GFA fed donor mice prevented recipient mice from developing an acute allergic skin reaction and e*x vivo* depletion of CD25$^+$ Treg prevented this without affecting Ig. The protection by the GFA diet was allergen specific since sham sensitized donor mice fed the control diet did not protect recipient mice from developing an allergic skin response. In conclusion, CD25$^+$ Treg were found to transfer tolerance, resulting in suppression of the allergic effector response occurring after dietary intervention with GFA in casein-sensitized mice.

P56

Cow's milk allergy symptoms are reduced in mice fed dietary synbiotics during oral sensitization with whey

B. Schouten[1], B.C.A.M. van Esch[1,2], G.A. Hofman[1], S.A.C.M. van Doorn[2], J. Knol[2], A.J. Nauta[2], J. Garssen[1,2], **Linette E.M. Willemsen[1]** and L.M.J. Knippels[1,2]
[1]Utrecht University, Utrecht Institute for Pharmaceutical Sciences, Pharmacology and Pathophysiology, the Netherlands and [2]Danone Research - Centre for Specialised Nutrition, the Netherlands; l.e.m.willemsen@uu.nl

Cow's milk allergy is the most common food allergy in children. So far, no effective treatment is available to prevent or cure food allergy. The purpose of this study was to compare effects of dietary supplementation with a prebiotic non-digestible carbohydrate mixture (Immunofortis®), a candidate probiotic strain (*Bifidobacterium breve* M-16V), or a synbiotic diet combining both, on the outcome of the allergic response when provided during oral sensitization with whey in mice. Mice were fed diets containing 2 w/w% Immunofortis® (a mixture of short chain galacto- and long chain fructo-oligosaccharides) and/or the *B. breve* M-16V (n=6 per group). The acute allergic skin response was determined by measuring ear swelling one hour after dermal injection with whey. Antigen-induced anaphylaxis was scored. Furthermore whey-specific serum immunoglobulins and mouse mast cell protease-1 (mMCP-1) were determined. In mice fed the synbiotic mixture the allergic skin response and the anaphylactic reaction was strongly reduced as compared to whey-sensitized mice fed control diet ($P< 0.01$). Immunofortis® or *B. breve M-16V* alone were significantly less effective in reducing the allergic skin response than the synbiotic diet and did not reduce the anaphylactic reaction. The whey-specific IgE and IgG_1 responses were not affected, however IgG_{2a} was significantly enhanced in all treated groups compared to control group ($P< 0.05$). Serum mMCP-1 concentrations, reflecting mucosal mast cell degranulation, were lower in mice fed synbiotics compared to those fed control diet ($P< 0.01$). In conclusion, dietary supplementation with Immunofortis® and *B. breve* M-16V and particularly the synbiotic mixture, provided during sensitization, reduces the allergic effector response in a murine model of IgE-mediated hypersensitivity that mimics the human route of sensitization. This model shows the potential for dietary intervention with synbiotics in reducing the allergic response to food allergens.

P57

Lipoteichoic acid from Gram-positive bacteria shows positivity in the *Limulus* amebocyte lysate assay and binds to polymyxin B

Zdenek Zídek and E. Kmoníčková
Academy of Sciences, Institute of Experimental Medicine, Czech Republic;
zidekz@biomed.cas.cz

Lipoteichoic acid (LTA) is an integral component of the cell walls of Gram-positive bacteria including the probiotic strains. Similar to lipopolysaccharide (LPS), expressed in outer membranes of Gram-negative bacteria, LTA has been reported to possess remarkable immunostimulatory properties. Credibility of the existence of their intrinsic immune potential has however been stigmatized by the reports suggesting that the commercial preparations may be contaminated with LPS. Yet, the source of LPS in LTA samples has never been satisfactorily explained. Therefore, we have addressed the question whether the positivity of LTAs in the Limulus amebocyte lysate (LAL) assay does represent the presence of LPS or rather is an artefact signalling the cross-reactivity of LPS and LTA in the LAL test. We found the LAL positive response of LTAs from *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis* and *S. pyogenes*. The findings could seemingly suggest that the samples would be contaminated with LPS reaching as high amounts as 605, 10.3, 6.2, and 127 pg per 1 µg of LTA, respectively. The effects of the LTAs on the *in vitro* production of nitric oxide (NO) were evaluated in the cultures of rat resident peritoneal cells ($2x10^6$/ml; 24 h) using the Griess reagent. All LTA samples were found to induce the high output NO production (approx. 80 µM). The most effective NO inducer proved to be the *B. subtilis* LTA (EC_{50} = 0.43 µg/ml) while the least effective was the *S. aureus* LTA (IC_{50} = 4.89 µg/ml). The LTA concentrations (µg/ml) were also expressed in terms of the amount of suspected LPS in the LTA samples ('sLPS/LTA', pg/ml). The NO-stimulatory effects of the accordingly diluted samples were compared with the effects of the equivalent doses of the reference LPS (*Escherichia coli* 055:B5; 0.1-1,000 pg/ml). The reference LPS was found to be much weaker inducer of NO than the equivalent concentrations of sLPS/LTA. Moreover, while the reference LPS-induced NO production was completely abrogated by polymyxin B (PMX) applied at the PMX:LPS ratio of 10,000:1, the NO production activated by identical concentrations of sLPS/LTA was only partially suppressed under these conditions. The inhibitory effects of PMX on the LTA-induced NO could only be enhanced with increasing doses of PMX. The estimates of the PMX:LTA ratio (µg:µg) necessary to inhibit by 50% the NO-stimulatory effects of LTA (IC_{50}s) reached the values of 0.3:1, 1.1: 1, 1.8:1, and 7.68:1 for the *S. aureus*, *S. faecalis*, *S. pyogenes* and *B. subtilis* LTAs, respectively. It can be concluded that LTA is a substrate exhibiting low binding capacity in the LAL assay. The positive response of LTA in this test is therefore not necessarily a measure of the contamination of commercially available LTA preparations with LPS. Similar to LPS, LTA is liable to the neutralizing activity of PMX. The capacity of PMX to bind LTA is much higher than that to bind LPS. The data provide the evidence showing unequivocally that LTAs *per se* possesses powerful immunostimulatory activity.

Acknowledgements
The work was supported by grant no. 305/08/0535 from GAČR.