INTERNATIONAL JOURNAL OF PROBIOTICS & PREBIOTICS
VOLUME 3 NUMBER 3

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ABSTRACT: The gut provides animals with the required energy for life. High selective pressures therefore act on the functionality of the gut. Despite these evolutionary pressures, very little changes in general gut anatomy is observed between taxa. In invertebrates and vertebrates three sections can be distinguished: 1) fore-gut for food-uptake and primary digestion, 2) midgut for further digestion and absorption, 3) hindgut for resorption of water and ions. Within this conserved anatomy, adaptation occurs by changes in absorptive area, retention time or volume for storage or fermentation. In species with low energy diets, or high-energy demands the digestive tracts is larger for better absorption and longer retention. In animals with high energy diets, or low energy demands the digestive tract is smaller. This change in gut-size is not only observed within evolutionary context, but also as phenotypic plasticity. When in some species the intestinal tract is temporarily in disuse, the intestinal tract becomes smaller and enlarges again when feeding is resumed. Although adaptation and plasticity of the gut seems to occur frequently, there are indications that it is limited by phylogenetic or ontogentic constraints. Despite these constraints it is shown that trophic plasticity may play a major role in speciation.

ABSTRACT: A wide range of age-related alterations in immune system function have been described which contribute to the high prevalence, the more severe disease course and the poorer prognosis of certain infectious diseases in the elderly population and the low efficacy of vaccinations. Moreover, the development and progression of other age-related diseases, such as certain cancers, atherosclerosis, dementia, osteoporosis and rheumatoid arthritis have been associated with altered immune function in old age. The most prominent event contributing to immunosenescence is the involution of the thymus which leads to a dramatic loss of T cell function. It is therefore of great importance to fully assess age-related changes within the immune system and to develop strategies aiming at restoring immune system function in elderly persons.

ABSTRACT: A continuous monolayer of gastrointestinal epithelial cells functions as a primary physical barrier against microbial invasion. In addition, one of intestinal epithelial lineages, Paneth cells produce antimicrobial peptides. Paneth cell α-defensins, cryptdins in mice, HD5 in humans are constituents of apically oriented granules with potent microbicidal activities against a wide variety of microbes. Paneth cells contribute to mucosal immunity by sensing bacteria and releasing microbicidal activities mostly form secreting α-defensins. It is known that HD5 peptides are protected from the enzymatic degradation by their disulfide bridges. Disrupted HD5 processing is suggested to be associated with Crohn's disease. Paneth cells and their antimicrobial peptides might contribute to maintaining intestinal integrity. Furthermore, the impaired innate immunity in Paneth cells and their α-defensins might associate with the pathology of Crohn's disease.

International Journal of Probiotics & Prebiotics 3(3): 127-132

127-132 EFFECTS OF PROBIOTICS AND COMMENSALS ON EPITHELIAL BARRIER FUNCTION

Jerry M. Wells, Sergey Konstantinov, Irene Konings and JurgenKarczewski

ABSTRACT: Increased permeability of the intestinal epithelium is now recognized as having a role in the pathophysiology of gastrointestinal diseases and is observed in inflammatory bowel disease, irritable bowel disease and celiac disease. Hyperpermeability of the intestinal epithelium is also associated with diabetes, diarrhea in HIV infected patients, atopic eczema and altered sensitivity to food-allergens. Consequently, modulation of epithelial permeability is an interesting target for novel therapeutic or preventative treatments against a range of diseases. Evidence for probiotic effects on barrier function has been demonstrated in humans as well as animal models of chronic stress, DSS-colitis, hemorrhagic shock and sepsis although the mechanisms have not been fully elucidated. Studies with polarized cell models of epithelium suggests that particular strains of probiotics can protect against barrier dysfunctions caused by invasive pathogens or pro-inflammatory cytokines. Here we review recent evidence for the role of innate signaling in the intestinal epithelium and the regulation of tight junction protein composition as probiotic mechanisms to enhance mucosal integrity.

International Journal of Probiotics & Prebiotics 3(3): 133-136

133-136 BACTERIAL METABOLITES AND IMMUNE MODULATION

Bernhard Watzl

ABSTRACT: The gut microbiota is important for the functioning of the intestinal immune system. It provides a specific set of pathogen-associated molecular patterns which activate the innate immunity. Further, through its metabolic activity it generates immunomodulatory active constituents such as the short-chain fatty acids (SCFA). Diet modulates these activities by providing non-digestible carbohydrates for bacterial fermentation. In addition, exogenous bacteria such as probiotics further affect the metabolic activity of the intestinal microbiota. SCFA are produced in the large intestine resulting in physiologically relevant concentrations in the lumen and the portal blood. Metabolic cross- feeding between different bacteria supports the synthesis of SCFA such as butyrate. Butyrate exerts specific effects on immune cells resulting in reduced cell proliferation and inhibited production of pro-inflammatory cytokines. Luminal fermentation further modulates the localization of immune cells such as NK cells in the large intestine as well as the functional activity of these cells. Intestinal leukocytes may sense SCFA via recently discovered SCFA-receptors. Optimal carbohydrate substrates and doses to support the gut microbiota and

to beneficially modulate the local immune system still have to be defined. Whether the systemic immune system is also affected by the intestinal SCFA synthesis is currently not known.

