FINAL PROGRAMME
&
ABSTRACTS OF LECTURES AND POSTERS

3rd TNO Beneficial Microbes Conference

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

26-28 March 2012
Noordwijkerhout, the Netherlands

3rd TNO Beneficial Microbes Conference

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

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WELCOME AT THE *3rd* TNO BENEFICIAL MICROBES CONFERENCE!

Dear participant,

It is with great pleasure that we announce the upcoming **3rd TNO Beneficial Microbes Conference**, to be held in Noordwijkerhout, the Netherlands, on 26-28 March 2012.

The microbiota in the gastro-intestinal tract of man and animals has been shown to be important for health and disease. Moreover, over the past decades the benefit of probiotics has been shown in various areas such as allergy, inflammatory disease, competitive exclusion of pathogens, stool habit, etc. 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. The role of prebiotics in directing the composition and activity of the endogenous microbiota is also studied widely.

The **3rd 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. Specific topic areas include beneficial microbes and the host metabolism, host-microbe communication, beneficial microbes and immune modulation, metagenomics and microbiome, application of beneficial microbes and prebiotics, and validated in vitro models.

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

***3rd* TNO Beneficial Microbes Conference**

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

3rd TNO Beneficial Microbes Conference

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

CONFERENCE PROGRAMME

Monday 26 March 2012

10.30 Opening of the ***3rd* TNO Beneficial Microbes Conference**

10.45 **Keynote lecture**
Setting the scene: How to define a beneficial microbe?
Prof.dr. Eric Claassen, VU University Amsterdam / Erasmus MC, the Netherlands

SESSION 1: BENEFICIAL MICROBES AND HOST-MICROBE COMMUNICATION

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

11.15 *Gate-keeper function of the epithelium*
Prof.dr. Per Brandtzaeg, University of Oslo and Oslo University Hospital, Norway

11.45 *Inter-kingdom chemical signalling in bacterial-host associations*
Dr. Vanessa Sperandio, University of Texas Southwestern Medical Center, USA

12.15 *Probiotic mechanism to enhance influenza vaccination*
Dr. Marjolein Meijerink, Host-Microbe Interactomics, Wageningen UR, the Netherlands

12.45 **Contributed paper**
Antimicrobial functions of intestinal Reg3ß
Linda Loonen, M.Sc., TI Food & Nutrition / Wageningen University, the Netherlands

13.00 **Lunch break**

Monday 26 March 2012

SESSION 2: EFFECT OF BENEFICIAL MICROBE METABOLITES ON THE HOST

Chair: Dr. Ger Rijkers, University Medical Centers of Utrecht and Nijmegen and St. Antonius Hospital Nieuwegein, the Netherlands

14.00 Speaker to be announced

14.30 *Effect of propionate on adipose metabolism*
Dr. Koen Venema, TNO Healthy Living, the Netherlands

15.00 *Administration of butyrate producing organisms in to treat IBD*
Dr. Hana Kozáková, Institute of Microbiology, Academy of Sciences, Czech Republic

15.30 **Contributed paper**
Lactate produced by Streptococcus thermophilus in intestinal tract of gnotobiotic rats may trigger goblet cells differentiation pathway: a new probiotic function?
Laura Wrzosek, Commensal and Probiotics-Host Interactions Laboratory, INRA, France

15.45 **Networking break**

SESSION 3: SHAPING AND MODULATING MICROBIOTIA COMPOSITION

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

16.15 *Evolution of the microbiota and the influence of diet*
Prof.dr. Michael Blaut, German Institute of Human Nutrition Potsdam-Rehbrücke, Germany

16.45 *Breast milk – the source of more life than we imagine*
Dr. Rocio Martin, Danone Research, the Netherlands

17.15 *A novel approach to microbial replacement: 30 strain transplant*
Dr. Elaine O. Petrof, Department Infectious Diseases, Biomedical and Molecular Sciences, Oueen's University, Canada

17.45 *Functional foods for digestive health in dogs*
Dr. Julie K. Spears, Nestlé Purina PetCare, USA

18.15 **Contributed paper**
The ontogeny of the intestinal IgA CDR3 repertoire and IgA production in response to the gut microbiota
Gerco den Hartog, Cell Biology and Immunology Group/Adaptation Physiology Group, Wageningen UR, the Netherlands

18.30 - 19.30
TNO's LOUNGE PARTY

Tuesday 27 March 2012

SESSION 4: MINING THE METAGENOME AND MICROBIOME OF THE GUT

Chair: Dr. Jiro Nakayama, Kyushu University, Japan

08.30 *Tools to display metagenomics data*
William A. Walters, Department of Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, USA

09.00 *Metagenomics screening (MetaHit)*
Dr. S. Dusko Ehrlich, MICA Department, INRA, France and Académie des Arts et Sciences, Croatia

09.30 *Asian Microbiome Project: a pilot study on the basal microbiota profile of healthy youngsters*
Dr. Yuan Kun Lee, Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

10.00 **Contributed paper**
A multiscale systems biology approach to the human Bacteroides thetaiotaomicron
Dr. Ines Thiele, Faculty of Industrial Engineering, Mechanical Engineering and Computer Science, University of Iceland, Iceland

10.15 **Networking break**

SESSION 5: CAN BENEFICIAL MICROBES PREVENT DISEASES?

Chair: Prof.dr. Michael Cabana, University of California, San Francisco, USA

10.45 *Bifidobacteria protect the host from enteropathogenic infection through production of acetate*
Dr. Hiroshi Ohno, Research Center for Allergy and Immunology, RIKEN Japan

11.10 *Beneficial microbes – the link between diet and cancer risk?*
Dr. Wendy S. Garrett, Department of Immunology and Infectious Diseases, Harvard School of Public Health and Dana-Farber Cancer Institute, USA

11.35 *Intestinal dysbiosis in coeliac disease: is there a role for probiotics?*
Dr. Yolanda Sanz, Institute of Agrochemistry and Food Technology, Spanish National Research Council, Spain

12.00 *The airway microbiome in asthma and COPD*
Yvonne J. Huang, Department of Medicine, University of California, San Francisco, USA

12.25 **Contributed paper**
Influence of human gut microbiota on the metabolic fate of glucosinolates
Vijitra Luang-In, Department of Life Sciences, Imperial College London, UK

12.40 **Lunch break** offered by Danone

Tuesday 27 March 2012

SESSION 6: VALIDATED *IN VITRO* AND *EX VIVO* MODELS

Chair: Dr. Koen Venema, TNO, the Netherlands

13.30 *Organotypic co-culture models to study the effect of beneficial microbes functionally*
Dr. Manfred Schmolz, EDI GmbH, Germany

14.00 *Use of an in vitro model to study fermentation by the microbiota*
Dr. Marjorie Koenen, TNO, the Netherlands

14.30 *Assessment of survival of probiotics under various conditions using a validated in vitro model*
Dr. Thomas D. Leser, Health & Nutrition Division, Chr. Hansen, Denmark

15.00 *A novel polarized ex vivo organ culture model to study beneficial microbes*
Dr. Katerina Tsilingiri, Department of Experimental Oncology, European Institute of Oncology, Italy

15.30 **Contributed paper**
Glycerol supplementation boots Lactobacillus reuteri's protective effect against Salmonella typhimurium infection in a 3-D organotypic model of colon epithelium
Rosemarie De Weirdt, Laboratory of Microbial Ecology and Technology, Ghent University, Belgium

15.45 **Networking break**

SESSION 7: TOWARDS SUCCESSFUL CLAIMS FOR PRE-AND PROBIOTICS

Chair: Dr. Gregor Reid, University of Western Ontario, Canada

16.15 *Beneficial microbes: claims and ethics*
Dr. Diane Hoffmann, School of Law, University of Maryland, USA

16.45 *Critical issues for successful claim applications*
Dr. Alwine F.M. Kardinaal, TNO, the Netherlands

17.15 *Why do we need claims on products? Consumers don't!?*
Dr. Ger Rijkers, University Medical Centers of Utrecht and Nijmegen and St. Antonius Hospital Nieuwegein, the Netherlands

17.45 **Contributed paper**
Primo-colonizing bacteria induce maturation of colonic epithelium in gnotobiotic rat models
Dr. Claire Cherbuy, Commensal and Probiotics-Host Interactions Laboratory, INRA, France

18.00 - 19.30
POSTER PRESENTATIONS + DRINKS

Wednesday 28 March 2012

SESSION 8: RECENT SUCCESSFUL APPLICATIONS OF BENEFICIAL MICROBES

Chair: Dr. Frédérique Chaucheyras-Durand, Lallemand, France

08.30 *Microbial mitigation: the Achilles' heel of allergy?*
Prof.dr. Harry Wichers, Food & Biobased Research, Wageningen UR, the Netherlands

08.50 *Effect of B. breve Yakult on childhood constipation – a case study*
Eline van Bel, Waterland Hospital, the Netherlands

09.10 *Probiotics for HIV subjects in the developing world*
Dr. Gregor Reid, Lawson Health Research Institute and University of Western Ontario, Canada

09.30 *Control of bacterial diseases in aquaculture with pre- and probiotics*
Prof.dr. Peter Bossier, Laboratory of Aquaculture & Artemia Reference Center, Department of Animal Production, Ghent University, Belgium

09.50 *Live yeast probiotics and immunomodulation in pigs*
Dr. Kenneth Mellits, Division of Food Science, School of Biosciences, University of Nottingham, UK

10.10 **Contributed paper**
Influence of probiotics on the intestinal epithelial barrier
Dr. Saskia van Hemert, Winclove, the Netherlands

10.30 **Networking break**

SESSION 9: FUTURE DEVELOPMENTS – NEW TOOLS

Chair: Dr. Koen Venema, TNO, the Netherlands

11.00 *Drosophila as a model system to study microbiota/gut interaction*
Prof. Dr. Julien Royet, Developmental Biology Institute of Marseilles – Luminy, CNRS, France

11.30 *A microfluidic platform for droplet-enabled co-cultivation of microbial communities*
Jihyang Park, M.Sc., Department of Chemical Engineering, University of Michigan at Ann Arbor, USA

12.00 *Single cell genomic sequencing of uncultured bacteria for the human microbiome project*
Prof.dr. Roger Lasken, J. Craig Venter Institute, USA

12.30 *From stem-cells to self-organizing organoids*
Prof.dr. Hans Clevers, Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Centre Utrecht, the Netherlands

13.00 Closing of the ***3rd*** **TNO Beneficial Microbes Conference**
(including a packed lunch to eat along the way!)

LECTURES

Gate-keeper function of the intestinal epithelium

Per Brandtzaeg
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A number of biological variables influence induction of oral tolerance and secretory IgA (SIgA)-dependent intestinal immunity, both of which are essential for mucosal homeostasis. There is currently a major focus on the important role of the gut barrier function in balancing immune responses. Increased epithelial permeability for exogenous antigens is clearly a crucial primary or secondary event in the pathogenesis of several disorders affecting body surfaces and beyond. The epithelial gate-keeper function is determined by the individual's age (e.g., preterm *versus* term infant), diet, genetics, mucus composition, interactions between mast cells, nerves and neuropeptides, concurrent infection, the commensal microbiota and the epithelium-shielding effect of SIgA provided by breast milk or produced in the individual's gut. The integrity of the epithelial barrier furthermore depends on homeostatic regulatory mechanisms, including mucosal induction of regulatory T (Treg) cells, where commensal microbial-host interactions apparently play decisive roles. There is considerable interest in how extrinsic and intrinsic variables impact on the intercellular tight junctions and thereby on the epithelial barrier in the gut.

The secretory immune system is critical for the mucosal barrier function because SIgA not only forms the first line of adaptive immune defence but also maintains mutualism with the indigenous microbiota. In mouse experiments it has indeed been shown that a single immunomodulatory molecule from a commensal gut bacterium can induce crucial modulation and homeostasis of the host's immune system. Also notably, the epithelial barrier depends on exposure to immune-modulating components with conserved microbe-associated molecular patterns (MAMPs) from the commensal microbiota and the environment, both by direct interaction with germline-encoded pattern recognition receptors (PRRs) on the intestinal epithelium and by induction of oral tolerance via mechanisms such as tolerogenic antigen-presenting cells and Treg cells. Before the adaptive immune system is fully developed with regard to SIgA production, breast-feeding is immunologically important, not only as a natural 'substitution therapy' which is integrated with the mother's mucosal IgA system, but apparently also by its numerous immune-modulating properties. Experiments have shown that SIgA normally cooperates with innate defence to protect the epithelium, but in the absence of SIgA the commensal gut bacteria overstimulate innate epithelial immunity at the expense of expression of genes that regulate fat and carbohydrate metabolism, resulting in a gene signature in the gut epithelium that correlates with the development of lipid malabsorption. This shows that the intestinal epithelial barrier is a cross-road between nutrition and defence, and that SIgA is essential to keep the balance between these two functions.

Inter-kingdom chemical signalling in bacterial-host associations

A.R. Pacheco[1], J. Ritchie[2], M. Waldor[1], C.G. Moreira[2] and **Vanessa Sperandio[1]**
[1]Departments of Microbiology and Biochemistry, University of Texas Southwestern Medical Center, USA and [2]Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, USA
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Enterohaemorrhagic *Escherichia coli* (EHEC) relies on two inter kingdom chemical sensing systems to regulate its virulence gene expression within the host intestine. Here we show that both of these systems also control the expression of a novel two component signal transduction system that senses fucose and controls expression of virulence and metabolic genes. This fucose-sensing system is required for robust EHEC intestinal colonization. During growth in mucus, the glycophagic *Bacteroides thetaiotaomicron* supplies fucose to EHEC, modulating its virulence gene expression. Our findings suggest that EHEC uses fucose, a host-derived signal made available by the microbiota, to modulate EHEC pathogenicity and metabolism and suggest a new layer of complexity in the inter kingdom signalling that underlies EHEC pathogenicity.

Translational research on probiotics: applications in vaccination and atopic disease

Marjolein Meijerink
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Beneficial effects of strains of probiotics have been established in the treatment and prevention of various intestinal disorders, including allergic diseases, colitis and diarrhoea. However, there are many negative or inconclusive studies on probiotics highlighting the need for a better understanding of the mechanisms and improved selection criteria. Data will be shown on the use of *in vitro* models to characterize the immunomodulatory potential of different lactobacilli and mechanistic research on the application of selected strains in different mouse models. In addition the predictive value of *in vitro* immune assays for the effects of probiotics *in vivo* will be discussed.

The immune profiles of 28 commercially available bacterial strains were obtained using co-culture assays with human PBMCs *in vitro* and selected strains were tested for their prophylactic effects in an established mouse peanut allergy model. The 28 probiotic strains induced highly variable cytokine profiles in PBMCs. Three lactobacilli strains were selected for further investigation due to their distinct patterns of IL-10, IL-12 and IFN-γ induction. Prophylactic treatment with both *Lactobacillus salivarius* strain HMI001 and *Lactobacillus casei* Shirota attenuated the Th2 phenotype indicated by the reduction of allergen-induced *ex vivo* IL-4 and/or IL-5 responses. In addition the mast cell response was reduced after challenge with the allergen. In contrast, *L. plantarum* strain WCFS1 augmented the Th2 phenotype (increasing mast cell and antibody responses and *ex vivo* IL-4 production). The result with WCFS1 was not predicted by the PBMC assay results but in a recently developed skewed Th2 *in vitro* assay (Nestec Research Centre) this strain enhanced the Th2 cytokine responses in contrast to strain HMI001 and other strains that were protective in allergic sensitization models *in vivo*.

Given the Th2 skewing effects of WCFS1 strain we considered that it might enhance humoral responses to vaccination. In addition to *L. rhamnosus* GG, 2 strains of *L. plantarum* which have strikingly different immune profiles in dendritic cell assays were tested in a mouse model of influenza vaccination. Strain WCFS1, significantly increased vaccine-specific antibody responses to the intranasal vaccine compared to the vaccine control group. The mechanisms were investigated by *ex vivo* stimulation of MLN cells and measurement of the DTH response. Additionally mutants of WCFS1 with altered cell envelope structures were tested in the same model. Further studies are in progress to investigate whether the effects of the probiotic impact on protection to influenza challenge. These results presented highlight the potential of different *in vitro* immune assays for selecting candidate probiotics for different applications.

Antimicrobial functions of intestinal Reg3β

Contributed paper

Linda Loonen[1,2], M. van Ampting[1,3], J. Dekker[1], R. van der Meer[1], I. Bovee-Oudenhoven[1,3] and J. Wells[1,2]
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The Reg3 protein family of antimicrobial peptides contains conserved C type lectin domains involved in carbohydrate recognition. In the mouse the Reg3β and Reg3γ family members are induced in the small intestine during the colonization of germ-free mice or in response to infection. At micromolar concentrations Reg3γ and the human homologue HIP/PAP are directly bactericidal to Gram-positive bacteria but the role of murine Reg3β in bacterial infection remains unclear. To investigate the role of Reg3β in intestinal infection, Reg3β knockout mice were infected with the Gram-negative *Salmonella enteritidis* or Gram-positive *Listeria monocytogenes*. *In vitro* produced Reg3β was used to unravel the antimicrobial mechanisms.

Eight week old Reg3β$^{-/-}$ or wild type animals were housed individually and fed a humanized diet. The animals were infected orally by gavage with $5x10^8$ bacteria. The animals were sectioned after 2 (*Listeria*) or 4 (*Salmonella*) days. Bacterial translocation was estimated by plating suspensions of liver, spleen, mesenteric lymph nodes, colon and ileum. Furthermore, the binding and antimicrobial properties of *in vitro* produced Reg3β were investigated by flow cytometry and killing assays with different bacterial strains.

Recovery of *Salmonella* or *Listeria* in the faeces of Reg3β$^{-/-}$ and wt mice was not significantly different during the first days of infection indicating that Reg3β did not influence colonization levels. Nevertheless, significantly higher numbers of viable *Salmonella* were recovered from the colon, mesenteric lymph nodes, spleen and liver of the Reg3β$^{-/-}$ mouse compared to the wt mice. In contrast, translocation of *Listeria* into these tissues was not significantly different between Reg3β$^{-/-}$ and wt mice, indicating that Reg3β inhibits translocation of *Salmonella* but not of *Listeria*. The mechanism by which Reg3β inhibits translocation seems not to be killing but rather binding. *In vitro* data confirms the binding of Reg3β to different bacteria without killing them.

Effect of propionate and other microbial metabolites on adipose metabolism

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Adipose tissue is a primary site of obesity-induced inflammation, which is emerging as an important contributor to obesity-related diseases such as type 2 diabetes (DB2). Dietary fibre consumption appears to be protective to DB2. Short-chain fatty acids (SCFA), e.g., propionic acid (PA), are the principal products of the fermentation of dietary fibre by the colonic microbiota and may have beneficial effects on adipose tissue inflammation. It has been demonstrated that PA lowers fatty acids content in liver and plasma, reduces food intake, exerts immunosuppressive actions and probably improves tissue insulin sensitivity. Thus increased production of PA (and perhaps other SCFA) by the microbiota might be considered beneficial in the context of prevention of obesity and DB2. Fatty acid ethanolamides (or N-acyl-ethanolamides), injected intraperitoneally, were reported to induce satiety and peripheral utilization of lipid substrates, thereby leading to reduction in body fat gain. N-arachidonoyl-ethanolamide (anandamide) is known to be one of the best characterized endocannabinoids. The endocannabinoid system (ECS) comprising the endocannabinoids, their receptors and the enzymes required for their synthesis and degradation is expressed in most human tissues. The ECS has been shown to be involved in many physiological and pathophysiological processes including energy balance and obesity. We hypothesized that SCFA-ethanolamines (SCFA-EA) might also contribute to this effect.

Human or pig omental and subcutaneous adipose tissue explants, human THP-1 monocytic cells, or mouse 3T3-L1 cells (with or without co-culture with mouse RAW macrophages) were incubated with PA in the presence or absence of LPS. Cytokine and chemokine production were determined by multiplex-ELISA, and mRNA expression of genes was determined by RT-PCR. Several experiments with other SCFA and SCFA-EA were performed as well.

Treatment of adipose tissue explants with PA in the presence of LPS results significantly in different expression of genes of several inflammatory cytokines and chemokines such as leptin, resistin, TNF-α and CCL5. In addition, expression of lipoprotein lipase and GLUT4, associated with lipogenesis and glucose uptake, respectively, increased. Similar effects on cytokine and chemokine production by macrophages were also observed. In the absence of LPS, PA appears to have a more inflammatory effect, at least on 3T3-L1 cells. Butyrate increased the CD86 activation marker on macrophages, similar to LPS. The butyrate-ethanolamide together with those of acetate and valerate also increased CD86. Expression of the G-protein coupled receptors GPCR41 and 43, for which the SCFA are ligands, was shown in adipose tissue.

We show that PA and other SCFA, normally produced by the colonic microbiota, may have a direct beneficial effect on visceral adipose tissue, reducing obesity-associated inflammation and increasing lipogenesis and glucose uptake. Effects on adipose tissue as a whole are at least partially explained by effects on macrophages but likely also adipocytes are involved. A contribution of SCFA-EA (either produced by the host or by the endogenous microbiota) is supported by the results as well. This suggests that, *in vivo*, PA and other microbial metabolites may have potential in preventing obesity-related inflammation and associated diseases.

Administration of butyrate producing organisms in treatment of experimentally induced IBD

Hana Kozáková[1], T. Hudcovic[1], J. Kolinska[2], J. Klepetar[2], J. Wells[3] and H. Tlaskalova-Hogenova[1]
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Inflammatory bowel diseases (IBD) refer to two chronic disorders that are manifested by intestinal inflammation: ulcerative colitis and Crohn's disease. One of the promising approaches in the therapy of IBD is administration of butyrate, an energy source for colonocytes, into the lumen of the colon. The aim of the study was to investigate the effect of butyrate producing bacterium *Clostridium tyrobutyricum* on dextran sulfate sodium (DSS) induced colitis in mice.

Two-month-old immunocompetent BALB/c and immunodeficient SCID mice reared in specific-pathogen-free conditions were treated intrarectally with live *C. tyrobutyricum* one week prior to the induction of DSS colitis and during oral DSS treatment. Clinical symptoms as changes in body weight, bleeding and rectal prolapse were evaluated. Mucin production, tight junction protein ZO-1 expression, the level of TNF-alpha and IL-18 were evaluated by ELISA and confocal microscopy. Study was completed by determination of short chains fatty acids in faeces of mice by gas chromatography.

Intrarectal administration of *C. tyrobutyricum* prevented appearance of clinical symptoms of DSS-colitis, restored normal MUC-2 production and did not change expression of TJ protein ZO-1. The production of IL-18 was dependent on immunocompetency of mice. *C. tyrobutyricum* treatment lead to decrease of TNF-α and IL-18 level in the descending colon of both SCID and BALB/c mice strains. Three-fold increase of n-butyric acid level and two-fold increase of propionic acid level were found in *C. tyrobutyricum*-DSS-treated SCID mice when compared with saline-DSS-treated mice.

This study demonstrates that in the DSS model, the severity of inflammatory symptoms depends largely but not exclusively on host immune functions. Thus, *C. tyrobutyricum* protection against destruction of mucosal barrier is equally effective in immunodeficient SCID mice and immunocompetent BALB/c mice. Manifestation of cytokines IL-18 and TNF-alpha in acute DSS-colitis depends largely on immune cell composition of the mouse host.

Acknowledgements
Supported by grants 303/08/0367 and 303/09/0449 of the Czech Science Foundation.

Lactate, produced by *Streptococcus thermophilus* in intestinal tract of gnotobiotic rats, may trigger goblet cells differentiation pathway: a new probiotic function?