International Journal of Probiotics & Prebiotics 3(3): 137-140

137-140 **POSSIBLE USE OF PROBIOTICS AS MODULATOR OF ALLERGIC DISEASE**
Claudio Nicoletti

ABSTRACT: Experimental evidence collected by using a combination of animal models and human studies is growing to support the hypothesis that host-microbe symbiosis is essential for driving the maturation and maintaining a correct homeostasis of the gastrointestinal (GI) immune system and that changes in composition of the commensal microbiota may lead to an increased susceptibility to allergy. Indeed, germ-free mice do not develop immunological tolerance in absence of the proper intestinal microflora and the composition of the latter is remarkably different in atopic and non-atopic infants. These observations have led to devise effective strategies to use microbial products for the prevention and/or treatment of allergic disorders. However, hitherto many basic aspects of probiotics-host interaction are still largely unknown. In this brief review, I will describe initially the interaction of probiotics with the gut epithelium in regard to their translocation across the epithelial barrier and interaction with Toll-like receptors (TLRs). This will be followed by the description of the immunoregulatory properties of probiotics in allergy; finally some of the most recent data on the effects of oral delivery of Lactobacillus casei on the immune system of individuals suffering from allergic rhinitis will be discussed.

International Journal of Probiotics & Prebiotics 3(3): 141-146

141-146 **NATURAL KILLER CELLS, PROBIOTICS, AND CANCER**
Carsten Watzl

ABSTRACT: Natural Killer (NK) cells were originally discovered because of their ability to kill certain tumor cells without the need for prior immunization. This anti- tumor activity of NK cells is under the tight control of inhibitory and activating signals. We are beginning to understand how a shift in the balance of activating and inhibitory signals enables NK cells to recognize and lyse tumor cells. Enhancing and modulating NK cell activity is therefore the goal of several NK cell-based anti-cancer immunotherapies. Probiotics can enhance the activity of NK cells. It is therefore interesting to speculate that this enhanced NK cell activity may help NK cells in their daily fight against transformed cells.

International Journal of Probiotics & Prebiotics 3(3): 147-148

147-148 **ROUND TABLE DISCUSSION: HOST IMMUNE SYSTEM AND COMMENSAL FLORA AS THE STIMULUS TO THE GUT**
Thomas T. MacDonald

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Thomas T. MacDonald

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The session on the host immune system and commensal flora was chaired by Dr. Ger Rijkers from University Medical Centre Utrecht, the Netherlands who focused on the establishment of gut microbiota, its interaction with the host and the maintenance of its balance via these interactions.

Oral presentations were given by Doctor Dietmar Herndler-Brandstetter (Austrian Academy of Science, Austria) on Changes within the immune system from Birth to Old Age; Professor Tokiyoshi Ayabe (Hokkaido University, Japan) on Antimicrobial Peptides in the Gut Innate Immunity and Professor Jerry Wells (Wageningen University and TNO, the Netherlands) who discussed Maintaining a Peaceful Relationship with our Symbionts.

There then followed a discussion on Maintaining a Peaceful Relationship with our Symbionts, chaired by Dr. Dietmar Herndler-Brandstetter (Austrian Academy of Science, Austria) who focussed on new scientific highlights from in-vitro, in-vivo, animal and human studies about the effect of probiotics on immune modulation in the gut and its evaluation by using biomarkers. Oral presentations included: Bacterial Metabolites and Immune Modulation from Dr. Bernhard Watzl (Federal Research Centre for Nutrition, Germany) and Prof. Carsten Watzl (University of Heidelberg, Germany on NK cell Activity as a Biomarker for Risk Reduction of Cancer?

A key part of the session was a round table discussion chaired by Prof. Thomas T. MacDonald (Barts & the London School of Medicine and Dentistry, University of London), involving the speakers, and with substantial audience participation, which attempted to achieve a consensus on several topics.

The concept of 'suboptimal health' in the context of the benefits of probiotics

There was essentially unanimous support from the panel and the audience that many apparently healthy individuals suffer suboptimal health. Supporting evidence comes from the large number of individuals with functional bowel disease such as irritable bowel syndrome, and in whom probiotics can be of benefit. Likewise being overweight, not just obese, has detrimental health effects because accumulations of fatty tissue contain inflammatory cells which can secrete cytokines with deleterious systemic effects, such as Interleukin 6. The potential link with some individuals having an "obesogenic" microbial flora, and its potential manipulation by probiotics was well received. Other conditions where the microbial flora might be altered include autism and type II diabetes and again probiotics may have a role to play.