Contributed paper

Laura Wrzosek[1], F. Rul[2], M.-L. Noordine[1], S. Bouet[3,4], V. Robert[1], P. Langella[1] and M. Thomas[1]

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The lactic acid bacterium *Streptococcus thermophilus* is the archetype of lactose-adapted bacteria and so far, its sugar metabolism has been mainly investigated *in vitro*. *S. thermophilus,* consumed by Humans for centuries, is widely and traditionally used in food industry for yogurt- and cheese-making but few data are available about its behaviour in the gastrointestinal tract (GIT). In this context, we studied the crosstalk between *S. thermophilus* and GIT in gnotobiotic rats. Recently, we showed that *S. thermophilus* was able (i) to adapt its global metabolism to the gut environment toward carbohydrate metabolism and (ii) to induce a host response [1]. We thus proposed that lactate, resulting from the adaptive metabolic activity of *S. thermophilus*, may serve as a biological signal to communicate and modulate colonic host epithelium. Moreover, we have shown that the presence of lactose acts as a prebiotic by enhancing *S. thermophilus* level and kinetic of implantation, and fermentative activity in the GIT [2]. In a context where probiotics are claimed to have a protective action toward GIT, our aim was to evaluate the ability of *S. thermophilus* in enhancing mucosal protection through the goblet cell production.

Each germ-free (GF) rat was inoculated orally with $5x10^9$ *S. thermophilus* and was euthanized 30 days later. Goblet cells on colon sections were stained with Alcian blue or periodic acid-Schiff and Mucin2 (MUC2) immunohistochemistry was performed. Krüppel Like Factor 4 (KLF4) protein, a goblet cells differentiation marker, was quantified by Western blot. We showed that *S. thermophilus* significantly increased the percentage of goblet cells per crypt compared to GF rats (41% and 29%, respectively) and the amount of KLF4 protein (2.2 ±0.5-fold). Quantification of goblet cells markers mRNA is currently in progress. Our hypothesis was that lactate produced at high level (from 15 to 50 mM) by *S. thermophilus* in GIT might be involved in the induction of goblet cells. To test this hypothesis, confluent HT29-MTX cells (a cell line producing mucus) were incubated with 20 or 50 mM lactate for 17 hours and we observed that lactate significantly increased the amount of KLF4 protein *in vitro* (2.3 ±0.3-fold).

To conclude, we proposed that lactate produced by *S. thermophilus* in the GIT of mono-associated rats may act as a bacterial signal to trigger goblet cells differentiation pathway suggesting a novel protective function of this probiotic bacterium.

References

1. Rul, F., Ben-Yahia, L., Chegdani, F., Wrzosek, L., Thomas, S., Noordine, M.-L., Gitton, C., Cherbuy, C., Langella, P. and Thomas, M., 2011. Impact of the metabolic activity of *Streptococcus thermophilus* on the colon epithelium of gnotobiotic rats. Journal of Biological Chemistry 286: 10288-10296.
2. Thomas, M., Wrzosek, L., Ben-Yahia, L., Noordine, M.-L., Gitton, C., Chevret, D., Langella, P., Mayeur, C., Cherbuy, C. and Rul, F., 2011. Carbohydrate metabolism is essential for the colonization of *Streptococcus thermophilus* in the digestive tract of gnotobiotic rats. PLoS ONE 6: e28789.

Evolution of the microbiota and the influence of diet

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Diet is a major force that shapes the composition and activity of the gut microbiota. This is evident from alterations in gut microbiota composition after weaning or after drastic dietary changes. Not only type and amount of fermentable dietary fibre but also the macronutrient composition of the diet affect the intestinal microbiota. Metagenomic analysis by Turnbaugh and colleagues revealed that genes encoding enzymes involved in the degradation of indigestible polysaccharides are enriched in the microbiome of obese mice as compared to lean mice.

Owing to the complexity of the microbial community in the gastrointestinal tract, the interactions of the intestinal microbiota with the host are difficult to study. Therefore we established an *in vivo* model of a simplified and defined human intestinal microbiota in gnotobiotic *Sprague* Dawley rats. We selected bacteria that represent dominant species in the human gut, whose genome sequence is available and that mimic at large the metabolic activity of the human gut microbiota: *Bacteroides thetaiotaomicron, Bifidobacterium longum, Anaerostipes caccae, Blautia producta, Clostridium butyricum, Clostridium ramosum, Lactobacillus plantarum* and *Escherichia coli*. Our analyses show that all these species were able to establish in the gastrointestinal tract of previously germfree rats. We observed the same colonisation pattern in the caecum, the colon and in the faeces. The microbiota responded to dietary modifications by changes in the relative proportions of the community members. Such changes were observed in response to inulin, pectin, resistant starch or a Western style diet.

Little is known on how dietary composition influences bacterial activities in the intestine and how this in turn affects the host. We used mice mono-associated with *Escherichia coli* MG1655 as a simplified model for host-microbiota interaction to investigate the influence of dietary factors on bacterial protein expression in the intestine. The mice were fed three different diets: a carbohydrate (lactose)-rich diet, a protein-rich diet and a diet rich in starch. Two-dimensional difference gel electrophoresis followed by electro-spray ionization-tandem mass spectrometry was used to identify proteins differentially expressed in *E. coli* cells recovered from the mouse intestinal tract. The lactose-rich diet led to an induction of proteins involved in *E. coli*'s oxidative stress response (FUR, AhpCF, DPS). The corresponding genes are under control of the OxyR transcriptional dual regulator which is activated by oxidative and other forms of stress. Using luciferase reporter gene assays we demonstrate that osmotic stress exerted by various sugars such as glucose, lactose, sucrose and sorbitol as well as by sodium chloride activates genes of the oxyR regulon. We propose that feeding the mice the lactose-rich diet increased the intestinal osmolality which in turn triggered the upregulation of OxyR dependent proteins, which enable intestinal *E. coli* to better cope with diet-induced osmotic stress.

Breast milk – the source of more life than we imagine

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Human milk is not sterile, it has been shown to harbour a variety of bacterial genera. Aseptically collected human milk contains approximately 10^3 colony-forming units per ml, including *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Peptostreptococcus*, *Staphylococcus*, *Bifidobacterium*, *Enterobacteriaceae* and/or *Corynebacterium* [1-5]. A recent study has shown that it even contains a more diverse bacterial community than was previously reported [6]. Human milk therefore serves as a source of bacteria to the developing infant gut.

The origin of bacteria in human milk remains controversial. It has been suggested that the maternal skin could be the source of coagulase-negative staphylococci, with *Staphylococcus epidermidis* as the predominant species, while the infant mouth could be the source of those bacteria that are rarely found as skin residents, such as viridans streptococci [3,7]. More recently, another hypothesis, involving cells of the immune system within the mucosal-associated lymphoid tissue (MALT) system has been proposed. It has been shown that dendritic cells can penetrate the gut epithelium to directly take up bacteria from the gut lumen, and that antigen-stimulated cells move from the intestinal mucosa to colonize distant mucosal surfaces [8,9]. Moreover, it seems that during late pregnancy and lactation, cells of the immune system selectively colonize the mammary gland (the so-called entero-mammary pathway), explaining the presence of bacteria in human milk [10,11]. This mechanism could also explain why oral administration of specific strains of lactobacilli to pregnant women, leads to their detection in the faeces of breastfed infants that are born by caesarean section [12]. Several studies have shown the mother-to-child transmission of different bacterial groups, such as lactic acid bacteria, bifidobacteria and *StaphylococcusI* [13,14]. However, the question remains what role this community plays in the colonization of the developing infant gastrointestinal tract, and the overall maintenance of mammary health.

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A novel approach to microbial replacement: 30 strains transplant

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Clostridium difficile infection (CDI) accounts for 15-25% of antibiotic-associated diarrhoea and occurs most commonly when patients receive antibiotics which eradicate their normal enteric gut bacteria, allowing overgrowth of *C. difficile*. Usual treatment consists of either metronidazole or oral vancomycin to eradicate the CDI. However, once a patient fails oral vancomycin therapy, treatment options for recurrent CDI are extremely limited. One effective treatment for recurrent CDI is faecal bacteriotherapy, or 'stool transplant' (infusing donor stool into the intestine of the recipient to re-establish normal bacterial microbiota), but concerns of donor infection transmission and patient acceptance limit its use. Our aim was to determine whether a multi-species mixture of intestinal bacteria, isolated in culture from stool of a single healthy donor, would be effective as an alternative to faecal bacteriotherapy for treatment of recurrent *C. difficile* infection.

We describe the development of a 'synthetic stool' culture preparation, made from over 30 purified intestinal bacterial strains derived from a single healthy donor, to treat 2 cases of recurrent CDI that failed repeated standard therapy with metronidazole and vancomycin. The study protocol was approved by the Human Research Ethics Boards at Queen's University and the University of Guelph. Both patients were found to be infected with a Ribotype 078 strain of *C. difficile*. Patients were cured of recurrent CDI within 72 hours of having received the synthetic stool preparation, and both patients remained symptom-free at 6 months of follow-up. Bioinformatic analysis demonstrated that the microbiota of both patients adapted characteristics of the synthetic stool mixture, yet still retained some of their original microbiota. This pilot study demonstrates that use of 'synthetic stool' may be a successful strategy for eradicating recurrent CDI and hence, may be an effective and feasible alternative to the use of defecated donor faecal matter (stool transplant) in the treatment of recurrent CDI.

Functional foods for digestive health in dogs

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The link between gastrointestinal health and overall wellbeing is a topic of great interest in companion animal nutrition. In addition to being the gateway for nutrient absorption, the gut plays a vital role in immune function and houses a community of beneficial and potentially harmful microbiota. Gut associated lymphoid tissue (GALT) forms a sizable portion of the immune system and is the first line of defense against intestinal pathogens. GALT primes the mucosal immune system by maintaining tolerance to food antigens and commensal gut microflora while responding vigorously to pathogens. This priming leaves the pet able to cope with immune challenges when needed. The gastrointestinal tract of the dog contains an enormous volume of both beneficial and harmful bacteria that generally live in equilibrium. Shifts in bacteria populations to a more negative balance can lead to inconsistent faecal quality and increase the susceptibility of the pet to illness. A frequent cause of these shifts in dogs is naturally-occurring stresses such as travel, change in environment, life stage, and exercise. While not necessarily negative stimuli, these stresses can impact microflora balance and increase the chance of poor faecal quality and immune response. The addition of functional ingredients such as colostrum and probiotic bacteria to a complete and balanced diet has been demonstrated to positively impact gut health in healthy dogs by increasing faecal IgA concentrations, improving vaccine titres, increasing populations of beneficial bacteria, and maintaining microflora stability during times of naturally-occurring stress. Functional ingredients that enhance immune function and microbiota in healthy dogs include colostrum, encapsulated probiotics, and heat-treated probiotics.

The ontogeny of the intestinal IgA CDR3 repertoire and IgA production in response to the gut microbiota

Contributed paper

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Intestinal IgA production is initiated upon colonization of the intestines with microbiota, and vice versa intestinal microbiota are controlled by IgA. We aimed at identifying the kinetics of this interplay and development of homeostasis during ontogeny. Therefore we studied immunoglobulin CDR3 repertoire development, composition changes of the gut microbiota and functional coating of microbiota by host immunoglobulins during the first ten weeks of life.

Ileum tissue samples were collected from laying hens at different time points and analyzed for CDR3 repertoire development by spectratyping and sequencing. Ileum samples were also analyzed for the adherent microbiota diversity by Microbial Community Profiling. A redundancy analysis (RDA) was performed of the community composition of adherent microbiota in relation to immune gene expression data. In a separate experiment isotype specific staining of faecal microbiota during ontogeny was determined using flow cytometry.

Subsequently the IgM, IgG and IgA CDR3 spectratype profiles changed during the first 10 weeks post hatch. However, usage of V pseudogenes for IgA CDR3 repertoire diversification remained the same during this period. RDA analysis showed that the microbial composition was influenced by IL-10, IFN-γ, TGF-ß and IgA expression. The CDR3 repertoire was not correlated with the intestinal microbial composition. FACS staining of microbiota revealed that in the first weeks of life the intestinal microbiota was mainly coated with IgM, but from day 35 and later up to 70% of the microbial cells were opsonized by IgA. IgA expression and functional binding to intestinal microbiota was significant from day 35 on.

We conclude that humoral immunity in the gut is limited in the first four weeks post hatch and hypothesize that the commensal binding IgA CDR3 repertoire undergoes only minor changes during ontogeny.

Tools to display metagenomics data

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The radical decline in the cost of DNA sequencing poses substantial bioinformatics challenges, especially in the analysis of complex mixtures such as complete microbial communities. In a few short years, microbial community analysis has gone from a few dozen sequences in a handful of samples to billions of sequences from samples spanning the globe.

In this talk, I discuss the various tools developed by the Dr. Robert Knight laboratory and collaborators to aid in analyses of the massive number of microbial sequences being generated by various sequencing technologies. These tools include QIIME (Quantitative Insights Into Microbial Ecology), a pipeline of programs for processing raw sequence data into final alpha and beta diversity results, Site Painter, a tool for displaying spatial (biogeography) gradients, and Topiary Explorer, a phylogenetic tree viewing program that allows users to view large trees annotated with taxonomies and/or metadata.

Metagenomics screening (MetaHit)

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One of the major questions in human biology is the role of gut microbial communities in health and disease. To address this question, the MetaHIT consortium has developed a powerful molecular microscope allowing to view the gut microbial communities. The microscope comprises two main elements, a reference gene catalogue of the intestinal microbial genes [1] and a quantitative metagenomics pipeline, based on a very high throughput DNA sequencing, allowing us to determine the presence and abundance of each gene in an individual.

Use of the MetaHIT microscope has led to detection of three gut enterotypes to which humans belong [2], characterized by different bacterial communities. This basic feature of human biology remains to be elucidated, but the enterotypes will be crucial to stratify individuals and assess the gut microbial communities associated with health and disease.

Focusing the microscope on the gut microbes present in obese and lean individuals or IBD patients and healthy controls has revealed considerable differences in the microbial communities, in terms of overall diversity and the prevalence of particular bacterial species. The microscope could be used to examine water and generate a global picture of the intestinal microbial contamination and thus open avenues for a much broader control of microbial risk.

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Asian Microbiome Project: a pilot study on the basal microbiota profile of healthy Asian youngsters

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Gastrointestinal microbes play important roles in the health and disease of the host. There are many documented evidences which demonstrated that disturbance of intestinal microbiota is linked to the risk of developing infectious, inflammatory and allergic diseases. It is of great interest to characterize both composition and succession of the intestinal microbiota. Such perspective studies provide markers for the stage of health and positive guidance for microbial colonization through interference. Most of these studies were conducted in the West, with very different dietary and cultural habit from those of Asia. Since diet is a major factor in determining intestinal microbiota population, it is expected that basal microbiota profile among the Asian is different from that of the Westerner, and that may partly explain the differences in susceptibility to different diseases.

An Asian Microbiome Project was initiated to examine the microbiota profile among healthy youngster in Asian cities as the pilot study to provide the background for further perspective studies of disease population and age groups. It is an open trial involving 360 healthy youngsters aged 8-9 years of both sexes derived from the following 6 countries (11 cities): China (Beijing, Guangzhou), Indonesia (Yogyakarta, Bali), Japan (Tokyo, Fukuoka), Singapore, Taiwan (Taipei, Tainan) and Thailand (Bangkok, Khon Kean). The pairs of cities from each country represent the cosmopolitan and rural background. The profiling of the intestinal microbiota is determined by quantitative PCR (qPCR), quantitative RT-PCR (RT-qPCR), and pyrosequencing. Uniqueness in Asian microbiota profile will be presented.

A multiscale systems biology approach to the human-*Bacteroides thetaiotaomicron* symbiosis

Contributed paper

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The human gut microbiome consists of ten times more microorganisms than there are cells in our body, processes otherwise indigestible nutrients (e.g., starch and xylan), and produces important energy precursors (e.g., short chain fatty acids), essential amino acids, and vitamins (e.g., vitamin K). Studies have shown that the microbial composition varies among healthy individuals depending on sex, genotype, diet, etc. [1]. A prominent representative of the human gut microbiome is *Bacteroides thetaiotaomicron*, which is a Gram-negative obligate anaerobe [2].

Our group assembled a well-curated and validated genome-scale metabolic reconstruction of *B. thetaiotaomicron* (iAH853). The reconstruction consists of 1305 reactions, 1044 metabolites and 853 genes. To construct a metabolic model of host-gut microbe interactions, iAH853 was joined with an existing reconstruction of mouse metabolism, iMM1415 [3]. In our model setup, the two reconstructions are linked through a joint compartment, the lumen, which allows metabolite and secretion product exchange between mouse and *B. thetaiotaomicron* and also provides in inlet for simulated dietary nutrients to enter the joint model.

The resulting joint model consists of 6900 reactions, 4941 metabolites and 2596 genes. We modelled simultaneous growth of mouse and *B. thetaiotaomicron* while simulating growth on four different nutrient-limited diets. The joint model was shown to capture mutually beneficial cross-feeding between the two *in silico* organisms, as well as competitive interactions as growth-limiting nutrients are depleted. Furthermore, we examined qualitatively and quantitatively which metabolite species are exchanged between the two models and compared the results with previously published metabolomics studies [4]. Finally, we mapped a set of mouse genes, whose human homologues are known to cause inborn errors of metabolism onto the joint model. We demonstrate that this modelling approach is a powerful tool for modelling host-gut microbe interactions in health and disease.

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Multi-omics approach reveals that bifidobacteria protect from enterohaemorrhagic *Escherichia coli* infection through production of acetate: importance of sugar transporter

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The human gut is colonized with a wide variety of microorganisms, including some species, such as those belonging to the bacterial genus *Bifidobacterium*, that have beneficial effects on human physiology and pathology. Among the most distinctive benefits of bifidobacteria are a modulation of host defense responses and protection against infectious diseases. Nevertheless, the molecular mechanisms underlying these beneficial effects have barely been elucidated. To investigate these mechanisms, we used a simplified model of lethal infection with enterohaemorrhagic *Escherichia coli* O157:H7 (O157) of mice associated with certain bifidobacterial strains, together with an integrated 'omics' approach. We show that genes encoding a sugar transporter present in certain bifidobacteria contribute to protecting mice against death induced by O157. We found that this effect can be attributed, at least in part, to increased production of acetate and that translocation of the O157 Shiga toxin from the gut lumen into the blood was inhibited. We propose that acetate produced by protective bifidobacteria improves intestinal defense mediated by epithelial cells and thereby protects the host against lethal infection [1].

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Keystone species and beneficial microbes in colitis and colon cancer

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Investigations of the microbiota that are found throughout the human body are underway with the goal of elucidating the role of microbes in human health and disease. Inflammatory bowel disease (IBD) is an ideal setting for such studies as disruption of homeostasis between the host immune system and the gut microbiota is a central to IBD pathogenesis. Using experimental colitis models, we have identified 'keystone species', microbes of low abundance that are capable of driving significant effects on hosts or microbial communities, that appear to instigate chronic inflammation. We have also determined that deficiencies in bifidobacteria correlate with colitis. The reduced presence of these beneficial bacteria may impact not only host response to the microbiota but also the behaviour of the endogenous microbiota. Repleting this deficiency with a fermented milk altered the metabolic function of the endogenous microbiota, reduced intestinal inflammation, and markedly reduced levels of the colitiogenic keystone species, *Klebsiella pneumoniae* and *Proteus mirabilis.* Chronic inflammation in the intestine is also an important risk factor for colorectal cancer. Ongoing work on the colorectal microbiome using experimental models and human tumours will be discussed as well as the potential benefits for beneficial microbes and functional foods in the context of this disease. Collectively, our studies support the utility of wedding culture-independent and culture-dependent studies with mouse models for defining how the gut microbiota works in concert with the mucosal immune system to shape disease susceptibility for IBD and colorectal cancer.

Intestinal dysbiosis in coeliac disease: is there a role for probiotics?

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Celiac disease (CD) is an autoimmune enteropathy induced by dietary gluten proteins in genetically predisposed individuals. The disease is strongly associated with the Human Leukocyte Antigens (HLA) genes of the major histocompatibility complex, and around 95% of CD patients carry the HLA-DQ2/DQ8 molecules. Gluten is the environmental element causative of the signs and symptoms of the disease but other factors are thought to play a role, including milk-feeding type, incidence of infections and intestinal microbiota. CD patients have alterations in the composition of the intestinal microbiota, characterized by increases in numbers of Gram-negative bacteria and their virulence features (e.g. *Bacteroides* spp. and *E. coli*), and decreases in numbers of *Bifidobacterium* spp., in comparison with controls. Moreover, these alterations are not fully restored after patients followed a gluten-free diet, suggesting they are not only a secondary consequence of the inflammatory milieu characteristic of the active phase of the disease. A prospective study of healthy newborns at family risk of developing CD have also indicated that both the HLA-DQ genotype and the milk-feeding type influence the composition of the microbiota early in life and could contribute to the disease risk. Evidence from these observational studies has set a rationale for the evaluation of bifidobacterial strains as potential probiotics for CD management. Studies in intestinal loops have showed that *B. bifidum* CECT 7365 strengthens the gut barrier function, disrupted by CD triggers (gliadin and IFN-gamma), by increasing the number of goblet cells and the production of chemotactic factors and inhibitors of metalloproteinases. It has also been reported that *B. longum* CECT 7042 attenuates the production of inflammatory cytokines and the CD4+ T-cell mediated immune response in a gliadin-induced enteropathy animal model. Although evidence from human studies has yet to be reported, these findings suggest that specific bifidobacterial strains could exert protective effects in CD.

The airway microbiome in asthma and COPD

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Infection or colonization of the lower airways by particular microbial species has been implicated in the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD). Much of the existing evidence is based upon a view of airway microbiology that has been informed from culture methods, microbe-specific molecular identification, or serologic tests that infer infection by specific organisms. Recent studies applying newer tools for microbiome profiling have identified a more diverse airway microbial community in patients with asthma or COPD than previously appreciated. New evidence suggests that the composition of airway microbiota differs in states of pulmonary health or disease, and may correlate with clinical pathophysiologic features of asthma or COPD. Thus it is conceivable that the microbiome of the airways contributes to biologic processes that shape the development, persistence, or prognostic course in obstructive lung diseases by mechanisms yet to be fully understood.

Prior to more recent microbiomic studies, specific bacterial species that have been associated with asthma include *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*. Infection in early life by rhinovirus or respiratory syncytial virus also may confer an increased risk of developing asthma. In COPD bacterial colonization of the airways often is observed, although the role of infection in the development of disease remains unclear. However, acute respiratory exacerbations in established COPD have been causally related to new infecting strains of *H. influenzae, M. catarrhalis,* or *Pseudomonas aeruginosa.*

Recent 16S rRNA-based microarray or next-generation sequencing studies of the lower airway microbiota in patients with asthma or COPD have expanded the spectrum of bacterial microbiota potentially implicated in these diseases. In certain asthmatics, greater airway hyper-responsiveness is strongly correlated with greater bacterial diversity, including increased relative abundance of multiple bacterial phylotypes. In COPD multiple additional bacterial groups identified through culture-independent analysis demonstrate significantly increased prevalence during exacerbations, suggesting that changes in microbial community composition could perturb a clinically stable pulmonary state.

Much remains to be learned about the airway microbiota in respiratory health and disease, including attention to other microbial groups like viruses and fungi. Coupling knowledge of the airway microbiota with complementary insights into the functional microbiome will be important to truly understand links between the airway microbiome and chronic respiratory diseases, and therefore identify opportunities for microbial-directed interventions to ameliorate or prevent disease burden.

Influence of human gut microbiota on the metabolic fate of glucosinolates

Contributed paper

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Glucosinolates are secondary metabolites predominantly found in cruciferous vegetables such as broccoli, Brussels sprout, cabbage and cauliflower which upon chopping and chewing will release the indigenous plant thioglucosidase enzyme myrosinase that hydrolyses glucosinolates. This hydrolysis releases a range of breakdown products including isothiocyanates, which have been implicated in the cancer-protective effects of cruciferous vegetables. However, plant myrosinases are rapidly denatured by cooking, and thus the health properties of cruciferous vegetables are absolutely dependent upon bacterial myrosinase activity of the human gut microbiota. We isolated two bacterial strains of *Enterococcus casseliflavus* NCCP-53 and *Escherichia coli* O83:H1 strain NRG 857C from the faeces of a healthy volunteer and their identities were confirmed by sequencing of their 16S rDNA genes. *Lactobacillus agilis* R16 that was reportedly found to degrade sinigrin [1] was also included in the study. These three bacteria metabolised glucosinolates completely within 24 h of *in vitro* incubation. Their putative myrosinases are inducible by glucosinolates based on resting cells experiments. This is the first report to show the time-course degradation product profiles of different types of glucosinolates such as methylthioalkyl-, alkenyl-, aromatic-, indolic- and some of their corresponding desulfoglucosinolates by human gut bacteria. Each bacterium was capable of converting most of intact glucosinolates to both nitriles and isothiocyanates which were found at pHs between 3.0 and 8.0 in the growth media. By contrast, bacterial resting cells in citrate phosphate buffer pH 7.0 produced only isothiocyanates. This suggests that co-factors or co-proteins or perhaps both present in the media are important for nitrile production. Out of three bacteria, only *Escherichia coli* O83:H1 was able to metabolize glucoiberin which produced iberverin as isothiocyanate product. However, iberin was produced from the same substrate by the purified plant myrosinase from *Sinapis alba*. It is likely that different bacteria may have different mechanisms or different enzymes involving in the metabolism of different glucosinolates and also bacterial myrosinases may be different from plant myrosinases. Previous reports suggesting that desulfo-glucosinolates are possible nitrile precursors was the case with our bacteria as when added to the bacterial fermentation gave only a nitrile product. Sulfatase activity was detected in *Enterococcus casseliflavus* NCCP-53 and *Escherichia coli* O83:H1 strain NRG 857C. It remains to be determined whether these bacteria could convert intact glucosinolates to desulfo-glucosinolates.

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Organotypic co-culture models to study the effect of beneficial microbes functionally

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The dialogue between cells of the human immune system and cells of various tissues controls inflammation and is mostly mediated by numerous cytokines, chemokines and other messenger molecules. This signalling network can be influenced therapeutically not only by drugs and nutrients, but also by probiotic bacteria.

Particular care has to be taken when investigating the effects probiotic bacteria may have on cells of the human immune system. In this context, many in vitro test systems have been used in the past that did not pay attention to the fact that, under physiological conditions, immune cells in vivo are normally not directly exposed to such bacteria. Instead, the epithelia of the body surfaces (in particular: skin, gut, lung) separate in fact immune cells from commensal as well as probiotic bacteria. Thus, in vivo the interaction between the immune system and especially the beneficial microbes take more intricate routes than those available in leukocyte cultures, where these cells are incubated directly with the bacteria to be tested. In such systems, immune cell activation is generated by what must be considered a defence response, which, as a matter of fact, is far from what could be considered a therapeutically relevant immunomodulation.

Thus, in order to establish more life-like in vitro models, human organotypic co-culture systems have been developed in our labs, in which differentiated human epithelial barriers (intestinal, but also epidermal and bronchial) are incubated together with a particular type of human whole-blood culture. Especially the latter represents a far more complete model of the highly complex regulatory network within the human immune system than cultures of isolated leukocytes (e.g. peripheral blood leukocytes, isolated subpopulations, or even cell lines). In addition, these co-cultures also allow to investigate the cross-communication between the cells of the immune system and local tissues.

As a matter of fact, the complexity in cellular composition of these models must be reflected in the selection of assays used to evaluate whole effect profiles, rather than individual endpoints. Thus, one of the primary goals in these co-culture models is to obtain a comprehensive overview on reaction patterns test samples generate with regards to immunoregulation. Multiplexed cytokine arrays are therefore normally chosen as read-out systems for a broad profiling of the immunomodulatory activities of drugs, biologicals, or probiotics in these co-culture models.

The use of these physiological, human organotypic co-culture models will help to improve in future the relevance of in vitro results considerably.

Use of an *in vitro* model to study fermentation by the microbiota

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At TNO dynamic computer controlled *in vitro* models of the GI-tract have been developed, optimized and subsequently validated with *in vivo* data. Because the successive dynamic conditions in the GI-tract are accurately mimicked, these models have been shown to be better predictors of human clinical trials than simulations in flasks or test-tube

The TIM-2 model simulates the colon, and is validated with respect to pH, microbiota (composition and activity) and uptake of nutrients and microbial (fermentative) metabolites. The TIM-2 model can be programmed to simulate the colon of healthy and diseased humans, adults, elderly and infants, and even animals. Experiments with a microbiota from lean and obese individuals have been performed, as well as those with inflammatory bowel disease. In addition, situations of an unbalanced microbiota in healthy humans has been simulated, such as after antibiotic treatment, with concurrent overgrowth of *Clostridium difficile*.

The TIM-2 model is among others used for fermentation studies, to study the fermentation of a variety of fibres, prebiotics and other substrates by the microbiota and the effect on the activity and composition of the microbiota. An example of application is the use of stable isotope labelled (^{13}C) prebiotics, which enable specifically studying which microbes ferment this labelled prebiotic and which metabolites are formed in this process. The products formed during fermentation can be analyzed, as well as the microbiota composition. Nowadays the microbiota is being analysed by 454-pyrosequencing, which gives a huge amount of very specific and quantitative data about the microbiota. With this technique the shifts in the microbiota which are induced by for example different fibres can be monitored. The newest development is aimed at the different enterotypes, enabling to study the effects of nutrients or other (dietary) ingredients on these, and to study if it is possible to direct one enterotype into another.

Also, interactions of the microbiota and/or their metabolites with host tissue, such as epithelial cells and immune cells (see also abstract of Manfred Schmolz or adipose tissue (see abstract of Koen Venema) are studied. In development are interactions with whole gut-segments, from e.g., pigs or produced from stem-cells (see poster of Guus Roeselers *et al.* and abstract of Hans Clevers). Combinations of these technologies will lead to mechanistic insight in the mode of action of functional food components, essential for development of new products for health of man, health that goes beyond the gut!

Assessment of survival of probiotics under various conditions using a validated *in vitro* model

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Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. This not only implies that probiotics should be alive when ingested, but also when they reach their target sites in the GI-tract. Further, dose-response effects of probiotics have been reported and it is reasonable to anticipate stronger effects if more live probiotics reach their site of action in the GI-tract.

When passing through the proximal GI-tract probiotics are exposed to several limiting factors, of which stomach acid and bile salts are the most detrimental. Digestion is a dynamic process resulting in gradients of pH, digestive enzymes, and bile in time and space. These conditions are difficult to simulate *in vitro*. The TNO Intestinal Model (TIM-1) is a dynamic model of the stomach and small intestine that simulates important physiological conditions, including stomach shear forces and intestinal peristalsis, stomach and intestinal emptying, dynamic pH profiles, bile concentrations and bile absorption, and gastric and duodenal enzyme secretions. The model's capacity for predicting survival of probiotics has been validated against studies in humans.

We use TIM-1 to evaluate probiotic viability. We have found that viability after passage of TIM-1 differs substantially between probiotic bacterial species (0.01% to 78% of intake), and between strains of the same species (up to 50-fold). The bactericidal effects of stomach acid or bile salts also vary between strains reflecting differences in acid or bile tolerance between the strains. This knowledge is being used in a program aiming at improving strains to increase viability in the GI-tract. Further we have used the model to document effects of modifying fermentation and downstream processing on probiotic survival. We have shown for example that allowing acidification of the medium at the end of fermentation induces a stress-adaptation that improves survival in stomach acid.

A novel polarized *ex vivo* organ culture model to study beneficial microbes

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The use of probiotics as supplements in the food industry in recent years has been a massive breakthrough, as most consumers only have positive comments to make about how daily intake of probiotics affects their wellbeing and the probiotic market has known a spectacular 25% rise from 2004 to 2012 (data only from the US). Powered by this amazing success, as well as recent findings relating composition of intestinal flora with various diseases (EAE, IBD, allergies, susceptibility to gastro-intestinal infections, etc.) many researchers have proposed probiotics as potential therapeutic agents, especially for IBD. However, after recent unfortunate clinical trials, most groups are quite reluctant to take treatments to the clinics, especially to be administered to patients with acute disease for the induction of remission. Partly responsible for that is the lack of physiologically realistic models on which to acquire valid pre-clinical data, as only about 10% of treatments that give promising results on mouse models of colitis translate to any significant clinical effect.

To address this issue, we developed a novel organ culture system of human healthy and IBD intestinal mucosa. With our set up, we manage to maintain polarity of the tissue and thus apply the stimuli of interest only on the apical side of the mucosa. We have tested three different lactobacilli strains on this system and show that surprisingly, not all of them are beneficial even on healthy tissues. Importantly, all three lactobacilli appear to be detrimental on IBD tissue coming from patients in the acute phase of the disease. Moreover, we have demonstrated the anti-inflammatory activity of a probiotic's metabolic product (here called a 'postbiotic') both on healthy tissues in the presence of a highly pro-inflammatory agent (Salmonella) and on IBD tissues as an anti-inflammatory mediator.

With this work, we present a valid protocol to maintain human intestinal mucosa viable for at least 24 hours. We also show the importance of polarized stimulation for obtaining relevant and reproducible data. Finally, we propose that postbiotics could represent harmless alternatives for the treatment of acute IBD.

Glycerol supplementation boosts *Lactobacillus reuteri*'s protective effect against *Salmonella typhimurium* infection in a 3-D organotypic model of colon epithelium

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Lactobacillus reuteri's probiotic effects have been speculated to partly depend on its capacity to produce the antimicrobial reuterin by using glycerol. In this study, we investigated the potential of this process to protect human intestinal cells against infection by *Salmonella typhimurium*. We used a highly differentiated model of human colonic epithelium, which was previously found to reflect key characteristics of the *in vivo Salmonella* infection process. The model was infected with *S. typhimurium* (i) in the presence of an established *L. reuteri* community without or with glycerol, or (ii) under continuous exposure to the sterile *L. reuteri* ferment without and with reuterin (produced from glycerol) or pure reuterin.

The established *L. reuteri* populations were found to protect significantly better against the early stages of *Salmonella* infection when producing reuterin from glycerol. Additionally, the reuterin-containing glycerol ferment of *L. reuteri* caused a reduction of 1 log unit in *Salmonella* adherence and invasion (1h) and 2 log units for intracellular survival (4h). In contrast, the *L. reuteri* ferment without glycerol stimulated intracellular *Salmonella* growth with 1 log unit, demonstrating the importance of glycerol and reuterin for *L. reuteri*'s effects on pathogen survival. However, long-term exposure (24h) to reuterin induced a complete loss of cell-cell contact within the epithelial structure and compromised cell viability.

Collectively, these results shed light on a biological role for reuterin in inhibiting intestinal *Salmonella* infection and support the combined application of glycerol and *L. reuteri*, dependent upon future *in vivo* studies of reuterin on intestinal health. To our knowledge, this is the first report of a reuterin effect on an enteric infection process in any mammalian cell type.

Beneficial microbes: the ethics of claims and claim regulation

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Supermarkets and pharmacies are brimming with new products with live micro-organisms claiming that they improve health. Some have characterized manufacturers of these products as snake oil salesman who peddle products of questionable quality but tout them as having miraculous powers in order to make a profit. Assuming such products are safe, with regard to efficacy many of these claims are unethical from any perspective. Intentionally misleading consumers with fraudulent claims can cause harm by giving consumers a false sense of security, preventing them from pursuing proven remedies, and by financially exploiting them. Apart from this utilitarian view, from a deontological perspective such deception is also unethical and erodes consumer trust in industry. There may be a gray area, however, where there is some question about whether there is sufficient evidence to make the espoused claim. The ethical basis of beneficial claims that are based on insufficient research, underpowered studies, or mixed research results are more difficult to assess and some would argue that as an ethical matter, we should respect the autonomy of the consumer and let the consumer decide, based on the available information, if a product provides him or her with some benefit. This argument is more persuasive when discussing products that may have different impacts on different individuals, e.g., the foundation of personalized medicine.

While a debate about what constitutes a sufficient substantiation of claims to make the claim ethical could be the subject of a paper, I will focus my remarks more on the ethics and philosophical perspective of different approaches to claim regulation and touch on the different approaches of the US and the EU. The roots of the approach to regulation of claims in the U.S. and Europe appears largely to stem from a broader philosophical difference in the ways the two jurisdictions have regulated more generally and reflect a more general view of the role of government vs. individual autonomy. The US, since its early days, has largely been influenced by a strong libertarian streak giving deference to individual self determination and government minimalism. The hallmark of individual self determination goes to an individual's right to contract with others, including business, and determine the terms of arrangement between the parties. The view is grounded in the idea that free markets (operating competitively) are ethical because they allocate resources in ways that are just, that maximize economic utility and that respect the liberty of both buyers and sellers. Regulation is justified only when markets are not operating competitively, create negative externalities, or don't have the characteristics of competitive markets such as 'perfect knowledge' of the product and/or services provided. As a result, in many areas, self regulation of business is relied upon. Yet, ethical lapses in corporate behaviour have led some sectors of the US public to question this strategy urging greater regulation in some areas.

Europe, in contrast to the US, has historically adopted a government philosophy of protectionism or benign paternalism, intent on protecting its citizenry from harms of all kinds. In terms of environmental regulation, another concept adopted in the late 1960s and 1970s, consistent with this general approach to regulation is the precautionary principle which has its roots in German and Swedish law. Under the principle, industry is required to demonstrate the safety of its products to regulators rather than regulators having the requirement to prove harm.

The current regulation of food and dietary supplement claims in the US, which generally does not require premarket claim approval (at least for structure/function claims), is considered

lax, in comparison to that of the EU. The EU, in contrast with the US, requires that companies submit evidence and obtain approval for claims that would be considered structure/function claims in the US and has approved virtually none for probiotics. This presentation will explore the current regulation of claims for products containing live micro-organisms in the US and Europe against the larger philosophical back drop of regulation in the two countries and propose that a 'middle way' for regulation of probiotics may be more appropriate than either region is now undertaking.

Critical issues for successful claim applications

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A health claim is a marketing tool, used on food products aimed at the general population, to help people make informed choices that may benefit their health. A health claim should be substantiated by sound scientific evidence and not mislead the consumer. This principle has been laid down in regulations worldwide, although details may vary. Health claims are different from dietary recommendations and, in general, are not applicable for (food) products aimed at specific patient populations.

All health claims for probiotics, submitted to EFSA so far, have received negative opinions: it was concluded that a causal relationship between the food product or probiotic ingredient, and a specific health benefit, had not been established. Reasons for failure of probiotic claim applications will be analysed in this presentation, and compared to some successful applications for other type of products. The chairman of the NDA Panel stated that the quality of studies was often a problem; scientists in probiotic research have accused EFSA of being overly critical and dismissing well performed studies. There clearly is a gap between credible scientific results and meeting regulatory requirements for scientific substantiation of a health claim. Research that is purely science-driven, often has too little focus on the specific claim that is aimed for. Companies need to combine both aspects in their research strategy, to be more successful in claim applications.

Guidance for claim substantiation in the area of gut health and immunity was provided by EFSA only after adoption of the claim regulation. However, it has clarified issues like appropriate characterization of strains, what is considered a health benefit, and what outcomes are acceptable to substantiate a specific claim. Scientists at the same time have published guidances on the appropriate design and conduct of clinical studies with probiotics. One of the most challenging aspects is to show that a probiotic contributes to improving health in a basically healthy population. Resistance to an infectious challenge is an acceptable outcome in this respect, and available research models will be discussed. It is conceivable that the effect of probiotics sometimes is more subtle, and not strong enough to be evaluated on the basis of established clinical outcomes. Such an effect may still be meaningful for maintenance of health, but, unless this has been properly validated, it is as yet unlikely to be accepted as evidence for substantiation of a health claim. Therefore, in addition to clinical endpoints, biomarkers of gut and immune health need to be validated in clinical trials. Preconsultation with EFSA on acceptability of specific models is not yet common practice, but seems crucial for stimulation of health claim development and innovation in research.

Beneficial effects of probiotics for human health: do consumers need claims on the product?

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Since Ely Metchnikov published his book 'Prolongation of life' in 1908, over 7,500 papers have been published in the biomedical literature on beneficial microbes: probiotics. The list of diseases and conditions for which the beneficial effects of probiotics have been proven, claimed, or merely hope for is long and diverse. It includes a great number of gut health and immune mediated diseases, but also asthma, obesity, autism and other conditions. Not all probiotics are the same, in fact they are all different. Therefore, the beneficial effects of a given species and strain cannot be extrapolated to other strains, let alone species. This great variety in probiotic strains hampers, would even preclude, meta-analysis of probiotic health effects. Yet, for antibiotic associated diarrhoea and for necrotising enterocolitis (a serious disease of premature babies with a high mortality rate), the scientific literature has been subjected to Cochrane review and clearly shows the protective effect of probiotics. The original hypothesis of Metchnikov has been difficult to demonstrate, although recent data from experimental animal models indicate that probiotics even may have the capacity to prolong life.

When probiotics are marketed as (functional) food, the communication of the health benefits to end-users is strictly regulated. Health claims for probiotics are evaluated by the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority (EFSA). Regulatory restrictions exist on the use of clinical endpoints when dealing with food for the general (healthy) population. This leads to the paradoxal situation that for a product, intended to reduce the risk for traveller's diarrhoea, the clinical studies which demonstrate a reduction of traveller's diarrhoea cannot be used as evidence. According to the regulations, and guidelines based upon those regulations, it should be demonstrated that a given probiotic has the ability to reduce the number of gastrointestinal pathogens by at least a log unit.

Whether supported by limited or by a substantial amount of basic and clinical research, all of the evaluated claim applications have received a negative opinion. With the restrictions on the use of clinical endpoints, validated biomarkers for gut health and immune health in relation to disease risk reduction are needed. It would be a joint responsibility of the (bio)medical and the regulatory science to draw such a list of validated biomarkers.

For a health claim to be approved, the evidence submitted (to EFSA) should be of the highest possible scientific standards. From the string of negative opinions on probiotics, it must be concluded that even research that is published in the highest possible scientific journals does not meet these standards. Not only the applicants of health claims for probiotics are left in the vague on the precise nature these standards, but also the scientific community. Clear-cut criteria for design as well as evaluation of future (clinical) studies are required. An open dialogue between basic and clinical scientists, regulatory authorities, food and nutrition industry, and consumers would be needed in order to bridge the gap between science and marketing of probiotics. Finally, whether consumers ultimately would need a health-claim on the label of functional foods is debatable. To that end we have conducted a survey among consumers on their motives for purchasing a probiotic product.

Primo-colonizing bacteria induce maturation of colonic epithelium in gnotobiotic rat models

Contributed paper

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Intestinal microbiota and their hosts have established symbiotic relationships. These interactions start immediately after birth once the progressive colonization of the sterile intestine has occurred. Our objective was to investigate the effects of primo-colonizing bacteria on the maturation of rat colonic epithelium.

In a first experiment, Germ Free (GF) rats were inoculated with intestinal contents of conventional 15d-old suckling rats (15d-suckling) and euthanized 2d, 7d and 14d after the transfer (respectively named Nb-2d, Nb-7d, Nb-14d). Caecal contents were analyzed for bacterial composition using quantitative PCR. In a second experiment, GF rats were inoculated with a mix of dominant bacteria isolated from 15d-suckling microbiota. Here, inoculated groups were euthanized 3d (EELCF-3d) or 21d (EELCF-21d) after the transfer. Isolated bacteria were characterized by 16S rRNA gene sequencing. Cell isolation and histological analyses were performed on the colon. The level and localization of PCNA, cyclin D2 and Ki-67; $p21^{Cip1}$ and $p27^{Kip1}$; and ß-catenin involved respectively in proliferation, cell cycle arrest and in cellular adhesion and Wnt signalling pathway were analyzed by immunohistochemistry or Western blot. The distribution of proliferative and goblet cells were studied by Ki-67 and Alcian blue co-staining.

Escherichia coli, *Enterococcus*, *Lactobacillus* and *Bacteroides* groups dominated the microbiota of suckling and Nb-2d-rats. In Nb-7/14d-rats these bacteria decreased in benefit of *Clostridium leptum.* EELCF microbiota consisted of *E. coli*, *E. faecalis*, *L. intestinalis*, *C. innocuum* and *Fusobacterium varium*. Histological analyses showed the crypt depth was higher in Nb- and EELCF-rats (+25%) than in GF. This is accompanied by a transient increase of the numbers of PCNA- and Ki-67-positive cells in Nb-2d and EELCF-3d (+60%). Higher amounts of PCNA and cyclin D2 proteins were also quantified in all groups when compared to GF (+3.0-fold for both proteins). $p21^{Cip1}$ was enhanced in Nb- (+2.5-fold) and EELCF- (+1.9-fold) rats suggesting it may counterbalance the extension of the proliferative zone. We observed discordant results for $p27^{Kip1}$as it increased in Nb- but not in EELCF-group. Co-staining of Ki-67 and Alcian blue revealed a sequential modulation of the proliferative/differentiated area in inoculated rats. ß-catenin was located at the intercellular zone of the colonic epithelium in all groups.

In our model, isolated primo-colonizing bacteria controlled both colonic cell proliferation and cell cycle arrest leading to a structured epithelium. The specific role of each isolated bacteria is under investigation. Such data will be relevant in Western countries where childbirth conditions (e.g., caesarian delivery) could impact implantation of these enteric bacteria.

Microbial mitigation: the Achilles' heel of allergy?

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The incidence and prevalence of allergic manifestations appear to have strongly increased over the past decades, now estimated to affect in total approximately 30% of the population in countries in the Western hemisphere, with incidence increasing in developing countries adopting a 'Westernized' lifestyle [1].

As a plausible explanation for this increase, an increased hygienic lifestyle and the success of vaccination campaigns are mentioned, which are supposed to have resulted in a delayed and likely persistently insufficiently developed Th1-associated immune compartment to achieve a healthy immune homeostasis. This hypothesis is commonly denoted as the 'hygiene hypothesis' [2]. Recently, an alternative version of this hypothesis has been formulated postulating that, during relatively recent human evolution (approx. in the last 10 000 years), major changes that have occurred in human living environment (notably co-evolution with domesticated animals and the development of a modern urban environment) have resulted in changes in intestinal microbiota and subsequent emergence of chronic adverse health conditions, including allergies [3]. These basic process may be amplified by e.g. changes in mobility of the population (resulting in changes in exposure to allergens), increased environmental pollution resulting in fine dust particles that may serve as vectors for allergen penetration into deeper airways, and changed medication patterns such as increased antacids and antibiotics usage.

Also microbiota composition has been found to be related to increased allergic manifestations. It is well recognized that microbiota are important for intestinal immune maturation, and that a disturbance in intestinal microbial homeostasis ('dysbiosis') is increasingly recognized to be associated to adverse health conditions, such as diabetes type 1 and 2, obesity, intestinal inflammation, etc.