Is immunology a component of this 'suboptimal health' and can it be modulated by probiotics

It was generally felt that the there was not enough evidence to link immunology directly to suboptimal health. In terms of probiotics boosting immune responses, there was discussion as to whether changes in immune parameters were meaningful, given that the immune system is highly redundant and that all of the changes are within the normal range. Theoretically one could argue that increasing NK cells through giving probiotics might give increased protection from cancer, but without an evidence base, NK cells cannot be used as a surrogate for resistance to cancer by probiotics. There was also discussion about how an evidence base can be generated when different studies use different combinations of probiotics, in different protocols in different groups of patients. A good point was made that the probiotics used may be chosen because animal and in vitro experiments suggested they had a particular function, such as increasing barrier function. However the large numbers of different probiotics being used in different conditions is rather confusing the area.

How to combine clinical studies and mechanistic studies to give important information

There was virtual unanimity in the panel and the audience that wherever possible studies should be done in vivo and in man. However this discussion again highlighted a major problem with this area which is that there are no reliable biomarkers for the health benefits of probiotics.

Are there any reliable biomarkers for the benefits of probiotics such as NK cells, butyrate, epithelial cell proliferation, carcinoembryonic antigen?

Going on from above, there was agreement that the absence of good biomarkers based on *in vivo* evidence in patients was a major impediment to progress on understanding how probiotics function in man. The rapid development of cheaper technology to measure the gut microbial flora at the genetic level means that it may soon be possible to determine if probiotics alter the gut flora. The ideal biomarker would be something in blood, and although studies are in progress, no reliable marker has been established. The capacity to take endoscopic biopsies from patients given probiotics opens up a new and potentially interesting area, where probiotic effects may be directly measured in the gut, and if it was possible to correlate this with something in blood or stool, it would be a major advance. It is very probable that a single biomarker may be insufficient and that panels of markers will have to be developed as surrogates for *in vivo* effects.

International Journal of Probiotics & Prebiotics 3(3): 149-152

149-152 **ANTI-INFLAMMATORY EFFECTS OF PROBIOTIC LACTOBACILLUS CASEI STRAIN SHIROTA IN CHRONIC INTESTINAL INFLAMMATORY DISORDERS**

Satoshi Matsumoto

ABSTRACT: IL6/Stat3 signals play key roles in inflammatory bowel disease. It is known that Lactobacillus casei strain Shirota (LcS) improves IL6 dependent inflammatory disorders. Here, we elucidate the effect of LcS on murine chronic inflammatory bowel disease and to clarify the mechanism of the effect in terms of the IL6 pathway. LcS inhibited the synthesis of IL6 in LPS-stimulated large intestinal lamina propria mononuclear cells (LPMCs) in vitro. LcS diet improved murine colitis with the repression of IL6 synthesis by LPMCs. The inhibition of IL6 synthesis in vitro is dependent on the presence of polysaccharide-peptideglycan complex (PSPG) on LcS. The safety of this probiotic strain has been confirmed in a clinical trial. LcS may be a useful probiotics for the treatment of human chronic inflammatory disorders.

International Journal of Probiotics & Prebiotics 3(3): 153-158

153-158 **IMPROVEMENT OF THE GUT ENVIRONMENT BY PROBIOTICS: POSSIBLE RISK REDUCTION OF CANCER DEVELOPMENT?**

K. Verbeke, V. De Preter and L. Cloetens

ABSTRACT: The colonic microbiota has been suggested to be involved in the etiology of colorectal cancer. Therefore, manipulation of the microbiota using probiotics might be useful to reduce the colorectal cancer risk. Several mechanisms by which probiotics improve the gut environment have been studied in vitro, animal models or human intervention studies. Administration of probiotics has been shown not only to change the composition of the predominant microbiota, but also its metabolic activity. The putrefactive proteolytic activity can be decreased whereas it is attempted to increase short chain fatty acid production. Besides, modulation of bacterial β-glucuronidase and β-glucosidase enzyme activity as

well as bile acid metabolism might improve the gut environment. Finally, probiotics can reduce the bioavailability of toxins and mutagenic compounds by simply binding them or by decreasing the colonic transit time. Whether these mechanisms effectively decrease the colorectal cancer risk in humans remains controversial. As yet, epidemiological studies do not provide direct evidence for reduced colorectal cancer risk in humans by consumption of probiotics. However, human intervention studies, most often using early markers of colorectal cancer risk, suggest beneficial changes in host-associated markers. Nevertheless, these data should be interpreted carefully since these markers are not yet fully validated.

International Journal of Probiotics & Prebiotics 3(3): 159-162

159-162 **DOES CYTOTOXIC ACTIVITY INFLUENCE THE HEALTH STATUS OF THE HOST?**
Paolo Boscolo and Mario Di Gioacchino

ABSTRACT: Blood cytotoxic activity is involved in a bi-directional network of immune and neuroendocrine mechanisms. Pro-inflammatory cytokines induce mood changes including depression and fatigue. On the other hand, blood NK activity is reduced by anxiety, depression, occupational stress, job insecurity and unemployment and is improved by physical activity as well as by a healthy lifestyle and mental status. Moreover, blood NK activity may be reduced by exposure to smoke and xenobiotics, along with a shift of the Th1/Th2 immune balance towards Th2; the impaired NK activity of smokers may be restored by fermented milk containing Lactobacillus casei in the diet.

International Journal of Probiotics & Prebiotics 3(3): 163-164

163-164 **LACTOBACILLUS CASEI STRAIN SHIROTA AND PREVENTION OF RECURRENCE OF BLADDER CANCER**
Seiji Naito

ABSTRACT: Although superficial bladder cancer can be successfully treated by a transurethral resection (TUR), the high frequency of intravesical recurrence remains a concern. Lactobacillus casei (LC) preparation, a powdered preparation containing about 1x1010 cells of LC strain Shirota per gram, has been safely used as a probiotic agent for more than 30 years in Japan. When orally administered, LC preparation has been reported to act as an immunomodulator and to potentiate antitumor responses in mice. Based on these reports, two randomized, controlled clinical trials have been conducted, and the oral administration of LC preparation has shown to be a safe and effective modality not only as monotherapy, but also as a combination therapy with intravesical instillation chemotherapy, for preventing recurrence after TUR of superficial bladder cancer. Furthermore, a case-control study has also been conducted and the habitual intake of LC preparation was shown to reduce the risk of bladder cancer. Thus, LC preparation is considered to be effective for preventing not only the occurrence, but also recurrence after TUR of superficial bladder cancer.

International Journal of Probiotics & Prebiotics 3(3): 165-168

165-168 **PROBIOTICS AND CANCER - FROM IN VITRO TO HUMAN STUDIES**
Ian Rowland

ABSTRACT: Studies in cell cultures and animal models provide evidence that probiotics can beneficially influence various stages in development of colon cancer including tumor initiation, promotion and metastasis. For example, oral administration of Lactobacillus and Bifidobacterium strains can prevent

genotoxic damage to the colonic epithelium (considered to be an early stage of the carcinogenic process). Administration to rats of probiotics reduced the incidence of carcinogen-induced pre-cancerous lesions (aberrant crypt foci) in the colon. Furthermore a combination of Bifidobacterium longum and inulin (a prebiotic) was more effective than either treatment alone. In this latter study, the dietary treatments were given after exposure to the carcinogen, which suggests that the protective effects were being exerted at the promotional phase of carcinogenesis. L. acidophilus feeding has been shown to decrease the incidence of colon tumors in rats challenged with a carcinogen and B. longum reduced the incidence of carcinogen-induced colon, liver and mammary tumors. There is limited evidence from epidemiological studies for protective effects of products containing probiotics in humans, but a number of recent dietary intervention studies in healthy subjects and in polyp and cancer patients have yielded promising results on the basis of biomarkers of cancer risk and grade of colorectal tumors.

International Journal of Probiotics & Prebiotics 3(3): 169-170

169-170 ROUND TABLE DISCUSSION: NUTRITION AND CANCER PREVENTION
Janusz Jankowski

ROUND TABLE DISCUSSION: NUTRITION AND CANCER PREVENTION

Janusz Jankowski

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1. OVERVIEW OF GENETIC AND EPIGENETIC FACTORS IN CANCER BIOLOGY

The paradigm of various premalignant lesions progressing to cancer is now well established. Particularly good examples of this are the oesophagitis-metaplasia-adenocarcinoma sequence in the oesophagus and the adenoma-carcinoma sequence. Even here where the progression sequence is well known the use of prognostic and predictive biomarkers is far from established.

2. NUTRITION AND BIOMARKERS

The panel and then the audience were asked 4 questions:

a. What nutritional factors are important in cancer development?

Obesity, mentioned by Professor Collins, was the single biggest risk factor for cancer development. Obesity has multiple confounding factors as it is caused by poor diet, increased fat, decreased exercise as well as resulting in increased BMI and hormonal and cytokine imbalances.

Professor Rowlands explained that lifestyle had modest risks for cancer, fibre modest risks (but it was not clear whether fermentable or non-fermentable fibre was best). The benefits of organic vegetables are unclear as there are considerable variation of organic and non-organic polyphenols and flavanoids in the effects on intermediate markers.

Professor Naito explained that lactobacilli bacteria had shown considerable benefits in intermediate biomarkers.

Professor Verbeke indicated that fruit and vegetables had the best evidence for cancer prevention.

Professor Guarner said that many studies indicated many studies indicated biotics had effects, but no clear evidence for one particular food.

Professor Matsumoto indicated that epidemiological evidence indicated switch from Mediterranean or Japanese diet to western diet was the most important risk factor for cancer. Even here confounding issues such as decreased exercise and increased fat and insulin could not discriminate clearly between diet and lifestyle factors.

b. What are the best endpoints for randomised controlled trials?

Professor Matsumoto felt that using high-risk cohorts such as familial adenomatous polyposis (FAP) patients was best. In this way polyp size and number could be assessed.