A number of reports indicates that application of microbe-derived bioactive preparations, amongst which probiotics, and specific fungal preparations (including non-digestible polysaccharides and specific proteins) exhibit allergy mitigating properties. It is tempting to speculate that this is achieved via activation of innate immune receptors, recognizing such bioactive preparations via binding to pattern recognition receptors, and subsequent amelioration of Th1-driven immunity. A number of observations supporting this hypothesis will be discussed.

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Effect van *B. breve* Yakult on child-hood constipation – a case study

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Chronic functional constipation is a chronic debilitating disorder associated in the more severely affected individuals by significant impairments in quality of life and social functioning [1]. There is some evidence that supplementation of probiotics, such as *Bifidobacterium* species, when ingested in adequate amounts, exert a health benefit e.g. increased stool frequency and reduction of abdominal complaints, to the host. Compared to adults, in paediatric patients with chronic constipation research with supplementation of probiotics is sparse. The aim of this study was to determine if *B. breve* is an effective alternative in the treatment of chronic constipation where other drug treatments have failed. Our second aim was to assess if there is a difference in the microbial flora of the gut before and after treatment.

A 10-year old boy with chronic constipation according to the Rome III criteria was treated with *B. breve* for two months. He received one sachet of powder daily, containing 10^8-10^{10} cfu *B. breve*. His healthy sister served as a control. In both children faeces samples were taken at the start of treatment, and at 1 and 2 months after starting *B. breve* suppletion. Faeces samples were investigated on colonization of *B. breve* species using16s rDNA barcoded pyrosequencing. The results were compared with defaecation frequency and stool consistency during treatment. Pyrosequencing of the faeces samples of the boy showed an increase of *Bifidobacterium*-OTU 3 (which includes *B. breve*) already after one month of treatment, whereas the *Bacteroidetes* species almost disappeared. In faeces samples of his healthy sister *Bifidobacterium*-OTU 21 (containing *B. adolescentis*) was dominantly present, but this OTU was completely absent in the faeces of the boy. Furthermore the ratio of *B. breve* to total number of faecal bacteria increased in the boy and became comparable to his sister's. In two separate studies defaecation frequency, stool consistency and reduction of abdominal discomfort improved remarkably in the boy during treatment period.

In conclusion, this case report suggests that treatment with *B. breve* improves stool frequency and consistency in children with functional constipation. A randomized placebo-controlled trial is required to confirm this suggestion.

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Probiotics for HIV subjects in the developing world

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Clinically proven probiotics are, for the most part, not available in the developing world and certainly not affordable for the majority of people infected with HIV. Management of the infection and AIDS relies upon governments or aid agencies providing anti-retroviral therapies (ART) free of charge. For example, of the 5.6 million people living with HIV and AIDS in South Africa in 2009, more than in any other country in the world, only 1.4 million have access to ART, even with major government initiatives. In countries where access is far less, the burden of suffering is enormous. To put this into context, for every Dutch person infected there are 78 in Tanzania. The question is, what can be done to reduce the burden of HIV complications, which include super-infections, chronic diarrhoea, wasting, loss of energy, plus side effects of the toxic drugs in those who do gain access to ART.

In 2004, a program termed Western Heads East set up a community kitchen in the Mabatini District of Mwanza, Tanzania, in which local mothers (yogurt mamas) were taught how to make probiotic yogurt, using *Lactobacillus rhamnosus* GR-1 and locally sourced milk. Eight years later, kitchens in Tanzania, Kenya, Rwanda and soon to be Burundi and Malawi provide daily probiotic yogurt to over 2,700 men, women and children, an estimated one third of whom HIV positive. Twelve studies, some using encapsulated probiotics but most with yogurt, have been published so far. Collectively, these show safe use of the yogurt, allaying fears that HIV populations should not receive probiotics. Furthermore, many benefits have accrued, including alleviation of diarrhoea, reductions in infections, increased energy levels and in some cohorts an increase in CD counts.

Meanwhile, studies from other countries, particularly Brazil have also shown benefits of probiotic use against HIV. A critical factor is sustainability of the treatment, rather than simply going to a region, doing a study, showing benefits then not making that product available to the population as too often occurs. Secondly, investments are needed to perform larger studies that look at responders and non-responders, subjects who require closer monitoring or who should not receive probiotics due to other clinical complications, and which probiotic strains work most effectively at what time and for what conditions. To not do this, is to deny millions of men, women and children access to products that could improve their quality of life.

Control of bacterial diseases in aquaculture with pre- and probiotics

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Due to large-scale use of antibiotics, many pathogenic bacteria have acquired (multiple) resistance, which render antibiotic treatments ineffective. Therefore, alternative treatments to control aquaculture disease are urgently needed [1]. In general terms either the overall robustness of the host can be increased or intestinal microbial numbers or activity can be manipulated. The presentation deals with two techniques to manipulate micobial numbers or activity.

The disruption of quorum sensing, bacterial cell-to-cell communication, has been suggested as an alternative strategy to control infections caused by antibiotic-resistant bacteria in aquaculture [2]. Quorum sensing has been shown to regulate virulence expression in many bacteria *in vitro* (i.e., in bacteria grown in synthetic growth media). However, microbiologists are becoming more and more aware of the fact that bacteria behave differently in different environments [3]. Hence, the question that arises is whether and how quorum sensing regulates virulence of pathogens where it really matters: *in vivo* during infection of a host.

We found that quorum sensing regulates the virulence of *Vibrio harveyi* towards gnotobiotic brine shrimp larvae [4] and rotifers [5]. Very recently, we developed a method to monitor bacterial gene expression in vivo, during infection of gnotobiotic brine shrimp. Using this method, we found that there is a significant difference in the expression of quorum sensing-regulated virulence genes between virulent and non-virulent isolates [6]. Finally, we found that quorum sensing also affects survival of burbot challenged to *Aeromonas hydrophila* and *Aeromonas salmonicida* (Natrah et al., unpublished). The most important quorum sensing-disrupting agents reported thus far include compounds that interfere with quorum sensing signal detection and signal transduction, and signal molecule-degrading bacteria. We found that signal molecule-degrading bacteria isolated from aquaculture settings have a positive effect on survival of turbot and giant river prawn larvae cultured in non-gnotobiotic conditions [7]. We are currently also studying the impact of metabolites produced by micro-algae that are frequently used in aquaculture on quorum sensing activity of Gram-negative bacteria [8]

Poly-β-hydroxybutyrate (PHB) is a bacterial energy and carbon storage compound which exhibits a controlling effect on microbiota associated with aquaculture species. By means of PHB it was for example possible to control pathogenic activity in gnotobiotic brine shrimp [9] and to decrease the number of Vibrios associated with the larval culture of giant freshwater prawn [10], resulting in higher survival of the trial animals. However, the influence of PHB on the intestinal microbial community as a whole has not yet been studied in depth. Indeed, the question remains if PHB allows managing the intestinal microbial community as a whole and if this can result in advantages to the host. Therefore, the effects of PHB on the microbiota composition in the intestinal tract of juvenile sea bass were examined by means of PCR-DGGE. It was found that juvenile sea bass cohabiting in the same tank were on average 87% similar regarding the intestinal microbiota. When subjected to the same treatment and environmental conditions but reared in different tanks, the compositions of the enteric communities diverged. The provision of PHB overruled this tank effect by sustaining a microbial core community in the gut that represented 60% of the total bacterial diversity at the highest PHB level of 10% [11].

In conclusion, the data we obtained thus far indicate that quorum sensing disruption and PHD feeding are valid alternative biocontrol strategies for aquaculture.

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The effect of live yeast probiotics on peri-weaning pigs

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The weaning pig suffers physical, physiological and psychological stressors leading to changes in immune status and bowel dysfunction via the HPA axis. This contributes to the 'post-weaning growth check' where pigs may show poor appetite, and infectious diarrhoea, due to multiple and successive enteric infections. Antibiotics are commonly used to combat this, and we have studied the effect of antibiotic use and, as an alternative to antibiotic use, the probiotic *Saccharomyces cerevisiae Boulardii* (SB) on various health parameters in the peri-weaning pig. Continued use of antibiotics in commercial units causes genes for antibiotic resistance to be selected for in the intestinal microflora and these persist; in addition, antibiotic induces changes in microflora diversity and thereby may allow infection by opportunistic pathogens. At weaning, SB had the effect of stabilizing the intestinal microflora. The expression of innate immune genes was measured by quantitative PCR and showed that compared to control pigs SB supplementation could up-regulate Toll-like receptor (TLR)2 and TLR4 expression in the small intestine. This modulation was also associated with the increase of the anti-inflammatory cytokine Interleukin-10 in the SB group showing evidence of the immunomodulatory properties of SB in the intestinal mucosa. Our results have implications for the understanding of the effect of SB on innate immunity, microflora, and pathogen load in pigs and other monogastric animals, including man.

Influence of probiotics on the intestinal epithelial barrier

Contributed paper

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The epithelial cell layer in the intestine has to protect the body against intraluminal toxins, antigens and the endogenous microbiota while allowing entrance of nutrients. A disruption of this epithelial barrier leads to an increased permeability, allowing the entrance of endotoxins to the body. Factors influencing the epithelial barrier are for example by heredity, bacterial microbiota, diet, psychological stress, oxidative stress, exercise, and drugs.

Increased permeability of the epithelial barrier has been associated with many gastrointestinal inflammatory disorders, like inflammatory bowel diseases, celiac disease and food allergy. An increased permeability also lead to increased levels of endotoxins in the blood, which are linked to systemic inflammatory diseases, like metabolic syndrome, diabetes, atherosclerosis, chronic fatigue syndrome, autism, migraine and rheumatoid arthritis.

Probiotics have been shown to influence the epithelial barrier function. They can have direct effects on barrier function, by modifying expression and localization of tight junctions proteins as shown both *in vitro* in Caco-2 cell-lines as well as *in vivo*. These effects are strain specific. Beside direct effects, probiotics can also indirectly influence the epithelial barrier. Examples of these mechanisms are inhibition of mast cell activation, inhibition of pro-inflammatory cytokines, production of short chain fatty acids and decreasing the LPS load. Also these characteristics have shown to be strain dependent. Based on different *in vitro* screenings, a multispecies probiotic product has been specifically designed to strengthen the epithelial barrier. Currently this product (Ecologic®Barrier) is tested in a trial with migraine patients and investigations in other areas with this product are considered. So although at present *in vivo* evidence is still limited, strengthening the epithelial barrier with probiotics seems to offer new therapeutic options in diseases related to barrier dysfunction.

Drosophila as a model system to study microbiota/gut interaction

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Metazoans carry microbial communities on their mucosal surfaces with which they establish complex interactions that are essential to many aspects of the normal mucosal physiology. In return, the microbiota derives benefit from the association with its host by inhabiting a protected nutrient rich environment. Recently, the role of the host microbiota in metabolic disease has been revealed suggesting a contribution of the microbiota to systemic physiological output in healthy condition. However the precise interactions that take place between microbiota and host remain poorly understood at the molecular level.

In order to study the functional impact of the microbiota on its host physiology, we used *Drosophila* as a host model. We identified some of few bacteria species that are associated with *Drosophila* and demonstrate that one of them, *Lactobacillus plantarum*, is sharing all the characteristics of a *bona fide* commensal bacteria. At the conference, we will present our data describing the impact on this bacteria species on the systemic growth of the host upon recolonisation of germ-free individuals. We will describe our results using the power of *Drosophila* genetic to dissect the molecular mechanisms underlying this systemic benefit.

A microfluidic platform for droplet-enabled co-cultivation of microbial communities

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The majority of existing microbial species, in particular bacteria living in synergistic communities, have not been cultured in the laboratory with conventional pure-culture oriented methods. This severely limits the characterization and understanding of various microbial systems, including those closely related to the human health. To address this issue, we have developed a microfluidic co-cultivation platform to expand the repertoire of cultivable species from natural microbial communities and to characterize co-cultivated communities. We fabricated a microfluidic device that could readily encapsulate and co-cultivate subsets of a community, using highly parallel nano-liter aqueous droplets dispersed in a continuous oil phase. To demonstrate the effectiveness of this approach in discovering synergistic microbial interactions, a synthetic model system consisting of cross-feeding *E. coli* mutants was co-cultivated. Our device was able to detect pair-wise symbiotic relationship when one partner accounted for as low as 1% of the total population or each symbiont was about 3% of the artificial community.

Different microbial species have different level of oxygen tolerance and preference. To further enhance the likelihood of cultivating diverse species from a community, we have combined droplet co-cultivation and oxygen gradient to provide both the environment for microbial interactions and the optimal oxygen condition. Our prototype device is composed of two glass layers with fluid channels separated by a thin PDMS membrane. A linear oxygen gradient, covering the range of strictly anaerobic to fully aerobic conditions, is established and maintained via a tree-shaped channel mixing humidified nitrogen and air. The gradient is then transferred through the porous PDMS membrane to the chambers in the liquid channel incubating droplets. A murine faecal microbial sample, of which the bacteria lived with limited oxygen concentration in their native environment, was cultivated and different species were enriched in chambers featuring different oxygen conditions.

Sequencing of uncultured bacteria from single cells for the human microbiome project

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Uncultured bacteria comprise the majority of complex microbial communities, including the human microbiome. Single cell genomic methods allow extensive sequencing of these otherwise inaccessible organisms. Acquisition of single cell genomes relies on the isolation of single bacterial cells, robust genome amplification by multiple displacement amplification (MDA), deep sequencing of amplified DNA, and de novo assembly. We report on a programme for acquisition of MDA-amplified single cell bacterial genomes from the human microbiome for genome sequencing, including high throughput methods for sorting of single cells by flow cytometry, MDA, and 16S PCR for taxonomic identification. A quality control assay was developed based on shallow 454 Titanium sequencing of bar-coded MDA reactions. The QC assay was tested on a set of MDA-amplified genomes of known quality. It was then used to assess genome representation and purity of 95 GI tract single cell amplified genomes prior to deciding on which should proceed to deep sequencing and *de novo* assembly. Amplified DNAs are currently being distributed to the HMP sequencing centers for sequencing of reference genomes.

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Lgr5 stem cells in self-renewal and cancer

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The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined *Lgr5* as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of *Lgr5* in cycling, columnar cells at the crypt base. Using an inducible Cre knock-in allele and the Rosa26-*LacZ* reporter strain, lineage tracing experiments were performed in adult mice. The *Lgr5*$^{+ve}$ crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that it represents the stem cell of the small intestine and colon. Similar observations were made in hair follicles and stomach epithelium.

Single sorted *Lgr5*$^{+ve}$ stem cells can initiate ever-expanding crypt-villus organoids in 3D culture. Tracing experiments indicate that the *Lgr5*$^{+ve}$ stem cell hierarchy is maintained in these organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the *Lgr5*$^{+ve}$ stomach stem cells.

Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the 'cancer stem cell'-concept.

Fate mapping of individual crypt stem cells using a multicolour Cre-reporter revealed that, as a population, Lgr5 stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. Lgr5 cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or TA fates after cell division. Lgr5 stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express EGF, TGF-a, Wnt3 and the Notch ligand Dll4, all essential signals for stem-cell maintenance in culture. Co-culturing of sorted stem cells with Paneth cells dramatically improves organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous Wnt. Genetic removal of Paneth cells *in vivo* results in the concomitant loss of Lgr5 stem cells. In colon crypts, CD24+ cells residing between Lgr5 stem cells may represent the Paneth cell equivalents. We conclude that Lgr5 stem cells compete for essential niche signals provided by a specialized daughter cell, the Paneth cell.

POSTERS

P1 *Impact of genomic diversity of Lactobacillus rhamnosus probiotic strains on their immunomodulation effects – Cross Talk EU project*
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P2 *Faecal butyrate production is inhibited by the PUFA linoleic acid but not by its biohydrogenation products*
Rosemarie De Weirdt[1], B. Vlaeminck[2], E. Mees[1], V. Fievez[2], V. Eeckhaut[3], J. Vermeiren[1], S. Possemiers[1], W. Verstraete[1] and T. Van de Wiele[1]
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P3 *An in vitro fluorescent staining method for visualization of lactic acid bacteria attachment to enteric mucosal cells*
Matthew F. Faulkner, L.R. Bielke, C.J. Kremer, O.B. Faulkner, G. Tellez and B.M. Hargis
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P4 *Metabolomic profiling of probiotic bacterial cultures by untargeted high resolution mass spectrometry analysis*
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P5 *In vitro evaluation of the upper gastrointestinal passage of a novel butyrate producing isolate to counterbalance dysbiosis in inflammatory bowel disease*
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P6 *Spodoptera littoralis larvae as a model for microbe-host interactions in the gut*
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P7 *Characterization of a novel Clostridium species isolated from the gastro-intestinal tract of rats*
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P8 *Improved adhesion of recombinant Bifidobacterium strains expressing the lipoprotein BopA*
Marita Gleinser, D. Zhurina and C.U. Riedel
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P9 *Technologically processed soluble factors of Lactobacillus rhamnosus are protective against allergic airway inflammation in neonatal mice*
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P10 *Lactobacillus rhamnosus GG and its soluble factors protect against Cronobacter sakazakii invasion and related mortality in neonatal mice*
Gabriele Gross[1], A.K. Agyekum[2], K.M. Overkamp[3], E.A.F. van Tol[1] and M.A. Smith[2]
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P11 *Differential regulation of mucosal IgA1 and IgA2 production*
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P12 *Different effects of germinated Bacillus subtilis C-3102 fractions on microbiota in a dynamic model of the large intestine simulating human conditions*
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P13 *Metabolic reconstruction of prominent human gut microbe Bacteroides thetaiotaomicron*
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P14 *Influence of probiotic E. coli O83 strain on the development of acute intestinal inflammation induced by dextran sulfate sodium*
Tomáš Hudcovic, R. Stepankova, H. Kozakova, D. Srutkova, M. Schwarzer, M. Souckova M. and H. Tlaskalova-Hogenova
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P15 *Immunological effects of selected probiotic and potential probiotic lactic acid bacteria*
Hanne Jensen, M. Taraldrud, L. Axelsson and S. Grimmer
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P16 *Metagenomic analysis of human milk*
Esther Jiménez, I. Espinosa, R. Arroyo, L. Fernández, and J.M. Rodríguez
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P17 *Adverse effects of low doses exposure to chlorpyrifos in a human simulator of the intestinal microbial ecosystem (artificial intestine)*
Claire Joly, J. Gay-Quéheillard, S. Delanaud, V. Bach and H. Khorsi-Cauet
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P18 *Impact of oral low doses of chlorpyrifos on intestinal maturation after perinatal exposure in rats*
Claire Joly, J. Gay-Quéheillard, V. Bach and H. Khorsi
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P19 *A public-private partnership that aims to establish a novel, multivariate view of oral health*
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P20 *Electrochemical behaviour of commensal gut bacteria*
M. Tanweer Khan[1], S.H. Duncan[2], A.J.M. Stams[3], J.M. van Dijl[1], W.R. Browne[4], H.J. Flint[2] and H.J.M. Harmsen[1*]
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P21 *Lactobacillus plantarum isolated from ayurvedic medicine ameliorates cytotoxicity caused by Aeromonas veronii*
Himanshu Kumar, A.Y. Rangrez, K.M. Dayananda, A.N. Atre, M.S. Patole and Y.S. Shouche
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P22 *Enterococcus faecium NCIMB 10415 does not protect interleukin-10 knock-out mice from chronic gut inflammation*
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P23 *Modulation of inflammatory responses in vitro and in vivo by commensal bacteria*
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P24 *Metabolomic applications to decipher gut microbial metabolic influence in health and disease*
François-Pierre Martin[1], C.L. Boulangé[2], M.E. Dumas[2], S.Moco[1], S. Collino[1], E. Holmes[2], S. Kochhar[1], J.K. Nicholson[2] and S. Rezzi[1]
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P25 *In vitro inhibitory activity of probiotic strains against pathogens in aquaculture*
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P26 *Gut microbiota composition is associated with hepatic fat content in humans*
Evelina Munukka[1], P. Wiklund[1], S. Pekkala[1], X.W. Ojanen[1], S. Cheng[1], A. Lyytikäinen[1,2], M. Alen[3], R. Borra[4], J.-P. Usenus[5], J. Lavonen[5] and S. Cheng[1,6]
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P27 *Mechanisms of uncultivability in the oral microbiome*
Pallavi P. Murugkar[1], A. D'Onofrio[1], E. Stewart[1] and K. Lewis[1]
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P28 *Community structure of developmental GI tract microbiota in infants and its correlation with allergy development in later life*
Jiro Nakayama, Y. Korenori, T. Kobayashi, K. Sonomoto
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P29 *Effect of direct fed candidate Bacillus microbials on Salmonella infection and production parameters in commercial turkeys*
Chris M. Pixley, R.E. Wolfenden, N.R. Pumford, M. Morgan, S. Shivaramaiah, A.D. Wolfenden, L.R. Bielke, G.I. Tellez and B.M. Hargis
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P30 *Arabinoxylans show distinct prebiotic properties, as studied using a combination of in vitro, animal and human intervention studies*
Sam Possemiers[1,4], P. Van den Abbeele[1], J.-M. Lecerf[2], F. Depeint[3], A. Cayzeele[2], E.Clerc[2], Y. Dugenet[4], P. Pouillart[3] and T. Van de Wiele[1]
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P31 *Effect of nitrate plus a Lactobacillus probiotic on Salmonella colonization in chickens*
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P32 *A top-down microbial systems ecology view of the impact of prebiotic oligosaccharides on bifidobacteria in human gut microcosms*
Guus Roeselers, M.M. Ossendrijver, J.P.M. Coolen, S.E. Ladirat, R. Montijn, F. Schuren and B.J.F. Keijser
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P33 *Streptococcus thermophilus LMD-9 is advantaged over Lactobacillus delbrueckii ssp. bulgaricus ATCC11842 in different media and in the digestive tract of gnotobiotic rats*
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P34 *Rapid discrimination of Bifidobacterium animalis subspecies by matrix-assisted laser desorption ionization time of flight mass spectrometry*
Santiago Ruiz-Moyano[1,2], N. Tao[3], M.A. Underwood[4] and D.A. Mills[1,2]
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P35 *Fermented milk containing Lactobacillus casei strain Shirota reduces incidence of hard or lumpy stools in healthy population*
Takafumi Sakai, H. Makino, E. Ishikawa, K. Oishi and A. Kushiro
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P36 *Intestinal stem cell derived organoids as a novel tool for analyzing food microbiota host intestinal tissue interactions*
G. Roeselers, M. Hassan Zade Nahjari, B.J.F. Keijser, R. Montijn and
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P37 *Associating phenotypic characteristics of Lactobacillus paracasei and Lactobacillus rhamnosus to strain-specific variability in gene content*
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P38 *Combining ^{13}C-metabolic flux analysis and genome-scale modelling of short chain fatty acid production*
Marvin N. Steijaert[1,2,3], M.J.A. van Hoek[4], T.W. Binsl[5], J.H.G.M. van Beek[5], R.M.H. Merks[4], K. Venema[1,2] and A.A. de Graaf[1,2]
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P39 *Development of a dynamic in vitro model for the human ileum*
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P40 *Exploration of suitable terminal restriction fragment length polymorphism analysis of 16S rRNA genes from human faecal microbiota*
Hirosuke Sugahara, T. Odamaki, S. Takahashi, A. Horigome, J.-z. Xiao and K. Iwatsuki
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P41 *The effects of Lactobacillus brevis KB290 on menopausal symptoms with a tendency for constipation in women: an open trial*
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P42 *Cell surface exopolysaccharides of Lactobacillus brevis KB290 protect against artificial digestive fluids*
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P43 *Evaluation of the safety and potential probiotic properties of Lactococcus lactis subsp. lactis MK02R isolated from rocket salad (Eruca sativa Mill.)*
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P44 *Isolation of bacteriocinogenic strain of Lactococcus lactis subsp. lactis from rocket salad (Eruca sativa Mill.) and evidence for production of a variant of nisin*
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P45 *Development and validation of an apical anaerobic model of the intestinal barrier*
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P46 *In vitro evaluation of nutrients that selectively confer a competitive advantage to Lactobacillus acidophilus*
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P47 *Identifying mechanisms of bacterial unculturability in the human gut microbiome*
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P48 *Caenorhabditis elegans as model organism for host-pathogen interactions and probiotics research*
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P49 *Identification and evaluation of candidate Bacillus probiotics (DFM) isolates for use in commercial poultry*
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P50 Analysis of pilus-encoding gene clusters in two bifidobacterial species

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P1

Impact of genomic diversity of *Lactobacillus rhamnosus* probiotic strains on their immunomodulation effects – Cross Talk EU project

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The main goal of the Cross Talk project one is to determine the impact of the diversity within a probiotic *Lactobacillus* species in relation to its immune modulatory capacities and to identify functions involved in immune modulation. The experimental work of the project encompasses a diverse set of techniques that are meant to first supply information about the phenotype and genotype of the *L. rhamnosus* strains and then to verify the presence/absence of the interesting traits in the mutants. A substantial part of the work involves bioinformatics programs for corroborating the sets of data, investigating genes of interest and obtaining candidate genes for mutagenesis. The first step was obtaining sequence information for 27 strains of diverse origins from the Danone Research Culture Collection. In addition, the strains were subjected to a detailed characterization *in vitro* including immune activity, pathogen inhibition and carbohydrate utilization. The datasets will be provided to a gene-trait matching project to identify potential genes of interest. It is also possible to make an educated guess about other genes that can have an impact on immune modulation – exoproteome genes will be especially targeted. The gene candidates will be evaluated by mutagenesis studies *in vitro* and *in vivo* through collaboration. It appears that genetically closely related *L. rhamnosus* strains can be discriminated on basis of their capacity to elicit differential effects of pathogen inhibition, metabolisation of simple sugars and cytokine production levels or NFkB activation in different model systems. The differential immuno-modulation data can be used to identify the responsible molecules using a comparative genomics approach which is currently in progress. These studies will be complemented by validation experiments using gene-targeted mutagenesis approaches that will be supported by *in vitro* experiments with the immune models already set in place and *in vivo* experiments using animal models. In conclusion, the research presented summarizes the main work plan and results obtained in the first two years of project life. Our hypothesis that genetically related bacterial strains can elicit differential immune modulation is supported by the *L. rhamnosus* group. Identification of factors involved in these differential effects on the host is in progress by gene trait matching as well as comparative genomics. Validation tests for selected molecules or genes will be performed *in vitro* and *in vivo*.