Professor Jankowski indicated that assessing non-death endpoints meant that important side effects like cardiovascular system (CVS) might be missed using the analogy of COX-2 specific inhibitors preventing polyps, but increased cardiac side effects.

Professor Collins agreed that FAP patients would be the ideal population to study.

c. What are the best nutritional interventions to prevent cancer?

Professor Collins explained that while food was the ideal to test it was still too variable due to inconsistency between one batch and another. On the other hand, food additives also had various impurities when tested by NMR. This left tablets as the single easiest option to test especially calcium, vitamin D, selenium and non-steroidal anti-inflammatory drugs (NSAIDs).

Professor Rowlands said food was indeed difficult to test in trials and easier to assess in epidemiological studies. For this reason biotics were easier to test.

Professor Verbeke mentioned that tablets were not physiological, but admitted they were more feasible interventions for the purposes of clinical trials. A point from the floor also indicated that even if the ideal food could be found it would not be clear whether the food would be additive or replacement to the endogenous calorific intake.

d. What are the best biomarkers to assess nutritional risk and benefits?

Professor Boscolo indicated that genetic markers from genome wide sequencing could predict host susceptibility factors.

Professor Collins suggested that carcinogens or mutagens in faecal water were very useful.

Professor Rowlands suggested DNA damage markers, whereas Professor Verbeke suggested short chain fatty acids (SCFAs).

The audience were asked for their views to points *a-d* above.

There were clear majorities that felt high fat diets were important targets for cancer prevention. There was weaker evidence for fruits and vegetables and also only provocative data for biotics having a clear role.

Trial endpoints of all causes of mortality, cancer conversion or progression to dysplasia were the preferred events. There was little enthusiasm for biomarkers except for mucosal inflammation as a surrogate endpoint.

In summary, high fat diets were highlighted as major risk for cancer. Second, the best agents to be tested in trials were biotics and NSAIDs. Third, it was agreed large randomized controlled trials ending with death or cancer endpoints and polyp progression were the ideal. Fourth, biomarkers using mucosal inflammation were the best available.

International Journal of Probiotics & Prebiotics 3(3): 171-190

171-190 POSTER PRESENTATIONS

Abstract Presentations

International Yakult Symposium 2007

The Gut, Immune Modulation and Probiotics

Palazzo della Gran Guardia, Piazza Bra, 37121 Verona.

November 22nd - 23rd 2007

A-1

Relevance of probiotic strain naming: scientific and commercial aspects of nomenclature

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The reliable identification of probiotic strains is a fundamental prerequisite for correct labelling of probiotic products. Taxonomic analysis of bacterial strains consists in the attribution of genus and species names to microorganisms, and the lowest taxonomic rank recognized in bacterial taxonomy is the subspecies. However, development of investigation techniques results in a deepen comprehension of microbial biodiversity, thus evidencing the existence of intra- and sub-specific heterogeneity. Technical and taxonomic improvements are particular useful to highlight and to detect strain-specific peculiarities, characterising the properties enable to distinguish distinct probiotic strains. Therefore, taxonomy and nomenclature appear to be relevant scientific disciplines in order to undoubtedly link each bacterial species and strains with all data available related to each organism, from their isolation until the most recent investigations. From the scientific point of view, nomenclature undergoes changes in time, which are the result of improvements in taxonomic characterisation of bacteria and it may result in classification and/or name adjustments. However, changes of names might produce confusion in the consumer's perception of product reliability, in the tracking of literature data for probiotic strains and product patenting. Issues related to the scientific and commercial aspects of bacterial taxonomy and nomenclature are discussed.

A-2

Bifidobacteria play an important role in the cross-feeding between colon bacteria