P2

Faecal butyrate production is inhibited by the PUFA linoleic acid but not by its biohydrogenation products

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Daily, significant amounts of poly-unsaturated fatty acids (PUFA) and in particular linoleic acid (LA; c9, c12-18:2) reach the colon microbiota [1-3]. Although the colonic fat load is rich in PUFA, fat in the faeces is mostly saturated due to the existence of a microbial saturation process, named biohydrogenation [1]. Considering the higher antimicrobial effects of PUFA compared to their saturated counterparts, we investigated to what extent LA and its bio-hydrogenation products vaccenic acid (VA; t11-18:1) and stearic acid (SA; 18:0) may affect the activity and community structure of gut microbiota. Faecal slurries of two healthy volunteers were incubated during 48 h with or without 5 g/l oat β-glucan and 1 g/l LA, VA or SA. In addition, we enriched the 2 faecal inocula during 20 days on rolled oats and LA and monitored the microbial communities by PCR-DGGE and qPCR. In the presence of oat dietary fibre, LA was not biohydrogenated efficiently and significantly stimulated propionate production while decreasing butyrate production, without affecting total SCFA production. LA's hydrogenated counterparts VA and SA did not exhibit such effects. Moreover, in the absence of oat dietary fibre, significant LA biohydrogenation to VA occurred and no specific SCFA effects were observed, suggesting that LA biohydrogenation may be an important determinant of the SCFA profile in the gut. Following the enrichment of the two inocula on rolled oats and LA, a different butyrate production and LA biohydrogenation activity was found. In addition, community 1, which produced more butyrate and converted LA to VA, displayed higher and more stable qPCR counts of *Roseburia* spp. and the butyryl CoA:acetyl CoA transferase gene than community 2. No differences were found in the *Faecalibacterium prausnitzii* counts. Overall, the results of this study reveal important correlations between efficient LA biohydrogenation and the butyrate production process.

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P3

An *in vitro* fluorescent staining method for visualization of lactic acid bacteria attachment to enteric mucosal cells

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Carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) is an intracellular vital dye that fluoresces only in live cells, potentially allowing microscopic adhesion to enteric mucosal cells. *Lactobacillus salivarus* (LS) was stained with CFDA-SE and evaluated for *in vitro* ability to adhere to mucosal epithelial explants *in vitro*. In experiment 1, an overnight LS culture was combined with 0.1, 0.33, or 1 mM concentrations of CFDA-SE. Each concentration was incubated in fresh media with or without shaking for 2 h. After incubation, cells were washed to remove unabsorbed CFDA-SE and observed under UV light microscope for evaluation of fluorescence and background. LS combined with 1 mM CFDA-SE and incubated on a shaker provided the highest fluorescence with little background. This method was used to prepare a culture for experiment 2, in which CFDA-SE LS cells were combined with mucosal explants from the crop and ileum of neonatal chicks. Explants, consisting of epithelial or villus sheets, were immediately placed in cell culture medium, combined with CFDA-SE LS, and incubated at 37 °C. Adhesion of stained LS to cells of explants were qualitatively observed at 0, 1, 6, and 24 h. At 0 h, LS adhered to cells of the crop, however, the number of LS observed in association with explants decreased throughout the 24 h period. Adherence of CFDA-SE LS to ileal explants gradually increased through 6 h, with a substantial decrease at 24 h. Since CFDA-SE does not replicate, the 24 h decrease may be due to lower concentrations as a result of dilution by binary fission. For experiment 3, multiple methods of collecting cellular explants for co-incubation with CFDA-SE LS were tested to investigate possible methods for an *in vitro* test. Cellular scrapings incubated with CFDA-SE LS resulted in a high number of un-bound LS. Incubation of whole tissue with CFDA-SE LS had low binding to the cell layer but little un-bound LS. This low-cost rapid technique may provide for a rapid *in vitro* method for identifying beneficial probiotic candidates that adhere to specific sections of the gastrointestinal tract.

P4

Metabolomic profiling of probiotic bacterial cultures by untargeted high resolution mass spectrometry analysis

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Low molecular weight organic compounds produced by probiotic bacteria such as lipids, amino acids, carbohydrates, nucleotides, etc., participate not only in cell metabolic reactions, nutrition and in energy cycle but also interact with the environment for example, benefiting the host or having an antimicrobial activity. The task of analysing significant variations in this highly complex mixture of chemicals is only nowadays possible due to recent technological developments in analytical chemistry (NMR spectroscopy or mass spectrometry) and bioinformatics with software to handle and process large amounts of data generated from high-throughput analysis. Fourier-transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) 12 Tesla is a powerful technique that can be combined for metabolomic studies due to its (ultra)high resolution, accuracy and sensitivity. The purpose of the present study was to investigate the metabolomic profile of cell cultures from different probiotic bacteria species using a non-targeted approach and correlate their metabolic fingerprint to immune modulatory activity or inhibition properties against pathogenic microbial such as *Pseudomonas aeruginosa* infection in *C. elegans* model or *Escherichia coli* and *Salmonella* growth in pure culture. Probiotic bacteria strains were obtained from Winclove Bio Industries B.V. (Netherlands) and were cultivated at 37°C in defined medium CDM1 (without Tween 80 due to signal interference in mass spectrometry analyses). Cells were harvested by filtration after achieve the stationary phase (OD at 600nm) where supernatants and pellets were collected and kept in -80 ºC until sample preparation prior chemical analyses. Intracellular metabolites were extracted from the pellets using methanol followed by sonication and centrifugation. Metabolites in the extra-cellular medium of the bacterial cultures were extracted by SPE cartridges with two different sorbent materials (HLB and cyanopropyl) followed by methanol elution. After developing the respective analytical methods for each group of samples, FT-ICR-MS spectra were acquired in positive and negative electrospray ionization modes for all extracts. Thousands of signals have been detected from each probiotic strain and assigned in metabolic databases. Different statistical and visual techniques have been applied in order to extrapolate the main biological properties of the experiment. Moreover through the multivariate analysis it was possible to define structure of correlation present in the data and classify the samples (bioactive versus non-bioactive). At the end of the different processes we have as output a list of masses that are going to discriminate the two different groups.

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P5

In vitro evaluation of the upper gastrointestinal passage of a novel butyrate producing isolate to counterbalance dysbiosis in inflammatory bowel disease

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Inflammatory bowel diseases (IBD) are characterized by chronic inflammation of the gastrointestinal tract. The aetiology of IBD is still unclear but there is a well-established link between the intestinal microbiota and gut inflammation. Therefore, manipulation of the unbalanced microbial community in IBD is a very attractive therapeutic strategy. Butyrate has well described beneficial effects in IBD, but the delivery into the gut is still problematic. Administration of butyrate producing bacteria that are able to colonize the gut is therefore an elegant solution for the delivery problem. Recently, a novel butyrate producing isolate *Butyricicoccus pullicaecorum* (member of Clostridial cluster IV), was isolated and showed the production of exceptional high concentrations of butyrate (up to 18 mM) while consuming acetate. The aim of this study was to evaluate a novel butyrate producing isolate, *Butyricicoccus pullicaecorum*, for its survival during passage of the upper gastrointestinal tract by batch incubations. These properties are essential to make *Butyricicoccus pullicaecorum* a suitable probiotic strain. During different batch incubations with a 2 h gastric phase and 4 h small intestine phase the effect of different gastric pH, low oxygen concentrations, fed versus fasted conditions and the matrix on the viability of an overnight culture was evaluated. The viability of *B. pullicaecorum* was determined by plating serial dilutions on M2GSC agar plates. The concentration of colony forming units (cfu/ml) of the inoculum and at different times during gastrointestinal simulation (1 h, 2 h, 4 h and 6 h) was determined. The results showed that *B. pullicaecorum* is able to survive the passage of the upper digestive. No survival was observed during gastric phase at pH 2. During this acid stress the cells probably went into a viable but non-culturable state whereas they resuscitated under small intestine conditions. Low concentrations of oxygen during the gastric phase had no effect on the viability of *B. pullicaecorum*. It is expected that *B. pullicaecorum* will further grow under colon conditions and produce butyrate. In the near future, the colonization efficiency and metabolic activity of *B. pullicaecorum* will be evaluated under colon conditions with a dynamic *in vitro* model of the human gastrointestinal tract, the SHIME. These future experiments will allow us to evaluate if *B. pullicaecorum* will be a good candidate as a new probiotic strain.

P6

Spodoptera littoralis larvae as a model for microbe-host interactions in the gut

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Microbe-host interactions in the gastrointestinal tract (GIT) of humans play a role in different kinds of disorders. Due to the complexity of the human microbiota, the exact mechanism of those interactions remains unclear and there is a need for straightforward models that allow addressing fundamental research questions. Insects are a good alternative for the time- and labour-intensive and high cost vertebrate models of the GIT. Insects have a relatively simple microbial community in their GIT and their innate immune response to microbiota is similar to the one in humans [1]. In addition, they are easy to work with because of their short lifecycles and there are no ethical concerns as opposed to vertebrates. The aim of this study was to evaluate the larvae of the cotton leafworm (*Spodoptera littoralis*; *Lepidoptera*, moths/butterflies) as a potential model for microbe-host interactions in the GIT trough determination of the dynamics and colonization resistance of the endogenous microbiota. The microbial community was analysed based on the amplified 16S rRNA gene fragments by means of denaturing gradient gel electrophoresis (DGGE) and sequencing. The endogenous microbial community of *S. littoralis* mainly consisted of *Enterococcus* spp. and varied over time (correlated with molting) and place (midgut vs. peritrophic matrix vs. lumen vs. faeces). During a first invasion experiment caterpillars were fed continuously with bacterial suspensions of different monocultures, a polyculture and a mixed culture of human colon microbiota. Only *Lactobacillus brevis* was able to colonize the midgut of *S. littoralis* larvae after 6 days of inoculation and reside there until the last larval stage. The other species were eliminated from the GIT with the faecal material, but *Bacteroides thetaiotaomicron*, *Escherichia coli*, *Pseudomonas putida*, *Serratia marcescens* and the polyculture were able to induce alterations in the microbial community compared with the control. During a second invasion experiment, *S. littoralis* larvae were orally inoculated through a syringe with a suspension of *L. brevis* and the colonization efficiency was evaluated. The results showed that *L. brevis* was able to settle in the midgut 18 h after inoculation. In general, it can be concluded that larvae of *S. littoralis* offer a potential to be used as a model to study microbe host interactions in the GIT. They have a GIT that has a similar structure as the human GIT with an epithelial monolayer with microvilli and a peritrophic matrix with a similar function as the mucus layer. Further, the larvae have a relatively straightforward endogenous microbial community that can be eliminated with an antibiotic treatment resulting in germ free animals. Moreover, it is possible to separate the epithelium, the peritrophic matrix and the lumen during dissection. This offers opportunities for the study of species with the ability to adhere. Besides, it was possible to induce shifts in the microbial community through oral inoculation with different bacterial species. The model in *S. littoralis* can learn us more about the organization of a microbial community in the GIT and its colonization resistance against invading species.

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P7

Characterization of a novel *Clostridium* species isolated from the gastro-intestinal tract of rats

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Recently a novel bacterial phylotype was reported to be associated with probiotic-induced changes in the gut microbiota of rats in an experimental model for acute pancreatitis. A high relative ileal abundance of this phylotype, named CRIB, was correlated with an improved disease outcome. The aims of this study were to isolate CRIB from the gastro-intestinal tract of rats and to characterize this novel *Clostridium* cluster XI representative. For isolation of CRIB, serial dilutions were made in a bicarbonate-buffered medium of a sample obtained from the ileum of a healthy rat. Morphological, physiological, molecular and chemotaxonomic studies were performed with cells grown on a basal peptone-yeast extract (PY) medium. For characterization, the isolate was compared to the closely-related clostridial species *C. lituseburense* (DSM 797^T), *C. irregulare* (DSM 2635^T), *C. hiranonis* (DSM 13275^T) and *C. bartlettii* (DSM 16795^T). Transmission electron microscopy was performed for detailed characterization of cell morphology. The complete genome of strain CRIB^T has been sequenced. A gram-positive, rod-shaped, non-motile anaerobic bacterium, designated CRIB^T, was isolated from the gastro-intestinal tract of rats. Based on the results of the phenotypic and physiological characterization, phylogenetic analysis and DNA-DNA relatedness, strain CRIB^T represents a novel species within *Clostridium* Cluster XI. Free spores were seen occasionally after prolonged incubation at lower pH. The peptidoglycan type of strain CRIB^T was determined to be A1ð lanthionine-direct, which has not been previously described for *Clostridium* species. Besides glucose, L-fucose and saccharose strain CRIB^T is able to grow on raffinose, a substrate the host is not able to utilize itself. Characterization of this novel commensal gut bacterium has provided the first insights into the intestinal functionality of this bacterium. Future studies will focus on isolation of a human variant of this novel species and determining its role in human health and disease.

P8

Improved adhesion of recombinant *Bifidobacterium* strains expressing the lipoprotein BopA

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Bifidobacteria belong to one of the predominant bacterial groups in the intestinal microflora of infants and adults. Several beneficial effects on the health status of their human host have been demonstrated, making bifidobacteria interesting candidates for probiotic applications. Adhesion of probiotics to the intestinal epithelium is discussed as a prerequisite for the persistence and the colonization of the gut. In the present study, 15 different strains of bifidobacteria were tested for adhesion. *B. bifidum* was identified as the species showing highest adhesion to all tested intestinal epithelial cell (IEC) lines. Cell fractions of *B. bifidum* S17 were used in competitive adhesion studies with intact bacterial cells of *B. bifidum* S17 to IECs. Adhesion of *B. bifidum* S17 was strongly reduced after pre-incubation of IECs with cell wall fraction. Furthermore, after treatment of *B. bifidum* with pronase adhesion to IECs was significantly reduced. These results strongly indicate that a proteinaceous cell surface component mediates adhesion of *B. bifidum* S17 to IECs. *In silico* analysis of the currently accessible *Bifidobacterium* genomes identified *bopA* as a *B. bifidum*-specific gene, encoding a lipoprotein previously identified as an adhesin of *B. bifidum* MIMBb75 [1]. The *in silico* results were confirmed by Southern Blot analysis. Furthermore, Northern blot analysis demonstrated that *bopA* is expressed in *B. bifidum* strains under conditions used to cultivate the strains for adhesion assays. BopA was successfully expressed in *E. coli* BL21 (DE3) and purified by Ni-NTA affinity chromatography as a C-terminal His_6-fusion. Purified BopA had an inhibitory effect on adhesion of *B. bifidum* S17 to IECs. Moreover, BopA was successfully expressed in *B. bifidum* S17 and *B. longum/infantis* E18. Strains overexpressing BopA showed enhanced adhesion to IECs, clearly demonstrating a role of BopA in adhesion of *B. bifidum* strains. In summary, the results of this study are one of the first reports on improved adhesive properties of *Bifidobacterium* strains.

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P9

Technologically processed soluble factors of *Lactobacillus rhamnosus* are protective against allergic airway inflammation in neonatal mice

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The incidence of allergic diseases such as asthma is increasing in infants and children. Protective effects of probiotic supplementation have been demonstrated in experimental rodent models of allergic airway inflammation. We previously reported soluble factors from *Lactobacillus rhamnosus* GG (LGG) to have beneficial effects in this model. When applying these factors from LGG in nutritional formulations, processing aspects like spray-drying, ultra-filtration and lyophilisation may negatively affect their bioactivity. Therefore, the aim of this study was to validate the effects of differentially processed LGG-derived supernatant supplementation on allergic airway inflammation in a neonatal mouse model. Newborn Balb/c mice were orally supplemented with viable LGG or defined differentially processed LGG supernatants, from day 2 until 6 weeks of age. Acute allergic airway inflammation was subsequently induced by sensitization and challenge with ovalbumin. On day 71, airway reactivity was determined, and animals were sacrificed on day 72. Bronchoalveolar lavage (BAL) cell counts, BAL cytokines, serum ovalbumin-specific immunoglobulin isotypes as well as lung histology were analyzed. Confirming earlier findings, supplementation with viable LGG caused a significant decrease in BAL eosinophils, BAL IL-5 and both inflammation and goblet cell scores, but did not have an effect on airway hyperresponsiveness. Similar results were obtained after supplementation with ultra-filtered and lyophilized LGG supernatant, with an additional increase in BAL IL-10 and decrease in IL-9. In contrast, supplementation with the raw unprocessed LGG supernatant and LGG supernatant that was desalted using G25 column chromatography and subsequently filtered and lyophilized did not show similar effects. In conclusion, dietary supplementation of newborn mice with ultra-filtered, lyophilized LGG supernatant induces comparable protective effects on the development of allergic airway inflammation as viable LGG bacteria. Specific preparation of soluble factors from LGG ensures retention of bioactivity and may thus provide an opportunity for product application.

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P10

Lactobacillus rhamnosus GG and its soluble factors protect against *Cronobacter sakazakii* invasion and related mortality in neonatal mice

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Cronobacter sakazakii is an opportunistic pathogen that has been associated with outbreaks of infection in infants, especially in neonatal intensive care units. As a consequence of bacterial invasion to the brain, infections frequently lead to developmental delays and mental retardation. The aim of the current study was to assess whether supplementation with viable *Lactobacillus rhamnosus* GG (LGG) or soluble factors excreted by LGG (specifically generated LGG supernatant) could reduce *C. sakazakii* invasion and related mortality in neonatal mice. Neonatal CD-1 mice (cumulative n = ≥46 per group) were supplemented with viable LGG (10^5 CFU/dose) or a corresponding dose of specific LGG supernatant by oral gavage for four consecutive days starting on postnatal day 1. On day 2, pups received 0.1 ml of reconstituted powdered infant formula containing *C. sakazakii* strain 3290 (10^7, 10^8, or 10^{11} CFU/dose in three independent experiments) or vehicle control. Morbidity or mortality was monitored twice daily, and all living pups at day 7 post-infection were euthanized. *C. sakazakii* invasion into liver, caecum, and brain was determined by culturing tissue samples with *Enterobacter* enrichment broth on violet red bile glucose agar and sub culturing on TSA plates. *C. sakazakii* isolation was confirmed by RapID ONE Identification. Supplementation with LGG supernatant or viable LGG significantly decreased the percentage of animals with at least one organ invaded with *C. sakazakii* [26% (control) vs. 20% (LGG supernatant) vs. 17% (viable LGG)]. Moreover, both LGG supernatant and viable LGG significantly reduced *C. sakazakii* invasion into brain tissues (19% vs. 7% vs. 8%). Interestingly, *C. sakazakii* related mortality was completely prevented by supplementation with LGG supernatant (20% vs. 0% vs. 17%). In conclusion, probiotic LGG or its secreted soluble mediators can protect against *C. sakazakii* invasion and related mortality in neonatal mice. Follow-up studies are aimed at unravelling the underlying mechanisms of protection including the intestinal mucosal interface.

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P11

Differential regulation of mucosal IgA1 and IgA2 production

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IgA is a highly abundant antibody class at mucosal surfaces. IgA forms a first line of defense against pathogens, may confer protection against allergy and is involved in maintaining intestinal homeostasis by controlling the microbiota. Next to T helper cell dependent induction, IgA can be induced locally in the intestine independent from T cell help. Intestinal DCs have been proven to induce IgA. In contrast to mice, humans have two IgA subclasses. We aimed at identifying if mucosal IgA1 and IgA2 production could be differentially regulated. Peripheral B cells were isolated from healthy donors. B cells were cultured in the presence or absence of DCs and other cytokines. DCs were derived from peripheral monocytes and differentiated with IL-4 and GM-CSF with or without retinoic acid (RA) to obtain different intestinal DC subsets. B cells were analyzed by flow cytometry and IgA1 and IgA2 were measured by specific ELISA. The presence of monocyte derived DCs enhanced expression of Integrin ß7, which is involved in homing of B cells to the intestines. Also IL-4 induced ß7 expression on B cells in the absence of moDCs. RA differentiated moDCs enhanced CD38 expression levels, indicating differentiation of B cells into antibody secreting cells. Induction of similar levels of CD38 on B cells after co-culturing with non RA differentiated moDCs required cooperation between TSLP and IL-4. Addition of the TNF-α family member cytokines APRIL and BAFF induced IgA2 but not IgA1 when B cell were co-cultured with both types of DCs. Finally, the presence of DCs decreased numbers of IgA1+ B cells and increased numbers of IgA2+ B cells, especially for RA differentiated moDCs. We conclude that IgA1 and IgA2 are clearly differentially regulated by DCs and/or cytokines. Our data suggest that IgA2 but not IgA1 is inducible by intestinal DCs. In combination with the (RA) DC dependent induction of the plasma cell marker CD38 and intestinal integrin ß7 our data provide insight in the mechanisms of protective IgA production in the gut mucosa.

P12

Different effects of germinated *Bacillus subtilis* C-3102 fractions on microbiota in a dynamic model of the large intestine simulating human conditions

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Live spores *Bacillus subtilis* C-3102 having a potential to control microbiota has been used as probiotics for animal additive to improve animals' performance. We have been investigating its potential use for modulation of the human microbiota throughout *in vitro* gastrointestinal TIM model study. Previously, our studies using TIM suggested that germination of spores from C-3102 might affect the composition of the human originating microbiota. In the present study, fractionated germinated cell broth was introduced into TIM-2 simulating human large intestine and these effects on microbiota were evaluated to understand the active components of C-3102 in this model. The 30% germinated C-3102 cells were prepared by cultivation of spore cells in trypticase soy broth, and then the supernatant and the precipitate were prepared. Then, 8% germinated samples for both supernatant and precipitate were prepared by mixing of each 30% germinated cell fraction and a non-germinated sample. A standardized microbiota from human healthy adults was inoculated into the TIM-2 model. After adaptation of the microbiota for 16 h, the test samples (0, 8, and 30% germinated cell fractions) were added to the model at 0, 24, and 48 h. Then, sampling was conducted at the start (0 hour) and at the end of the experiment (72 h). Composition of microbiota in the collected sample was analyzed by real time-PCR method by using species and group-specific primers. As a result, germination dependent changes in the amount of some species were observed with both supernatant and precipitate fractions. In more detail, *Bifidobacterium* group was increased by addition of both the supernatant and the precipitate of the germinated culture. On the other hand, repressed effect of the supernatant on specific species was observed as well. These results suggest that there might be more than two kinds of different components in the supernatant and the precipitate of the germinated C-3102 fractions that act on specific members of the microbiota.

P13

Metabolic reconstruction of prominent human gut microbe *Bacteroides thetaiotaomicron*

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A common inhabitant of the human gut is *Bacteroides thetaiotaomicron*, a Gram-negative obligate anaerobe. This bacterial symbiont benefits the host by breaking down otherwise inaccessible dietary plant polysaccharides [1], leading to the production of short-chain fatty acids (SCFAs). SCFAs not only contribute to up to 10% of the host's daily caloric intake [2], but also regulate host energy balance [3] and have anti-inflammatory effects via activation of host signalling pathways [4]. Here we present a manually curated, genome-scale metabolic reconstruction of *B. thetaiotaomicron*. The reconstruction of the metabolic network was based on an automated reconstruction obtained from the web-based resource Model SEED [5], a pipeline that automates some steps of the reconstruction process. We then manually curated the draft reconstruction based on the supplementary methods provided by [5], as well as established methods in constraint-based reconstruction and analysis [6]. To ensure comparability with existing metabolic models, rBioNet, a tool designed for building high-quality reconstructions, was utilized for assembling metabolites and reactions [7]. The resulting *in silico* model accurately captures the microbe's *in vivo* behaviour, including the ability to utilize 49 carbohydrates as carbon sources, and production of known secretion products such as SCFAs. In 11 of 12 cased, the model correctly predicts known knockout phenotypes. Of the 925 non-exchange reactions included in the draft reconstruction, 786 were retained. While central biochemical pathways were well accounted for in the automated reconstruction, organism-specific pathways were poorly represented and required intensive manual curation based on available literature. This added a total of 315 reactions to the automated reconstruction. The resulting manually curated and validated reconstruction should provide a useful tool for studying the interactions between this bacterial symbiont and its host.

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P14

Influence of probiotic *E. coli* O83 strain on the development of acute intestinal inflammation induced by dextran sulfate sodium

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Escherichia coli is one the first bacterial species that colonizes newborns. The prophylactic colonization with *E. coli* O83:K24:H31 strain (Colinfant) has been shown to decrease the number of infections in premature babies and incidence of allergies later in adulthood. In this study we studied the effect of *E. coli* O83:K24:H31 strain on cytokine profile in *in vitro* spleen cultures of conventional (CV), germ-free (GF) and *E. coli*-monocolonized mice after stimulation with formolized *E. coli*. In *in vivo* experiments we studied the effect of *E. coli* O83 monocolonization on the development of acute ulcerative colitis induced by dextran sulfate sodium (DSS). Two-month-old mice were used in our experiments. Splenocytes of conventional (CV), germ-free (GF) and *E. coli*-monocolonized were cultivated 48 h with and without inactivated *E. coli*. Level of interleukin IL-10, IL-17, IFN-γ and TNF-α were measured by ELISA. Experimental colitis was evoked by an administration of 2.5% DSS in drinking water (7 days) in *E. coli*-monocolonized and in GF mice (controls). Colon morphology and mucin production were evaluated. The level of cytokines was determined in supernatant of cultivated intestinal pieces of colon descendens. We observed that monocolonization of GF mice with *E. coli* O83:K24:H31 has no pathogenic effect. Production of IFN-γ and TNF-α of *in vitro* unstimulated splenocytes of GF, CV and *E. coli*-monocolonized mice was very low, but cultivation with formolized *E. coli* lead to significantly stimulated IFN-γ and TNF-α production. The level of IL-17 was significantly lower in mice monocolonized with *E. coli* compared with GF and CV controls. On the other hand, the level of IL-10 increased markedly after cultivation with formolized *E. coli*. Mice monoassociated with *E. coli* strain developed mild intestinal inflammation in colon in DSS model in comparison with controls. The level of pro-inflammatory cytokines TNF-α and IL-6 was reduced markedly in colon compared with controls. In conclusion, we observed that monocolonization with *E. coli* O83:K24:H31 is safe and is able to ameliorate the intestinal inflammation induced by DSS treatment in mouse model of ulcerative colitis.

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P15

Immunological effects of selected probiotic and potential probiotic lactic acid bacteria

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Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Stimulation of immunomodulatory cells is among the suggested mechanisms by which probiotic bacteria can interact with the human host and mediate their health benefits. In this study seven *Lactobacillus* strains (*L. reuteri* DSM20016, *L. reuteri* DSM17938, *L. reuteri* ATCC PTA 6475, *L. plantarum* MF1298, *L. plantarum* NC8, *L. plantarum* 299v and *L. rhamnosus* GG), of which three are commercially marketed as probiotcis (DSM17938, 299v and GG), were tested for their ability to induce secretion of cytokines from the monocytic THP-1 cell line. All the strains tested increased the secretion of IL-8, IL-10 and TNF-alpha from monocytic THP-1 cells and the secretion of IL-6, IL-8, IL-10 and TNF-alpha from THP-1 derived macrophages. Generally, the three *L. reuteri* strains gave the highest induction of cytokine secretion, while *L. plantarum* NC8 gave the lowest induction. Interestingly, the effect of *L. rhamnosus* GG was dependent on whether it was UV-inactivated or not, where the UV treated GG gave a higher induction of the cytokine secretion than the non-treated GG. In conclusion, the seven *Lactobacillus* strains tested all have a potent stimulatory effect on immunomodulatory cells.

P16

Metagenomic analysis of human milk

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Human milk is an important factor in the initiation, development and composition of the neonatal gut microbiota but the knowledge of the bacterial diversity in breast milk is still limited. In this study, the metagenome of 5 human milk samples were analyzed. Samples were obtain from two healthy women (WH1 and WH2), one obese women with a body mass index above 30 (WO1) and, finally, two women with lactational mastitis; one of them suffered acute mastitis caused by *Staphylococcus aureus* (WM1) while the other suffered subacute by *Staphylococcus epidermidis* (WM2). Total DNA was isolated and submitted to 454 pyrosequencing (LifeScience-Roche) using a shotgun (SG) strategy in the facilities of Life Sequencing (Paterna, Valencia, Spain). Each read was faced against the NCBI nt database that includes nucleotide sequences from GenBank, EMBL and DDBJ, and assigned to the taxon corresponding to the best BLAST hit against nt. The bioinformatics analysis was performed in collaboration with Era7 (Granada, Spain). A total of 3,000,000 reads (approximately 250,000 reads per sample) were generated. The amount of non-microbial DNA (human, plants, ingested food) in the metagenomes was around 90% in all the samples. A total of 11,344 reads belonging to the Bacteria kingdom, representing 17 taxonomic phyla and 318 operational taxonomic units (OTUs) were obtained. The number of species detected in the samples varied from 33 to 210, being WH1 and WM1 the samples with the highest and lowest diversity, respectively. Globally, the 4 most predominant phyla were *Proteobacteria* (58.6%), *Firmicutes* (12.4%), *Bacteroidetes* (6.7%) and *Actinobacteria* (1.8%). *Alphaproteobacteria* was the predominant class in the samples (39-67%), except in WO1 and WM1 where clostridia and bacilli predominated, respectively. A core microbiome was detected in all the samples (with the exception of WM1), and included the following genera: *Pseudomonas* (*Gammaproteobacteria*), *Sphingomonas*, *Novosphingobium*, *Sphingopyxis* and *Sphingobium* (all *Alphaproteobacteria*). On a lower taxonomic level, the most prevalent species in the milk samples analyzed was *Pseudomonas aeruginosa* except in WM1, where *Staphylococcus aureus* was the predominant specie (75% of the sequences). Globally, although the top eight predominant species were identical in both healthy women, there was an inter-individual variability in the composition of the microbiota at the species level. In the WO1 sample, *Firmicutes* predominated followed by *Bacteroidetes*. In WM1 sample, it was detected a strong effect of the pathogen responsible for the mastitis (*S. aureus*) over the bacterial diversity while such effect was notably lower in the sample (WM2) of the women suffering from *S. epidermidis* mastitis. In conclusion, this study suggests that microbial community of breast milk may differ depending on the individual and on the health status of the lactating women.

P17

Adverse effects of low doses exposure to chlorpyrifos in a human simulator of the intestinal microbial ecosystem (artificial intestine)

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The excessive use of pesticides has become a public health problem. Although ethyl-chlorpyrifos (E-CPF), a commonly used organophosphorus insecticide, is described as a stressing agent disrupting body homeostasis, its impact on the digestive system, primarily affected by pesticides exposure, has been poorly described. This study aimed at evaluating the effect of chronic exposure to E-CPF on the evolution and development of human gut microbiota in a simulator of the human intestinal microbial ecosystem (VitroSim). VitroSim mimes the human digestive physiological conditions and allows to study microbial intestinal ecosystem behaviour. It is composed of six reactors, stomach, duodenum/jejunum, ileum/caecum, ascending colon, transverse colon and descending colon, respectively, numbered from F1 to F6 (constantly mixed by homogenizer and peristaltic pumps which transfer food and gases from a compartment to the other one. The system is checked by computer piloting. The three last reactors corresponding to colonic segments have been inoculated with healthy volunteers faeces (n=4). After a 15 day-long period necessary to stabilize the microbial community in the artificial system, the simulator was exposed to a daily dose of 1mg E-CPF during 30 days. The evolution of different aerobic and anaerobic bacteria populations was examined for samples taken from the caecum and colon reactors at 3 different times: D0 (Day 0, before pesticide exposure=control), D15 and D30. Our results clearly show microbial disorder during pesticide exposure. The number of aerobic and anaerobic bacteria in the last reactors, (D15 and D30 vs. D0, $p<0.001$) increased. More specifically, potentially pathogen bacteria such as enterobacteria ($p<0.05$), enterococci ($p<0.01$), *Clostridium* spp. ($p<0.05$) and *Bacteroides* spp. ($p<0.05$) increased (D15 and D30 vs. D0). In contrast, the number of beneficial bacteria such as bifidobacteria significantly decreased (D15 and D30 vs. D0, $p<0.05$). Therefore, our results suggest that a daily exposure to a low dose of E-CPF corresponding to daily food consumption, leads to intestinal dysmicrobism which increased potentially pathogen bacteria that might alter the human intestinal function and affect human health. Also, VitroSim is a good tool as an *in vitro* alternative model to study the human microbial ecosystem. It is very reproducible and exempt from ethical questions.

P18

Impact of oral low doses of chlorpyrifos on intestinal maturation after perinatal exposure in rats

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Currently, pesticides represent a real public environmental problem because of some impacts on human's health. Different studies demonstrate that residues of these substances can be find in our daily supply. The impacts might be particularly harmful when the exposure takes place during development of young organism and especially during pregnancy. Although the digestive system is the first compartment in contact with food contaminants, very few data are available to this respect; in particular, the effects of ethyl- chlorpyrifos (E-CPF), an organophosphate insecticide known to cross the placental barrier. This study aimed at assessing the impact of E-CPF *in utero* and during the postnatal period on intestinal maturation of juvenile rats. Three groups of pregnant rats (n=6/group) were gavaged with two doses of E-CPF (1mg/kg/day or 5 mg/kg/day), or its vehicle (controls), until pups (n=10/group) were weaned (21 days). After sacrifice, different pieces of intestine and organs were removed in order to assess microbiological and histological studies. Weaning rats are smaller when exposed to CPF (weight: 38.4g ± 3.4 vs. 54.1g ± 4.3; size: 17.7cm ± 0.7 vs. 20.3cm ± 0.7, exposed vs. controls, $p<0.001$). The morphology of structures involved in the absorption of food was altered (reduced duodenal, ileac and colic villosity height and width in exposed groups vs. controls, $p<0.01$). CPF exposure induced an intestinal microbial disorder, as evidenced by an overgrowth of anaerobic flora in ileum ($p<0.01$), as well as variations of aerobic and anaerobic sub-populations. More particularly, potentially pathogen bacteria such as *Clostridium* spp. increased in the ileum (exposed vs. controls; $p<0.05$) and in caecum (exposed vs. controls, $p<0.01$) to the detriment of beneficial bacteria in caecum (*Lactobacillus* spp.: exposed vs. controls, $p<0.01$). Moreover, E-CPF exposure increased bacterial translocation particularly in liver (aerobic bacteria, exposed vs. controls, $p<0.05$, and anaerobic bacteria, exposed vs. controls, $p<0.05$), in spleen (aerobic bacteria, exposed vs. controls, $p<0.05$ and anaerobic bacteria, exposed vs. controls, $p<0.05$) and kidney (aerobic bacteria, exposed vs. controls, $p<0.01$). In conclusion, *in utero* and postnatal exposures of E-CPF induce an effect on intestinal development of pups: alteration of structures involved in nutrient absorption (intestinal villi), an intestinal dysmicrobism and increased bacterial translocation. Gestational and lactational exposure could therefore have digestive impacts at short and long terms.

P19

A public-private partnership that aims to establish a novel, multivariate view of oral health

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The Dutch Top Institute for Food and Nutrition (TI Food and Nutrition) has launched a four-year public-private partnership on oral health. Core participants include the Dutch organization for applied scientific research TNO, the Academic Centre for Dentistry Amsterdam (ACTA), Wrigley, Cargill, GlaxoSmithKline, and Philips Research. The initiative will focus on novel approaches to maintain and promote oral health. In a multidisciplinary effort that brings together dentists, microbiologists, biochemists, and immunologists, the project aims to identify actors and processes that contribute to the maintenance of a healthy oral ecosystem. Furthermore, the initiative sets out to establish *in vitro* as well as *in vivo* approaches for the development and validation of preventative strategies to sustain oral health in humans. Central in the approach is the application of multivariate -omics-technologies, including metagenomics, proteomics, and metabolomics, to provide an integrated and comprehensive view of the oral ecosystem. While the field of oral health has traditionally dedicated its main focus to understanding the basis of diseases, the current initiative marks a paradigm shift, aiming to provide a conceptual view of oral health that goes beyond the 'absence of disease' definition.

P20

Electrochemical behaviour of commensal gut bacteria

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Gut bacteria are generally known to rely on fermentation for generating energy. However the energy gain in the form of ATP is relatively low as compared to the energy gain achieved through respiration. Bacteria would gain extra energy if they could use extracellular electron transfer (EET) to redox-active compounds. The environmental conditions prevailing in the gut are favourable for EET, since there is an excess of nutrients, a negligible oxygen tension and a wide variety of dietary or host-derived redox-active compounds. Hitherto, most of the bacteria that are capable of EET have been isolated from ecological niches such as the soil or marine sediments. In contrast, the data on EET by gut bacteria and the relevance of EET to human gut physiology are scarce. This research was focused on screening the EET potential of human gut bacteria, and to unravel their physiological relevance in their native niche. A two-chambered microbial fuel cell (MFC) was constructed to screen the ability of gut microbes to shuttle electrons to electrodes using flavins as redox mediators. Common gut microbes were selected for initial screening. Metabolic profiles of responding bacteria were assessed by estimating sugars and short-chain fatty acids. Interestingly, *Faecalibacterium prausnitzii*, *Enterococcus faecium* and *Enterococcus faecalis* produced currents in the MFC experiments using flavins as redox mediators. Despite the fact that *Bacteroides* spp. and *E. coli* were shown to produce extracellular flavins in the complex medium, they were not able to produce currents. The flavin-type compounds such as riboflavin are readily available in human gut lumen where they can be exploited by these and maybe other bacteria to shuttle electrons. EET may thus play a thus far under-estimated role in the energy-gain under conditions where terminal electron acceptors are difficult to access.

P21

Lactobacillus plantarum isolated from ayurvedic medicine ameliorates cytotoxicity caused by *Aeromonas veronii*

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Lactobacillus plantarum is widely accepted as a safe and effective probiotic microorganism. Antibacterial property of *L. plantarum* has been demonstrated against various enteric pathogens in both *in vitro* and *in vivo* systems. We have reported the isolation of *L. plantarum* from Kutajarista, an ayurvedic fermented biomedicine and characterised its probiotic potential. Among probiotic properties, *L. plantarum* was tolerant to pH 2, 0.3% bile salts and simulated gastric juice. Its adhesive capacity was established in HT-29 cell line. *In vivo* feeding trial in BALB/c mice also provided evidence of its colonization in mouse gut. In concordance to earlier reports, cell free supernatant (CFS) of *L. plantarum* was antagonistic to enteric pathogens like *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Aeromonas veronii* and clinical isolates of *P. aeruginosa* and *E. coli.* We detected the presence of bacteriocin (plantaricin) genes which has been reported for its antimicrobial activity. *L. plantarum* ameliorated vacuole formation and cytotoxicity caused by *A. veronii* toxins in Vero cells. In addition, *A. veronii* CFS caused disruption of tight junction proteins ZO-1 and actin in MDCK cell line, which was prevented in cells pre-incubated with CFS of *L. plantarum*. CFS of *L. plantarum* was also found to be anti inflammatory as it reduced the expression of pro-inflammatory markers like TNF-α, IL-1β, etc., activated by *A. veronii*. This study provides evidence that CFS of *L. plantarum* possesses active constituents which prevents cellular damage caused by enteropathogens like *A. veronii.* Moreover, Isolation of probiotic microorganisms like *L. plantarum* from ayurvedic fermented medicines suggests that these herbal medicines can be an alternative reservoir of potential probionts. In future, it may also help in formulating herbal medicines in conjunction with probiotics, which are safe and are effective alternative to antibiotics.

P22

Enterococcus faecium NCIMB 10415 does not protect interleukin-10 knock-out mice from chronic gut inflammation

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Enterococcus faecium NCIMB 10415 beneficially affects acute intestinal disorders in humans and piglets. We tested whether the strain also reduces chronic gut inflammation in interleukin-10 deficient (IL-10-/-) mice. Experimental and control IL-10-/- mice were fed a diet with or without NCIMB 10415 for 3, 8 and 24 weeks, respectively. Gut inflammation was evaluated histologically and by the measurement of mucosal cytokine expression. Gut epithelial barrier function was evaluated by measuring concentrations of isothiocyanatelabelled dextran (DX-4000-FITC) in plasma after oral application. Denaturing gradient gel electrophoresis (DGGE), bacterial 16S rRNA gene sequencing and quantitative real-time PCR were used for intestinal microbiota analysis. NCIMB 10415 successfully colonized the intestine of the experimental mice but was not detected in any of the control animals. After 3 weeks of intervention the mice treated with NCIMB 10415 were less inflamed in the caecum than the control animals. This effect was not observed in the colon and we observed no differences at any other time point. The application of the strain was associated with higher expression levels of interferon gamma and interferon-induced protein 10 after 3 and 24 but not after 8 weeks of feeding. No differences in the expression of tumour necrosis factor-α, interleukin (IL) 6, and IL-23 were observed. Similar plasma DX-4000-FITC concentration after oral application in all mice suggested that NCIMB 10415 did not influence epithelial paracellular permeability. We detected only minor differences between the animals in intestinal microbiota composition. However, 16S rRNA gene sequencing and specific quantification with real time PCR indicated a low abundance of the mucin-degrading bacterium *Akkermansia muciniphila* in the mice fed NCIMB 10415 for 8 weeks. These low cell numbers were associated with a significantly lower caecal inflammation score than in the NCIMB-treated mice that were killed after 3 and 24 weeks of intervention. We conclude that NCIMB 10415 does not alleviate chronic gut inflammation in our model. The exact role of *A. muciniphila* and of possible interactions between this bacterium, NCIMB 10415 and the host in gut inflammation requires further investigation.

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P23

Modulation of inflammatory responses *in vitro* and *in vivo* by commensal bacteria

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Host-microbiota interactions play a major role in maintenance of intestinal immune homeostasis. Accordingly, probiotics may display protective effects against intestinal inflammation. We have recently demonstrated that *L. plantarum* and VSL#3 render BALB/c mice partly resistant towards the induction of colitis[1], evident from less influx of macrophages, T cells and mast cells in the colon. These observations were substantiated by transcriptome analysis of colons showing decreased expression of genes encoding chemokines, cytokines and mast cell proteases. For a better understanding of the mechanism by which probiotics dampen intestinal inflammation, we investigated immunomodulation of dendritic cells by probiotics *in vitro.* Naïve DC derived from the bone marrow of C57BL/6 (Th1 polarized) and BALB/c (Th2 polarized) mice were co-cultured with VSL#3, LPS, or a combination of both. mRNA profiling of cultured DC with a dedicated array of genes involved in TLR signalling identified 3 major gene clusters: (I) a cluster of genes that was down-regulated (mainly pattern recognition receptors) or up-regulated (set of cytokines) irrespective of the stimulus, (II) a cluster of genes that was synergistically up-regulated by LPS and VSL#3 but only in C57BL/6 derived DC, (III) a cluster of LPS-induced genes that were suppressed by VSL#3, in particular chemokines. These data indicate that probiotics may act at different levels to contribute to the control of inflammation.

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P24

Metabolomic applications to decipher gut microbial metabolic influence in health and disease

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Dietary preferences and nutrients composition have been shown to influence human and gut microbial metabolism, which ultimately has specific effects on health and diseases' risk. In such a context, new concepts joining biomarkers development and health monitoring are increasingly recognized as plausible strategies to provide new health oriented solutions to the general population. In particular, major advances in immunology, metabolomics, and microbial ecology have shown that the contribution of the intestinal microbiota to the overall health status of the host has been so far underestimated. Tackling these challenges can be achieved through systems biology approaches to underpin the highly complex metabolic exchanges between diverse biological compartments, including organs, systemic biofluids and microbial symbionts. The current contribution will describe recent applications of metabolomics in clinical fields with insight into gastrointestinal health and obesity, and perspectives for future development. One example will demonstrate how metabolomics can be employed to monitor the metabolic adaption of C57Bl/6 mice fed with a high fat diet containing different blends of complex carbohydrates in relation to a beneficial improvement of body weight and composition. The metabonomic results highlight the existence of a metabolic signature comprising non-exclusively an alteration of lipid and amino acid metabolism, differentially modulated directly or indirectly by prebiotic modulation of the gut functional ecology. The prospective of preventing the progression of human diseases and promoting health by specific nutritional intervention programs, such as prebiotics, could benefit from the development of specific biomarkers for prediction of health and disease.