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Introduction: Changes in the nature or amounts of undigested carbohydrates available in the human colon are thought to start a chain of events involving a wide range of mutually influencing functional groups of gut bacteria. The bifidogenic and butyrogenic effects caused by the addition of prebiotic inulin-type fructans to the diet, for example, are probably only the most pronounced end-results of complex interactions involving a wide range of gut inhabitants. Cross-feeding between colon bacteria is considered to be one of the determining factors in these interactions. In this study, two mechanisms of cross-feeding with possible influence on stimulation of bifidobacterial growth and colonic butyrate production were investigated. **Methods:** Monoculture fermentations of *Bifidobacterium longum* BB536, *B. adolescentis* LMG 10734, *Roseburia intestinalis* DSM 14610, and *Bacteroides thetaiotaomicron* LMG 11262 were carried out in a medium for colon bacteria under anaerobic conditions, with fructose, oligofructose (BeneoP95), and inulin (BeneoHP) as the sole added energy sources. Bacterial growth was followed through plating on selective media. Metabolite formation was studied using HPLC, HPAEC, and GC. **Results:** *B. longum*, *B. adolescentis*, and *R. intestinalis* all grew on fructose and oligofructose, but not on inulin. *Ba. thetaiotaomicron* LMG 11262 was able to grow on all substrates. Detailed analysis of fructan degradation revealed preferential breakdown of shorter chains (linked with intracellular degradation) by the *Bifidobacterium* spp., whereas *R. intestinalis* and *Ba. thetaiotaomicron* metabolized all fractions simultaneously (extracellular degradation). *R. intestinalis* displayed an absolute requirement of acetate for growth. During inulin degradation by *Ba. thetaiotaomicron*, a release of free fructose and oligofructose in the fermentation medium occurred. A coculture of *B. longum* with *R. intestinalis* on oligofructose in initially acetate-free medium led to butyrate production by the latter strain, using acetate produced by the former. Detailed analysis of oligofructose degradation showed a profile in between preferential and simultaneous degradation, indicating that both strains were metabolizing the substrate simultaneously. A coculture of *B. adolescentis* and *Ba. thetaiotaomicron* with inulin as the sole added energy source allowed the former strain to grow on the shorter oligofructose chains released by the latter. As the ability to degrade inulin is not common amongst bififobacteria, this and similar mechanisms of cross-feeding might contribute to the bifidogenic effect of this type of prebiotics. **Conclusion:** These *in vitro* studies reveal two mechanisms of cross-feeding that might add to the *in vivo* observed bifidogenic and butyrogenic effect of inulin-type fructans. A better understanding of the underlying mechanisms of these effects will be crucial for further development of new prebiotics.

A-3

Evaluation of the impact of a contemporary administration of the antibiotic rifaximin and the probiotic strain *Bifidobacterium infantis* BI07 on the intestinal microbiota

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Introduction: Inflammatory bowel disease (IBD) is a diffused chronic inflammatory condition of the gastrointestinal tract, but its aetiology remains unknown. It has been shown that the intestinal microflora in patients suffering of IBD differed from the faecal microflora of healthy subjects in term of diversity and composition. In the last year, several clinical trials demonstrated the use of antibiotics and probiotics as an alternative, non-conventional therapy of IBD. In this study, we investigated the effects of the administration of the antibiotic rifaximin and *B. infantis* BI07 on the intestinal microbiota using a three-stage continuous culture model of the human gut. **Methods:** Rifaximin was added in three times at a concentration of 1800 mg/day, while the probiotic strain was added daily at a concentration of 10^9 CFU/ml. The treatment was carried out for a period of 10 days. Bacterial groups of interest were evaluated by cultivation methods and fluorescence in situ hybridization (FISH). Additionally, fermentation end products were analyzed by HPLC to assess short-chain fatty acid concentrations in the different vessels. **Results and conclusion:** This is the first study to investigate *in vitro* the possible effectiveness of the simultaneous administration of a probiotic strain and an antibiotic as an IBD treatment, using a continuous culture system to simulate the intestinal system. Preliminary data suggest that the concentration values of the most important bacterial groups can vary during the antibiotic treatment, but without a drastic decrease of their numbers. In addition, strains belonging to *Bifidobacterium* genus are particularly able to upsurge resistance to rifaximin and persist in higher titre in the intestinal ecosystem. Further studies are underway to evaluate the comparison of rifaximin administration on the intestinal microbiota and the administration of a cocktail composed of rifaximin and *B. infantis* BI07, a component of the probiotic preparation VSL#3.

A-4

Isolation and identification of probiotic lactic acid bacteria from raw donkey milk

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Introduction: Different bacterial species of gut microbial *consortium* exert positive effects in the host. Probiotic bacteria are involved in the metabolism of nutrients and in the control of pathogen growth and abdominal pain (1). Fermented dairy products, like yogurt, are useful in the diet for the reintegration of gut microbiota. Conversely, raw milk represents a relevant source for the isolation of probiotic strains. Donkey milk is very similar in composition to human one and has been utilized for feeding of children with severe allergy to cow milk proteins (2). Aim of this work was to identify probiotic strains of *Lactobacillus* from microbial cultures of raw donkey milk. **Materials and methods:** For the screening of probiotic strains 150 colonies of LAB from an organic breeding of raw donkey milk, were randomly picked, grown on MRS broth and tested for resistance to bile salt and low pH and for cell hydrophobicity (3) and antibiotics sensitivity. Biochemical identification was performed using the API 50 CH fermentation assay. API results were validated by strain-genotyping. DNA purification, DNA-DNA hybridization and RAPD-PCR fingerprint were performed as previously described (4-5). Amplimers from RAPD-PCR were analysed by microchip-electrophoresis (2100 Bioanalyzer, Agilent).
Results: Eight clones were selected for the resistance to bile salt and acidic pH (70-75% respect to controls). They revealed also a good surface hydrophobicity and a marked antimicrobial activity against different pathogen strains. By API fermentation assay, three of them resulted related to *L. plantarum* (clones 37, 38, 48), four exhibited a metabolic profile belonging to *L. salivarius* or *L. brevis* (clones 32, 34, 41 and 43), the last one (clone 57) was not identifiable by this approach. RAPD-PCR finger-print confirmed the API data. Clones 37, 38, 48 were confirmed as belonging to *L. plantarum* species by DNA-DNA hybridization. Identification of the other clones and 16S RNA sequencing are in progress. **Conclusions:** Identification of probiotic strains in donkey milk allows to hypothesize their use to formulate donkey yoghurt and dairy products. Fermentation of donkey milk with natural occurring probiotic ferments could have noticeable effects on sensorial and texture properties and could constitute the basis for a new line of health-functional dairy products. **References: 1)** Shah., N.P. Probiotic bacteria: selective enumeration and survival in dairy foods. J. Dairy Sci. 2000, 83, 894-907. **2)** Chiofalo, B., Salimei, E., Chiofalo, L.,: Acidi grassi del latte d'asina:proprietà bio-nutrizionali ed extranutrizionali. Large Animals Review, 2003, 5,1-6, **3)** Ljuingh, A., Hjerten, S., Wadstrom, T. High Surface Hydrophobicity of Autoaggregating Staphylococcus aureus Strains Isolated from Human Infections Studied with the SaltAggregation Test Infection and Immunity, 1985, 47, 522-526. **4)** Poli, A., Romano, I., Caliendo, G., Nicolaus, G., Orlando, P., Falco, A., Lama, L.,