P25

In vitro inhibitory activity of probiotic strains against pathogens in aquaculture

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Members of the genus *Vibrio* as well as other facultative anaerobic bacteria (such as *Yersinia ruckeri, Streptococcus agalactiae, Aeromonas veronii*) are among the most common pathogenic species in shrimp and fish causing serious economic losses in the hatchery and grow out phases throughout the world. Disease outbreaks, however, can be prevented by the prophylactic use of beneficial bacteria (probiotics) of different origins. Various modes of action of probiotics have been described including their antagonism towards pathogens (competitive exclusion), attachment to intestinal mucus and production of beneficial compounds. Among such beneficial bacteria are enterococci, pediococci and cactobacilli, Gram-positive, facultative anaerobic lactic acid producing bacteria which are widely distributed in nature. *Bacilli* are Gram-positive bacteria, which grow aerobically. In the current study the use of the bacteriocin-producing *Enterococcus faecium* (BIO 34) and other beneficial intestinal and biodegrading bacteria was investigated *in vitro* regarding their inhibitory activities against aqua-pathogens. *In vitro* studies using the agar spot test on MRS agar showed that after 22 hours incubation at 37 °C *Pediococcus acidilactici* (BIO 69) had the best inhibition properties against the aquatic pathogens *Y. ruckeri*, *Vibrio harveyi, S. agalactiae* and *A. veronii* (Table 1), followed by *Lactobacillus reuteri* (514). *E. faecium* (BIO 34) showed good inhibitory activities against all these pathogens. *Bacillus subtilis* (BIO 56),

Table 1. Inhibitory activity of the microorganism combinations in the agar spot test on MRS agar at 37 °C and the BLIS test at 25 °C.

	Probiotic			
Pathogen	*E. faecium BIO 34*	*P. acidilactici BIO 69*	*L. reuteri 514*	*B. subtilis BIO 56*
V. harveyi 751	++	+++	++ to +++	-
Y. ruckeri 752	+	++	+	++
S. agalactiae 754	+	+	+	+++
A. veronii 755	+	++	++	++

The symbols +++, ++ and + indicate highest modest and low inhibitory activity, respectively, and - indicates no response.

used in aquaculture for bioremediation as well as for intestinal application, was evaluated in a BLIS test. Results showed that *B. subtilis* (BIO 56) could inhibit the pathogens *Y. ruckeri, S. agalactiae* and *A. veronii* very well. From these studies it was concluded that *Enterococcus faecium* (BIO 34), *Pediococcus acidilactici* (BIO 69), *Lactobacillus reuteri* (514) and *Bacillus subtilis* (BIO 56) inhibit the growth of pathogenic bacteria and therefore, are good probiotic candidates for aquatic species.

P26

Gut microbiota composition is associated with hepatic fat content in humans

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The aim of the cross-sectional study was to evaluate whether there are differences in the overall faecal microbiota composition of subjects with high hepatic content (HHFC, hepatic fat accumulation exceeding 5 % of liver weight, n=9) and subjects without high hepatic fat content (LHFC, n=21). In addition, the relationships between the faecal microbiota, body composition and non-alcoholic fatty liver (NAFL) related biochemical parameters and nutrition intake were investigated. Gut microbiota was profiled from faecal samples by 16S rRNA fluorescence *in situ* hybridization and flow cytometry. Body composition was assessed from dual-energy X-ray absorptiometry (DXA) scan images and dietary intakes were collected via food diaries. Hepatic fat content was measured with magnetic resonance spectroscopy (^{1}H MRS). Standard procedures were used to assess plasma glucose, serum insulin, lipids, hormones and inflammatory status. Insulin resistance (IR) was estimated by homeostasis assessment (HOMA) score. The proportion of *Faecalibacterium prausnitzii* and so-called Microbial Balance Index (MBI) indicating overall healthy gut microbiota composition was lower in HHFC subjects compared to LHFC (p=0.023 and 0.014, respectively). In addition, MBI correlated negatively with liver fat percentage (p=0.007) and HOMA-IR (p=0.032). HLFC group consumed more carbohydrates and sucrose but less polyunsaturated fatty acid (PUFA) than the LLFC group (p<0.05 for all). In conclusion, subjects with HHFC had dysbiotic, i.e., unbalanced gut microbiota composition described as lower anti-inflammatory *F. prausnitzii* proportion and MBI value compared to their LHFC counterparts. At the same time, the HHFC subjects had higher insulin resistance than LHFC.

P27

Mechanisms of uncultivability in the oral microbiome

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Our knowledge of the bacteria that make up the human microbiome and the roles they play in health and disease is severely limited, and one of the greatest causes of that limitation is the inability to culture many of these organisms. It is estimated that 50% of the human oral flora is uncultured and the essential challenge is to develop methods for cultivating these elusive organisms, in order to understand the role of the oral microbiome in human health. We have previously discovered that many natural bacterial isolates from environments outside of the human body were uncultured due to their dependence on growth factors that are normally provided by other organisms in the environment. We hypothesized that similar interactions are responsible for the failure to culture many of the organisms that make up the human microbiome. In this study, we isolated several dependent bacteria from the oral cavity using co-culture techniques. To identify their growth factors, a screen using knockout mutants of *Escherichia coli* (*E. coli*) was developed. Menaquinone 4 (MK4) was identified as the growth factor for inducing growth of one previously uncultured bacterium, KLE1280. KLE1280 is closely related to *Porphyromonas catoniae*. KLE1280 requires both MK 4 and haem (in the form of haemin, blood or haemoglobin) for its growth. MK4 is an important part of the electron transport chain in bacteria and the data suggests that this bacterium is missing a part of the electron transport chain. Adding MK4 and haem restores this function. Other uncultured bacteria might be deficient in the same or similar growth factors. Using this growth factor may allow us to isolate many more uncultured organisms.

P28

Community structure of developmental GI tract microbiota in infants and its correlation with allergy development in later life

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After the birth, intestinal microbiota transits from germ-free state to adult-type complex community including several hundred species through *Bifidobacterium*-dominated flora in early infancy. The bacterial colonization in the early life is believed to have great influence on the development of host immune system. To address this notion, we have collected faecal samples from more than two hundreds of infant subjects including more than fifty subjects who developed any allergic symptom of food allergy, atopic dermatitis, or asthma until two years old. The GI-tract bacterial composition of each subject was analyzed by massive pyrosequencing of 16S rRNA genes in the faecal bacterial community. Then, the population of each bacterial group was compared in between allergic and non-allergic groups. As a result, relative ratio of phylum *Bacteroidetes* and phylum *Proteobacteria* at one month old were positively and negatively correlated with the allergy development in later life, respectively. It is suggested that the early colonization of *Bacteroidetes* which is generally colonized after weaning and the less LPS stimulation by *Proteobacteria* in early life may be risk factor for the allergy development in later life. The comparative analysis at lower levels in taxonomy is now undergoing.

P29

Effect of direct fed candidate *Bacillus* microbials on *Salmonella* infection and production parameters in commercial turkeys

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As effective probiotic *Bacillus* spores are identified, these may offer advantages of in terms of stability, cost, and feed application over current probiotics. Presently, environmental samples were pasteurized, plated, and evaluated for anti-microbial activity using soft agar overlays containing target bacteria. Colonies which produced anti-*Salmonella* activity were selected for isolation and then evaluated for *in vitro* anti-clostridial and anti-*Campylobacter* activity using similar soft agar overlays under appropriate atmospheres. Polyvalent isolates were speciated and GRAS or non-pathogenic species were further evaluated for resistance to high temperatures (survival of spores in boiling water for 10 minutes with only minimal loss of viability), and for ability to grow to high numbers with high sporulation efficiency (10^{10} spores per gram or greater). Isolates PHL-MM65 and -NP122 (a *Brevibacillus laterosporus* and *Bacillus subtilis*, respectively) were further evaluated using poults raised under commercial conditions. After 7 days of conventional brooding, 600 poults from within the house were tagged, weighted, and placed into one of four replicate pens for each treatment group (negative control, histostat, PHL-MM65 10^6 spores/g feed, or -NP122 10^6 spores/g feed). After 23 days the poults were weighed and BWG calculated, PHL-NP122 (728 g), and histostat (709 g) were found to be heavier (p<0.05) than the negative control (664 g), while PHL-MM65 (668 g) was not significantly heavier (p<0.05). Also at day 23 of the trial, the caeca were aseptically removed from 5 poults per pen and cultured for recovery of *Salmonella.* Treatment with *Bacillus* isolates PHL-NP122 and PHL-MM65 resulted in a significant reduction (p<0.05) in the percentage of poults colonized by *Salmonella* (2% and 6%, respectively) as compared to histostat (33%) and the negative control (47%). These data may suggest that this method of screening and evaluation could lead to commercially-useful *Bacillus*-based probiotics.

P30

Arabinoxylans show distinct prebiotic properties, as studied using a combination of *in vitro*, animal and human intervention studies

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Arabinoxylan (AX), is a dietary fibre 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, mainly related to specific prebiotic modulation of the intestinal microbiota. In this work, we describe the prebiotic properties of a concentrate of water-extractable AX, either long-chain or enzymatically hydrolyzed into short fragments, which were studied using *in vitro*, animal and human intervention studies. Prebiotic properties of long-chain AX were investigated first using the dynamic *in vitro* SHIME (Simulator of the Human Intestinal Microbial Ecosystem) and *in vivo* in gnotobiotic rats, with inulin acting as control. In addition to luminal effects, prebiotic effects on the mucus-associated microbiota were investigated using a new *in vitro* adhesion model and gut fragments from the rat study. To test the effect of short-chain AX in humans, a placebo-controlled study was performed in which either 5g/d short-chain AX or maltodextrin was provided to 20 individuals per group in a parallel design. Whereas inulin increased butyrate production both *in vitro* and in the rat intestine, AX significantly increased the production of propionate, known to beneficially regulate cholesterol and fatty acid synthesis in the liver. Molecular analysis of the lumen microbiota showed that 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. Administration of 5 g/day short-chain AX did not induce negative side effects, such as flatulence, bloating and intestinal cramps. In contrast, positive effects on the intestinal environment were indicated by a decreased faecal pH, increased levels of butyrate and propionate and decrease in acetate and p-cresol levels, selective stimulation of bifidobacteria and induction of specific enzyme activities. Combined, these data confirm the potent prebiotic properties of water-extractable AX, either as long-chain or enzymatically hydrolyzed, and suggest that the mode-of-action behind the previously observed biological activity profile in relation to metabolic homeostasis includes an important role of the intestinal microbiota.

P31

Effect of nitrate plus a *Lactobacillus* probiotic on *Salmonella* colonization in chickens

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In our previous studies, we have found a consistent reduction of *Salmonella enteritidis* (SE) using a lactic acid probiotic culture (B11) in neonatal chicks. *In vitro* studies suggest nitrate (NO_3) may potentiate this effect. An *in vitro* crop assay model was used to evaluate the effect of NO_3 in combination with B11 against SE. B11 plus NO_3 at 1000 ppm reduced SE by 6.54 log_{10} as compared with non-treated control during a 24 h incubation at 40 °C. Two *in vivo* studies were initiated to determine if addition of NO_3 in feed could further reduce SE in crop and caecal tonsils of chicks. Briefly, 180 day-of-hatch chicks were randomly assigned to 6 groups: control, B11, or B11 with NO_3 at 1, 10, 100 or 1000 ppm. All groups were challenged by oral gavage with ~10^4 cfu SE (n = 30/group). One hour later all groups, except control, were treated by oral gavage with ~10^7 cfu B11. Twenty-four hours and 72 h after treatment chicks were humanely killed (n=15/group), and crop and caecal tonsils were cultured for SE. In experiment 1, at 72 h there was a significant ($p < 0.05$) decrease in the percent of SE positive crops from the B11+NO_3 100 ppm (57%) and B11+NO_3 10 ppm (67%) as compared with the control (93%). A significant reduction in the percent positive caecal tonsils in all groups was observed at 24 h, however at 72 h, only the B11 (47%) and B11+NO_3 100 ppm (14%) were different from the control (93%). Experiment 2 was similar to experiment 1 with the following exceptions: chicks assigned to 3 groups (n=40/group): control, B11, B11+NO_3 100 ppm. In experiment 2, significant reductions of SE positive crops in both treated groups compared with control were noted: control: 24 h 100%, 72 h 100%; B11 24 h 10%, 72 h 65%; B11+NO_3 100 ppm 24 h 60%, 72 h 10%. Similar results were noted in the caecal tonsils: control 24 h 95%, 72 h 95%; B11 24 h 10%, 72 h 35%; B11+NO_3 100 ppm 24 h 15%, 72 h 25%. These experiments indicate that the addition of NO_3 potentiated the effects of the lactic acid bacteria probiotic *in vitro* and *in vivo*. It is postulated that this effect is through increased production of nitric oxide by the beneficial bacteria.

P32

A top-down microbial systems ecology view of the impact of prebiotic oligosaccharides on bifidobacteria in human gut microcosms

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The human gastrointestinal tract (GIT) hosts a microbial diversity of astonishing density and complexity that is fundamental to host health. It protects against infections and exerts beneficial effects on the host immune system and energy metabolism. Prebiotics are non-digestible carbohydrates, which allow selective modulations of the composition and activity in the GIT microbiota that confer benefits upon host health. Species from the genus *Bifidobacterium*, predominant in the human GIT microbiota during the neonatal period, are the main target for prebiotics as numerous clinical trials have demonstrated their bifidogenic nature. This study aims to characterize the transcriptional response of *Bifidobacterium* sp. incubated in the presence of prebiotic oligosaccharides. Using a high-throughput microcosm system designed to mimic the human colon and a multi-species microarray covering the complete genomes of *Bifidobacterium breve*, *B. longum*, and *B. longum* subsp*. infantis*, we show how these bacteria exhibit differential transcription profiles when exposed to the non-digestible fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS). In line with predicted prebiotic effects of FOS and GOS, these bifidobacteria showed an increased transcription of genes involved in their unique carbohydrate metabolism that occurs through a phosphoketolase pathway (bifid shunt), when exposed to these oligosaccharides. Notably, exposure to GOS invoked an earlier transcription response than FOS. In addition, GOS was consumed at a higher rate than FOS. *B. breve* exhibited a faster transcription response when exposed to both FOS and GOS than the known probiotic species *B. longum* and *B. longum* subsp. *infantis*. Characterization of these transcription dynamics will expand our understanding of the ecology of these important constituents of the GIT microbiota and reveals the molecular mechanisms of the prebiotic functionalities of FOS and GOS.

P33

Streptococcus thermophilus LMD-9 is advantaged over *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC11842 in different media and in the digestive tract of gnotobiotic rats

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Yogurt bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus,* are alleged to have beneficial effects on human health. Our objective was to determine the growth and the metabolic activity of the two yogurt bacteria in simplified environments, *in vitro* and *in vivo*. *S. thermophilus* LMD-9 and *L. bulgaricus* ATCC11842 were monitored in different synthetic media and in gnotobiotic rat gastro-intestinal tract (GIT). *In vitro*, and particularly in milk, *S. thermophilus* had a growth selective advantage at the expense of *L. bulgaricus*. *In vivo*, *L. bulgaricus* did not efficiently colonize GIT of germ free rats in absence of lactose. When germ-free rats were inoculated with a mixture of two yogurt bacteria and lactose (45 g/l), *S. thermophilus* showed a rapid and maximal colonization (10^{10} cfu/g of faeces) at the expense of *L. bulgaricus* which remained in most cases below $<10^2$ cfu/g of faeces. Furthermore, we showed that *S. thermophilus* specifically produced L-lactate, while *L. bulgaricus* produced only D-lactate. Considering its competitive and numerical advantages, its capacity to adapt and to produce L-lactate in the rat GIT, *S. thermophilus* seems to be the major contributor to the health effects of yogurt.

P34

Rapid discrimination of *Bifidobacterium animalis* subspecies by matrix-assisted laser desorption ionization time of flight mass spectrometry

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The species *Bifidobacterium animalis* consists of two subspecies, *B. animalis* subsp. *lactis* and *B. animalis* subsp. *animalis*. Of these two subspecies, *B. animalis* subsp. *lactis* is especially important because it is widely used in the manufacture of probiotic dairy products. The application of these microbes in the food industry demands fast, accurate and low cost methods to differentiate between species and strains. Although various genotypic methods have been employed to discriminate between these two subspecies, they are not easily adapted for rapid identification in the industry. The purpose of this study was to evaluate the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) to differentiate between the two subspecies of *B. animalis*, and for discrimination at strain level. A total of 23 *Bifidobacterium animalis* strains assigned as *B. animalis* subsp. *lactis* and *B. animalis* subsp. *animalis* were obtained from type culture collection (ATCC and JCM), dairy products, and human faeces. The *B. animalis* subsp. *animalis* strains were classified by genotypic methods, at subspecies level by the sequence of the genes *tuf* and *atpD* as described by Ventura and Zink [1] and Masco *et al.* [2], and at the strain level by single-nucleotide polymorphisms (SNPs), insertions, and deletions (INDELs) as described by Briczinski *et al.* [3], and finally by proteomics using MALDI-TOF MS. The proteomics identification by MALDI-TOF was nearly identical to that obtained by genotypic identification using comparison of *tuf* and *atpD* genes sequences as well as SNPs and INDEL analyses. We identified four protein markers which are useful for discriminating between both subspecies. Proteomics identification using MALDI-TOF MS is an accurate method for identifying and discriminating these bacterial strains. Given the speed in which this method is achieved (~20 min including sample preparation), MALDI-TOF MS is promising as a tool for rapid discrimination of starter cultures and probiotics

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P35

Fermented milk containing *Lactobacillus casei* strain Shirota reduces incidence of hard or lumpy stools in healthy population

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Constipation is a common problem affecting approximately 17% of the general population in Europe. Several reports suggest that production of hard or lumpy stools (HLS) is strongly associated with the development of constipation. HLS are produced not only by chronic constipated patient but also by healthy population. Thus, reducing the incidence of HLS might be beneficial in terms of reducing the incidence of constipation in healthy population. Koebnick *et al.* [1] found that fermented milk containing *Lactobacillus casei* strain Shirota (LcS) significantly reduced the incidence of HLS and reduced the severity of constipation in patients with chronic constipation. On the basis of the evidence, we hypothesized that the same probiotic product could have a similar positive effect in reducing the incidence of HLS in healthy population. In order to verify the hypothesis, we have conducted an intervention study. Forty healthy subjects who tend to produce HLS were recruited to investigate the efficacy. The subjects were randomly assigned to either the fermented milk treatment group (intake 1 bottle of Yakult Light per day) or the non-intervention control group (no intake of fermented milk). The study consisted of a 1-week observation period (baseline) and a 3-week treatment period. In order to evaluate the stool consistency, the Bristol Stool Form Scale (BS), a validated seven-point scale was applied. Subjects evaluated their stools with BS every bowel movements during the study period. BS scores 1 or 2 were considered to be HLS. The proportion of subjects that produced HLS in at least 25% of their weekly BMs (high incidence of HLS; H-HLS) was compared between the groups as the primary endpoint. After 3 weeks of treatment the proportion of H-HLS subjects had significantly decreased from 73.7% to 36.8%, whereas in the control group the proportion did not decrease during the same period (p=0.002). The average BS score was significantly improved after the treatment compared with the control (p<0.001). In conclusion, daily consumption of fermented milk containing LcS reduced the incidence of HLS in healthy population.

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P36

Intestinal stem cell derived organoids as a novel tool for analyzing food microbiota host intestinal tissue interactions

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In vitro analysis of the effects of food ingredients on intestinal tissues currently depends on using cultured cell lines. Layers formed by these cell lines hardly resemble intestinal epithelia and therefore are not very representative for the processes occurring *in vivo*. Organoids derived from culturing intestinal stem cells have recently become available and much better resemble the intestinal epithelium. All cell types and structures occurring in the intestinal epithelium are present in these structures. We have explored the potential applications of these gut epithelial organoids as an enhanced *host-microbe* interaction screening platform. It was shown that the injection of the attaching and effacing mouse gut pathogen *Citrobacter rodentium* results in significant morbidity within the crypt structures of the organoids. We are currently performing experiments with known commensal microorganisms, dead *C. rodentium* cells, and microbial LPS fractions. The effect of these injection experiments will be documented by fluorescent microscopy. Furthermore we have conducted experiments in which we exposed the gut organoids to short chain fatty acids (propionate and butyrate). RNA extracted from these organoids has been subjected to microarray analysis. Results of this SCFA exposure experiment will be presented.

P37

Associating phenotypic characteristics of *Lactobacillus paracasei* and *Lactobacillus rhamnosus* to strain-specific variability in gene content

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Probiotic bacteria can persist and adapt in the gut environment where they can inhibit the growth of pathogens and interact with the host, Bacterial characteristics are strain-specific and may confer health beneficial effects to the host when measured clinically. However, evidence on the molecular mechanisms involved is scarce, though recently specific bacterial factors interacting with host–cell receptors have been identified [1,2]. In this study we identify genetic loci involved in the interaction with the host in a set of diverse strains of two *Lactobacillus* species. Forty strains of *Lactobacillus paracasei* and *Lactobacillus rhamnosus*, respectively, have been selected from Danone's large strain collection. Strains were subjected to complete genome sequencing using the GSF-FLX high-throughput platform. After assembly and annotation, comparative genomic analysis was performed to define the distribution of orthologous genes resulting in the two pangenomes of the species. In addition, for each strain, phenotypic data such as sugar utilization profile, mucus adhesion, Nf-KB/IL8 modulation in epithelial cells, gut-barrier protection and inhibition of pathogen growth were collected. Using the classification algorithm 'Random Forest' [3] we associated these phenotypic traits with the presence and/or absence of variable genetic loci. The discovery of mechanism-associated genetic loci is the first step to elucidate the molecular mechanism behind a probiotic feature. The pangenomes of *L. paracasei* and *L. rhamnosus* were indentified using genome sequencing approaches of multiple strains. Specific genetic loci were associated to phenotypic traits, aiding future host-effectors molecule discovery in probiotic strains.

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P38

Combining ^{13}C-metabolic flux analysis and genome-scale modelling of short chain fatty acid production

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Non-digestible dietary carbohydrates are fermented by colon microbiota yielding short chain fatty acids (SCFAs) such as acetate, propionate and butyrate. SCFAs are a source of energy for the host and play important roles in the maintenance of gut health and the overall metabolic health of the host. Consequently, SCFAs are regarded as important targets for pre- and probiotic modulation. In this study we aim to quantitate bacterial metabolic fluxes involved in SCFA production. We used the TNO *in vitro* colon model (TIM-2) to determine how carbohydrate substrates affect the production of SCFAs. This system allows the controlled application of a uniformly ^{13}C-labelled compound (in this case starch or lactose) as a substrate for fermentation by a microbial community derived from human faecal samples. The concentrations and ^{13}C-labelling of major fermentation products and intermediates were determined at various time points up to 8 hours after substrate administration. From the measured concentrations and ^{13}C-labelling, the underlying metabolic fluxes were determined using ^{13}C metabolic flux analysis (^{13}C-MFA). In addition, we developed a metabolic model to perform genome-scale flux balance analysis (GS-FBA), incorporating the individual reactions of the relevant metabolic pathways. By adding a constraint on the total enzyme level in individual cells and varying protein costs per cell, the individual metabolism of multiple bacteria was modelled. In this project, the use of two different models has yielded complementary information about the same fermentation process. ^{13}C-MFA successfully quantitated the overall fluxes by restricting the possible overall fermentation patterns to those that best explain the measured ^{13}C labelling. On the other hand, GS-FBA shows how the metabolism of individual species and the cross-feeding between those species can yield the observed overall fermentation patterns, including the transient accumulation of lactate during fast fermentations. As a next step, we will further integrate both approaches to synergistically exploit these unique capabilities. In particular, the results of ^{13}C-MFA will be used as additional constraints for GS-FBA. In this way, GS-FBA is expected to only find solutions that reflect the measured ^{13}C-labelling dynamics. These solutions will then be compared with independently determined genetic information about the presence and growth of bacterial species in the TIM-2 experiments.