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A-5

Isolation of lactic acid bacteria from chickens that demonstrate probiotic properties of autoaggregation and coaggregation with *Salmonella enteritidis*.

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Introduction: The use of probiotics to promote gut health and prevent enteric disease is gaining interest in the poultry industry. The probiotic properties of lactic acid bacteria have been widely studied. Their ability to auto-aggregate and potential for co-aggregation with pathogenic bacteria are potential mechanisms for providing a competitive advantage in the intestinal microbiota and forming a barrier that prevents colonization of pathogenic microorganisms. **Materials and methods:** Fifty three lactic acid bacteria (LAB) isolated from the gastrointestinal tract of organically farmed chickens were examined for auto-aggregation (method of Kmet and Lucchini 1999) and co-aggregation with *Salmonella enteritidis* (method of Drago *et al* 1997). Aggregation within 15 min was scored as rapid, within 15-30min as normal and within 30-60 min as slow. Positive reactions were observed by scanning electron microscope (JEOL 5600 Low Vacuum SEM). **Results:** Of the 53 LAB tested, 20 were non-aggregative, 11 showed a rapid auto-aggregation, 12 had a normal reaction and the remainder showed weak auto-aggregation activity. Of the 23 LAB that showed normal and rapid auto-aggregative activity, one strain showed maximum co-aggregation with *S. enteritidis* (<15min), two rapid co-aggregation and six showed normal co-aggregation. Results were confirmed by scanning electron microscopy to exclude organisms with only auto-aggregative properties from co-aggregation scores. **Conclusions:** It has been suggested that there is an association between the aggregation and epithelial adhesion ability of bacteria, which may contribute to the exclusion of pathogenic bacteria. Therefore the LAB strain that gave a rapid auto-aggregation activity and a maximum ability to co-aggregate with *S. enteritidis* could be further screened as a potential probiotic in chicken nutrition. **Acknowledgements:** Greek State Scholarships Foundation for financial support. **References:** Drago, D., et al. 1997. *Fems Microbiology Letters* **153**: 455-463. Kmet, V. and R. Lucchini 1997. *Fems Immunology and Medical Microbiology* **19** (2): 111-114.

A-6

Screening and selection of porcine lactic acid bacteria as potential GI tract colonisers

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Introduction: There is a growing interest in the use of probiotics in livestock production to improve gut health and to offset in part the reduction in animal performance resulting from the withdrawal of antibiotic growth promoters. Colonization of the GI tract is an important characteristic of probiotic organisms. Adhesion to the mucus layer and/or the epithelial cell surface are useful characteristics to assess in determining the potential probiotic properties of bacteria. Adhesion to, or association with, these intestinal compartments by probiotic organisms may help protect against pathogen invasion by interfering with pathogen adhesion and subsequent invasion. In this study lactic acid bacteria of porcine origin were screened for potential probiotic properties. **Methods:** Ten strains of acid tolerant, bile resistant lactobacilli, of porcine origin, were screened for their ability to bind to porcine mucin and collagen IV and for adherence to CaCo2 and IPEC-I cells. In addition, strains were assessed for their ability to inhibit the adherence of the porcine ETEC pathogen *E. coli* K88.
Results: Two strains (C28 and NFD4) bound to mucus and three strains (VD36, C28 and NFD4) bound to collagen-IV. The majority of lactobacilli tested adhered to some extent to both epithelial cell lines regardless of their origin. However, adherence was generally weak. The inoculation rate for the lactobacilli was ~$\log_{10}$ 9 c.f.u/ml with adherence ranging from 4.5 – 6.8 and <4 to 6.46 $\log_{10}$ c.f.u./ml for CaCo2 and IPEC-1 cells respectively. Strains NFD4 and P14 showed the lowest percentage of adherence on both epithelial lines. Three of the strains tested (C28, VD36 and P14) significantly ($P < 0.05$) reduced the binding of E. coli K88. **Conclusions:** Of the strains tested, C28 showed the most promising suite of probiotic properties as it bound to

both mucin and collagen, adhered to IPEC-1cells and reduced the adherence of *E.coli* K88. Supported by EC FW6 programme Feed for Pig Health