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Development of a dynamic *in vitro* model for the human ileum

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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, but remain largely unexplored due to the poor accessibility of the small intestine in healthy individuals. To determine the impact of a variety of factors that shape the phylogenetic and functional composition of the small intestinal microbiota, we aim to develop, validate and apply a dynamic *in vitro* model simulating the human distal small intestine. The model prototype was developed on basis of insights obtained in *in vitro* intestinal models (TIM models) that simulate the small and the large intestine. To determine if a stable and complex microbiota can be obtained in this *in vitro* ileum model-system, faecal and ileostomy samples were used as inoculum and the microbiota composition was followed in time by 16S ribosomal RNA gene profiling using Human Intestinal Tract Chip (HITChip). The microbial profiles obtained with HITChip analyses indicate that the microbial diversity and complexity within the *in vitro* ileum could be maintained for a long time-period. In specific, a complex and stable microbiota composition was observed after model inoculation with faeces and ileostomy effluent for 10 and 3 days, respectively. The observed composition reflected those found in small intestinal samples, indicating that the model conditions resemble those of the small intestine. To determine the stability and resilience of the model, the impact of specific dietary components and internal intestinal factors on microbiota composition and activity will be monitored by phylogenetic fingerprinting, short chain fatty acid profiling and meta-transcriptomics.

P40

Exploration of suitable terminal restriction fragment length polymorphism analysis of 16S rRNA genes from human faecal microbiota

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Terminal restriction fragment profiles (T-RFLP) analysis is a convenient, semi-quantitative, high-throughput fingerprinting technique used to monitor changes in the composition of various microbial communities and is being usually applied to investigate human faecal microbiota. However, suitable combination of primers and restriction enzymes for analyzing human microbiota using this technique has not yet been thoroughly evaluated. At first, we checked the T-RFLP profiles when amplified with modified fluorescently labelled primers (FAM-27MF: AGRGTTYGATYMTGGCTCAG, VIC-806MR: TTAGATACCCYDGTAGTCC) for a total of 169 restriction enzymes based on 16S rRNA gene sequences of 68 species commonly colonized in human gut to determine the best combination of primers and restriction enzymes. This simulation allowed us to select 15 restriction enzymes being able to distinguish most of the species. Then, we performed T-RFLP analysis on faecal samples obtained from 32 healthy subjects digested with each of these enzymes for understanding the characteristics of T-RFLP pattern of each enzyme. Cluster analysis based on the clustering patterns of T-RFLP obtained by each restriction enzyme led to the formation of three clusters (Cluster 1: *Nci* I, *BsrF* I, *Msp* I, *Hinf* I and *Alu* I, Cluster 2: *Rsa* I, Cluster 3: *TspR* I, *Hpy166* II, *Hpy188* I, *Sau96* I, *Nla* IV, *Hae* III, *Hha* I and *HpyCH4* III). These results enabled us to choose enzyme combinations composed of more than three kinds of restriction enzymes suitable for analyzing human microbiota using T-RFLP method with high discriminates.

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The effects of *Lactobacillus brevis* KB290 on menopausal symptoms with a tendency for constipation in women: an open trial

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Bowel movement disturbances such as awareness of constipation are more frequently shown among women having menopausal symptoms than those without the symptoms. Recently, it was reported that the consumption of a prebiotics might improve not only constipation but also some pathogenomic symptoms observed at menopause such as feeling of cold and stiff shoulders. *L. brevis* KB290 (KB290) isolated from Japanese traditional pickles named 'Suguki' has been demonstrated to improve human bowel movement and to affect intestinal microflora favourably as a probiotic. Thus, we evaluated the effects of KB290 on menopausal symptoms with a tendency for constipation in women. This study was performed as an open trial. Twenty-two, 45-55 years old, Japanese women having menopausal symptoms with a tendency for constipation were recruited as subjects. Written consent was obtained from all subjects. Following an observation period for a week, the subjects consumed a test capsule containing about $1.6x10^{10}$ cfu of KB290 per day for 4 weeks (consumption period). Subjects completed a daily questionnaire about their bowel movements throughout the study. Menopausal symptoms of the subjects were examined by 'modified Kupperman Menopausal Index adapted to Japanese women' (mKMI) as described by the subjects at the end of each period. No relevant adverse effects were observed during this study. Nine subjects were omitted by conflicting with the factors for patient exclusion and thirteen subjects remained for the following analyses. Frequencies and amounts of stool during the consumption period were significantly higher than those during the observation period. The following scores of the mKMI were significantly lower at the end of the consumption period than those at the end of the observation period: total menopausal index, symptom complex scores of 'vasomotor symptoms', and 'joint and muscle pain'. Furthermore, the frequency of stool tended to correlate statistically with the symptom complex score of vasomotor symptoms (R=-0.55, p=0.054). These results suggested that KB290 consumption might improve bowel movement and some menopausal symptoms mentioned above. Elucidation of the correlation between them is also required.

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Cell surface exopolysaccharides of *Lactobacillus brevis* KB290 protect against artificial digestive fluids

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Probiotic bacteria are required to survive in the gastrointestinal tract (GIT) and confer health benefits on the host. *Lactobacillus brevis* KB290 was isolated from Japanese traditional pickles 'Suguki' and has shown a high survivability in the artificial digestive fluid. In a clinical study, improvement of bowel movement, enhancement of NK activity, improvement of acnes, amelioration of menopausal problems, restful sleep promotion and interferon-α producing activity by intake of KB290 have been reported. In addition, KB290 produces exopoly-saccharides (EPS) and shows significant coagulability by severely mixing in broth media. In this study, we focused on EPS which exist on the KB290 surface and demonstrated the contribution of EPS to survivability in the artificial digestive fluid and bile acid. To indicate that deficiency of EPS attenuates the survivability of KB290 in the artificial gastric fluid and the bile acid, we compared EPS-deficient KB290 with intact KB290 under the following conditions. (i) Survivability of KB290, its mutant (KB392; fewer EPS production than KB290) and *L. brevis* ATCC367 in artificial digestive fluid or bile acid was studied. Cell surface EPS of KB290, KB392 and ATCC367 were extracted using EDTA-treatment. The amounts of crude EPS isolated from those strains were measured by the phenol-sulfate method. (ii) Survivability of those strains treated with EDTA was analyzed. (iii) Survivability of those strains homogenized by a Polytron homogenizer to detach EPS from cell surface was analyzed. (iv) Morphological features of cell surface treated with EDTA or homogenization were observed using transmission electron microscopy (TEM). The results can be summarized as follows. (i) KB290 showed the highest survivability in both the artificial digestive fluid and bile acid. The amounts of crude EPS from 10^9 cells surface of KB290, KB392 and ATCC367 were approximately 13, 3 and 6 μg, respectively. (ii) EDTA-treatment reduced the survivability of all strains in the artificial digestive fluids or bile acid. (iii) Homogenization reduced the survivability of KB290 significantly, but not for KB392 in the artificial digestive fluids. (iv) Shadow of EPS on KB290 was observed by TEM using uranyl acetate staining. Fewer shadows of EPS were observed in EDTA-treated KB290 and KB392 than in KB290. Shadow of EPS was not detectable in homogenized KB290. In conclusion, these results suggested that the EPS of KB290 might play a critical role in protection against artificial digestive fluids. This might lead to the unique character of KB290, a high survivability in the gastrointestinal tract.

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Evaluation of the safety and potential probiotic properties of *Lactococcus lactis* subsp. *lactis* MK02R isolated from rocket salad (*Eruca sativa* Mill.)

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Bacteriocins produced by lactic acid bacteria (LAB) are ribosomal-synthesized antimicrobial peptides usually active against closely related organisms and some pathogenic bacteria such as *Listeria monocytogenes*. Bacteriocinogenic *Lactococcus* (*L.*) *lactis* subsp. *lactis* MK02R was isolated from rocket salad and identified using morphological, biochemical and biomolecular techniques (including species specific PCR and 16s rDNA sequencing). The bacteriocin production by probiotic LAB may have an advantage for these strains in competitive interactions with the pathogenic bacteria from the GIT. The objective of this study was to determine the resistance of the strain MK02R to the different conditions represented in the GIT (low pH, bile salts), antibiotics and medicaments, production of bacteriocin in presence of 2% glucose, inuline or fructo-oligosaccharides (FOS) in modified MRS broth at different temperatures, PCR screening for genes encoding virulence factors, antibiotic resistance or biogenic amines. Good growth was recorded for *L. lactis* subsp. *lactis* MK02R in MRS broth supplemented with 0.1% to 0.1% ox bile. However, even at ox bile concentrations of 2.0% and 3.0%, strain MK02R was able to survive. *L. lactis* subsp. *lactis* MK02R grew well in MRS broth adjusted to pH 5.0 to 11.0. Strain MK02R showed resistance to metronidazole, trimetoprim, nalidix acid and sensitivity to commercially available medicaments such as amoxil (<0.39 mg/ml), atlansil (5.0 mg/ml), cataflam (0.62 mg/ml), potasium diclofenac (1.25 mg/ml), dramin (20.0 mg/ml), spidufen (15.0 mg/ml) and urotrobel (5.0 mg/ml). We could not detect any significant influence on bacterial growth and bacteriocin production when prebiotics (inulin or FOS) were added to the MRS broth. From the tested adhesion of collagen protein, aggregation substance, cytolisin, endocarditis antigen, enterococcal surface protein, gelatinase, hyaluronidase, histidine decarboxylase, ornithine decarboxilase, tyrosine decarboxylase, vancomicin A and vancomicin B genes only PCR targeting enterococcal surface protein and histidine decarboxylase genes generated positive results. Besides being active against several human pathogens, the survival of *L. lactis* subsp. *lactis* MK02R in the presence of ox bile and low pH, low presence of genes related to virulence factors, antibiotic resistance and biogenic amines, resistance to several drugs used in human therapeutics evidenced the probiotic potential of this bacteriocinogenic strain.

Acknowledgments
CNPq and CAPES.

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Isolation of bacteriocinogenic strain of *Lactococcus lactis* subsp. *lactis* from Rocket salad (*Eruca sativa* Mill.) and evidence for production of a variant of nisin

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Strain MK02R, isolated from rocket salad, was identified as *Lactococcus lactis* subsp. *lactis* based on a biochemical and biomolecular approach. The analysis of the bacteriocin produced by MK02R revealed the presence of a nisin variant, however, genes for plantaricin NC8, plantaricin S and pedicoin PA-1 were detected as well on the genome of *L. lactis* subsp. *lactis* MK02R. The bacteriocin MK02R showed heat stability at 60°C and 100°C and it was inactivated in the presence of proteolytic enzymes. The antimicrobial activity did not change with pH adjustment between 2.0 and 9.0 and the production was stimulated by the addition of 0.5 and 1.0% cysteine in MRS broth at 37°C. Bacteriocin MK02R inhibited the growth of *Enterococcus faecium, Lactobacillus* (*Lb.*) *sakei, Lb. sakei* subsp. *sakei, Listeria* (*L.*) *innocua, Lb. delbrueckii* and *L. monocytogenes* from different serological groups. The addition of bacteriocin MK02R to actively growing cultures of *L. monocytogenes* Scott A and *Lb. sakei* ATCC 15521 caused a complete inhibition of the test microorganisms over the period of 12 hours. Low levels of adsorption of bacteriocin MK02R were detected in the cell surface of the producer cells suggesting that bacteriocin MK02R remains bound to the outer surface and that it is released when the pH of the environment increases. This is an indication that, for practical application, the peptide will be more active in less acidic food products. The chromatographic studies of purified samples of the antimicrobial peptide and the genetic tests using primers *nis*F and *nis*R related to specific genes of nisin production confirmed that the antimicrobial MK02R is a natural variant of nisin. The partial sequencing of the purified protein showed that the peptide has an amino acid change in the sequence of the leader peptide compared with nisin A, Z, Q, U and F, but the structure is homologous to that of nisin F.

Acknowledgments
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Development and validation of an apical anaerobic model of the intestinal barrier

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The human intestinal epithelium is formed by a single layer of epithelial cells, and the space between these cells is sealed by 'tight junctions', which regulates intestinal barrier permeability. Increased permeability can result in the entry of unwanted antigens and pathogens into the body, and is implicated in autoimmune, inflammatory and atopic diseases. The intestinal tract is inhabited by 10^{14} microbes and it is becoming increasingly evident that they affect intestinal barrier function. However, >99% of commensal bacteria are obligate anaerobes, making it is difficult to co-culture them with oxygen-requiring mammalian cells *in vitro*. To investigate the interactions between obligate anaerobes and epithelial cells that regulate the intestinal barrier, an *in vitro* model, which utilised a proprietary dual-environment co-culture chamber, was developed. The chamber allowed for the intestinal cell-line Caco-2 to be grown such that the apical (luminal) side was exposed to an anaerobic environment, while maintaining an aerobic basal side. The prototype chamber, used inside an anaerobic workstation, contained 12 individual wells, each equipped with a pair of electrodes for automated measurement of transepithelial electrical resistance (TEER), and a port sealed with a septum for sampling of basal media for measuring molecule permeability. The viability of Caco-2 cells grown in the apical anaerobic chamber for 12 hours measured using the neutral red uptake assay, was unaffected compared with cells grown in standard aerobic conditions. Oxygen depletion in the aerobic compartment was slow (-1.5 % saturation/hour), and dissolved oxygen in the anaerobic compartment was low (0.15 ± 0.14 saturation at 12 hours). In the absence of a Caco-2 monolayer however, it was 16-fold higher, suggesting the Caco-2 monolayer prevented oxygen diffusion to the anaerobic compartment. The barrier function of the Caco-2 monolayer, measured using TEER and ^{3}H-mannitol flux over 12 hours, was not different when exposed to an aerobic or anaerobic apical environment. The localisation of tight junction proteins occludin and ZO-1 was also unaffected in Caco-2 monolayers in the apical anaerobic environment compared to a standard aerobic environment. This model can now be used to study the mechanisms of regulation of intestinal barrier integrity by live obligate anaerobes *in vitro*. Furthermore, as the Caco-2 cells are exposed to an anaerobic environment in the apical side, they are arguably 'more' representative of human intestinal conditions than traditional cell culture models in a solely aerobic environment.

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In vitro evaluation of nutrients that selectively confer a competitive advantage to *Lactobacillus acidophilus*

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The vaginal microflora is composed of various strains of bacteria and fungi. Studies have shown that healthy women have a lactobacilli dominant vaginal microflora. Lactobacilli are thought to suppress the growth of harmful bacteria in the urogenital tract by producing hydrogen peroxide, lactic acid, and bacteriocins. The balance of beneficial and harmful bacteria can be regulated by delivering nutrients that selectively confer a competitive advantage to beneficial bacteria. Prebiotics are one such nutrient that upon digestion selectively stimulates beneficial microbes in the intestinal tract. However, carbohydrates that have a beneficial effect on vaginal flora have not been well studied. Therefore a need exists to define nutrients that have the potential to balance the flora of the vaginal tract. This study describes an in-vitro screening method to evaluate nutrients that have the potential to enhance lactobacilli's ability to out-compete an exogenous challenge from a pathogen. A co-culture assay was developed using *Lactobacillus acidophilus* and *Escherichia coli.* It was used to screen carbohydrates for the ability to confer a competitive advantage to L. acidophilus. *L. acidophilus* was cultured in a glucose deplete medium in the presence or absence of a soluble carbohydrate as part of a feminine health formulation. After 6 hours, *E. coli* was added to the culture to simulate a pathogenic challenge. After 18 hours the ability of *L. acidophilus* to out-compete the challenge of *E. coli* was determined by enumeration of each bacterium. In the media alone samples, *E. coli* consistently outnumbered *L. acidophilus*, however the addition of fructo-oligosaccharide to the culture significantly inhibited the growth of *E. coli* while *L. acidophilus* grew to similar numbers as a sample that was unchallenged. Oligofructose also stimulated a drop in the pH of the culture unlike the media control sample. Growth curves generated for *E. coli* in a single culture showed that fructo-oligosaccharide did not affect the growth of *E. coli* indicating that this carbohydrate was not toxic to *E. coli*. This result indicates that fructo-oligosaccharides may increase the ability of beneficial microbes to out-compete a pathogenic challenge. This study shows a co-culture method may be used as a screening tool to define nutrients that confer a competitive advantage to beneficial flora specific to the female urogenital tract.

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Identifying mechanisms of bacterial unculturability in the human gut microbiome

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The microbial flora of the digestive system contributes to human health and disease, but understanding the role of particular microorganisms remains elusive. This is mainly due to the fact that a majority of the intestinal bacteria remain uncultivated. Metagenomic studies provide important information about the microbiome, but to understand the role of individual microorganisms, it will be necessary to culture them. The goal of this study is to provide tools for the cultivation of uncultured microorganisms from the human gut microbiome and to identify their required growth factors. We previously developed a co-culture method to grow uncultured bacteria from marine sediment. These organisms could be grown on rich medium only in the presence of other bacteria from the same environment. We determined that these previously uncultured bacteria were dependent on siderophores from neighbouring organisms. Using this co-culture technique and applying it to the human gut microbiome, we have successfully cultivated several previously uncultured bacteria from human faeces in the presence of a cultivable helper organism. One of these isolates, KLE1255, is related to *Faecalibacterium prausnitzii*, an important commensal of the human gut flora that has been described to have anti-inflammatory properties. KLE1255 can be grown with *Escherichia coli* as a helper. In order to identify the growth factor provided by the *E. coli* helper, we screened an *E. coli* deletion library and found that an *E. coli* mutant lacking menaquinone biosynthesis does not induce the growth of KLE1255. This, in combination with bioassay-driven purification of helper supernatant, has led to the discovery of menaquinone-like molecules as growth factors for the previously uncultured isolate. These results show that previously uncultured microorganisms from the human gut microbiome can be cultivated in the laboratory when provided with the correct growth factors from other bacteria. The described techniques in combination with the identified growth factors will promote the future cultivation of uncultured bacteria from the human microbiome. Cultivation will facilitate the determination of their role in human health and disease.

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Caenorhabditis elegans as model organism for host-pathogen interactions and probiotics research

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Caenorhabditis elegans is a widely used model organism, which was introduced in the 1960s by Sydney Brenner. Its sequenced genome, the easy cultivation, the short reproduction cycle and transparency make it an ideal model organism. We use *C. elegans* to explore the effects of pathogens, *like Pseudomonas aeruginosa*, or beneficial bacteria, like probiotics on the metabolome. For non-targeted metabolomics studies ultrahigh performance liquid chromatography – ultrahigh resolution time of flight mass spectrometry (UPLC-UHR-ToF-MS) and ion cyclotron resonance – Fourier transform mass spectrometry (ICR-FT/MS) are conducted. Both techniques allow precise measurement of up to 5000 features or over 20000 masses, for UPLC-UHR-ToF-MS or ICR-FT/MS respectively on a routine basis. Together with sophisticated extraction methods this yields are broad coverage of the *C. elegans* metabolome. First work shows that this methodology is able to discriminate between different states in *P. aeruginosa* infection and other stresses. To evaluate if probiotic feeding offers benefits in *P. aeruginosa* infection*, C. elegans* was pre-fed with different strains of probiotics prior to infection. Survival was compared against *Escherichia coli* OP50, a standard laboratory food for *C. elegans*. Most of the probiotics strain did not show any effect, whereas two showed a positive and one a negative effect on survival*. C. elegans* fed with these strains are currently under metabolome measurement. First results show that our platform is able to discriminate between different infection states, proofing that it is applicable to *C. elegans* biology. Previous research already discovered positive effects of *Lactobacilllus* isolates in a *C. elegans – S. thyphimurium* model. Our first results from *C. elegans* killing assays show that two probiotics offer protection also in *P. aeruginosa* infections. Metabolome analysis will show which metabolic pathways are affected by infection and how probiotics can alter the metabolism to increase resistance. Together with bioactivity guided fractionation of small molecules from probiotic cultures, this will allow identification of active compounds offering positive effects produced by probiotics.

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Identification and evaluation of candidate *Bacillus* probiotics (DFM) isolates for use in commercial poultry

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Bacillus-based direct-fed microbials may be an effective alternative to antibiotic growth promoters. Environmental samples were pasteurized to remove vegetative cells, plated onto TSA or SPA for 24 or 72 h and overlayed with soft agar containing *S. enteritidis* or *C. perfringens*. Isolates which produced antimicrobial activity against both pathogens were used to inoculate a solid state fermentation media and allowed to sporulate, to numbers greater than 10^9 spores/g and subjected to *in vivo* testing in both poults and chicks. In experiment 1, chicks fed isolates PHL-RW35 and PHL-RW41, at doses of 10^7 and 10^5 spores/g feed, respectively, showed significant increases ($p<0.05$) in both body weight (BW) and body weight gain (BWG). No significant differences in BW or BWG were noted in poults for any treatment. In this experiment, all groups were challenged with 10^5 cfu of *S. typhimurium* at day-of-hatch, no significant differences in *Salmonella* were noted between groups. In experiment 2, PHL-RW41 fed at 10^5 spores/g of feed significantly increased BWG by 8.3 and 11.7% in chicks and poults, respectively. Isolate PHL-RW35 also increased BW and BWG in poults. These data indicate this approach for *in vitro* selection may be effective for screening and selection of *Bacillus* direct-fed microbials capable of causing an increase in BW and BWG in commercial poultry.

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Analysis of pilus-encoding gene clusters in two bifidobacterial species

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Certain bifidobacterial strains are reported to have beneficial effects on the health status of the human host. Tight adhesion to the intestinal epithelial cells (IECs) is considered to be an important prerequisite for host-colonisation. Recently multiple strains of bifidobacteria were shown to possess different types of pili on their cell surface. Since pili were shown to be important for adhesion of pathogens to host tissues, expression of pili might contribute to the adhesion of probiotic bifidobacteria to the intestinal epithelium. Here we have investigated expression and organization of gene clusters encoding for pili under *in vitro* conditions in *Bifidobacterium bifidum* S17 and *B. breve* S27. *Bifidobacterium bifidum* S17, a promising probiotic candidate, is able to tightly adhere to the IEC and has a potent anti-inflammatory *in vitro* and *in vivo*. By contrast *B. breve* S27 does not adhere to IECs. The genomes of *B. bifidum* S17 and *B. breve* S27 were sequenced, assembled and manually annotated. Genome sequencing revealed differences in amount and structure of the potential pili-encoding clusters between *B. bifidum* S17 and *B. breve* S27 under *in vitro* conditions. *B. bifidum* S17 harbors four potential pili-encoding gene clusters: three *fim* clusters and one *tad* cluster. All genes of the *fim2* and *fim3* clusters are expressed in bacteria under conditions of standard cultivation indicating that functional pili might be expressed on the surface of *B. bifidum* S17. Moreover, RT-PCR analysis indicates that the genes of the *fim2* and *fim3* clusters are organized in operons. Analysis of the *B. breve* S27 genome revealed the presence of two *fim* clusters and one *tad* cluster. Our results indicate that the genes of only one cluster are expressed. The gene encoding the minor pilin protein might be non-functional due to a frame-shift mutation. No transcripts were detected for the gene encoding the pre-pilin precursor of the Tad pili in both strains ruling out their presence on the surface under *in vitro* conditions.