A-7

Differences in faecal bacterial communities in coeliac and healthy children as detected by PCR and denaturing gradient gel electrophoresis

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Coeliac disease (CD) is a chronic inflammatory disorder of the small intestinal mucosa. Scientific evidence supports a role of the gut microbiota in chronic inflammatory disorders, yet information is not specifically available for CD. In this study, a comparative DGGE analysis of faecal samples from coeliac children and age-matched controls was carried out. The diversity of the faecal microbiota was significantly higher in coeliac children than in healthy controls. The presence of the species *Lactobacillus curvatus, Leuconostoc mesenteroides* and *Leuconostoc carnosum* was characteristic of coeliac patients while that of *L. casei* group was characteristic of healthy controls. *Bifidobacterium* population showed a significantly higher species diversity in healthy children than in coeliacs. In healthy children, this population was characterized by the presence of *B. adolescentis*. Overall, the results highlighted the need for further characterization of the microbiota in coeliac patients, and suggested a potential role of probiotics and/or prebiotics in restoring their gut microbial balance.

A-8

Analysis of *in vivo* active genes of *E. coli* Nissle 1917 by using a promoter trap library

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Introduction: *Escherichia coli* Nissle 1917 (EcN) is a fecal isolate which is used as a probiotic and comprises a therapeutic alternative for the treatment of inflammatory bowel diseases. Clinical trials comparing EcN to standard medication used for therapy of ulcerative colitis revealed an equal effectiveness of the probiotic in maintaining remission, associated with less or no side-effects. EcN is a good colonizer in the gut of humans and animals. Specific characteristics of the strain are for example the production of microcins or a semirough LPS. However, the molecular mechanisms which are responsible for the beneficial behaviour of EcN during gastrointestinal passage and colonization are not very well understood. Investigation of *in vivo* gene regulation in EcN will provide important information for a better understanding of the strain´s probiotic traits. **Methods:** For construction of a promoter-trap-library mechanically sheared fragments of EcN genomic DNA were randomly cloned in front of a promoterless *gfp* gene on a plasmid and transformed into EcN. Plasmids containing functional promoters would lead to GFP expression in these clones. To identify i*n vivo* active promoters, the library was fed to Balb/c mice followed by FACS sorting of green fluorescent bacteria in the feces and subsequent sequencing of the cloned promoters. **Results:** A nucleotide BLAST search of the identified promoter fragments against the EcN genome as well as a computer based analysis of the promoter regions revealed genes with potential *in vivo* activity. **Conclusion:** This technique is a very useful tool to identify genes that are positively regulated under defined *in vivo* conditions. Based on their frequency in the hit list or on their

presumed importance for the probiotic nature of EcN, selected genes from this collection of *in vivo* regulated ones were chosen as candidates for further analysis.

A-9

Different phenotypes in *rpoS*-mediated acid resistance of probiotic and pathogenic bacteria

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Introduction: Shiga toxin (Stx) producing enterohemorrhagic *E. coli* (EHEC) can cause severe enteritis and as a complication also hemolytic uremic syndrome. EHEC are transmitted orally via contaminated food or smear infection. The very low infection dose requires survival of the bacteria during uptake and passage through the gastric acid barrier. Therefore, acid resistance is an important mechanism of virulence. But, acid resistance should also account for the fitness of the probiotic *E. coli* strain Nissle 1917 (EcN) which is increasingly used as a therapeutic for the treatment of various intestinal disorders. Acid resistance as well as resistance to distinct environmental stress factors in *E. coli* and related enteric bacteria is mediated by the *rpoS* encoded alternative sigma factor sigmaS, acting as a master regulator. **Methods:** *rpoS* deletion mutants of a highly virulent EHEC O26:H11 patient isolate and the sequenced EHEC O157:H7 strain EDL933 (ATCC 700927) were investigated for the impact of a functional *rpoS* gene to acid stress. Then, *rpoS* genes of probiotic EcN and five Stx producing *E. coli* isolates, pre-investigated for acid resistance, were functionally characterized.
Results: We found out that ATCC isolate 700927 of EHEC EDL 933 has a point mutation in *rpoS*, not present in the published sequence, leading to a premature stop codon. Moreover, to our surprise, one STEC strain as well as EcN was acid sensitive in our test environment, although their cloned *rpoS* genes could effectively complement acid sensitivity of an *rpoS* deletion mutant.**Conclusion:** Our data demonstrate that individual *E. coli* isolates are able to significantly modulate their acid resistance phenotype by regulatory mechanisms independent of their *rpoS* genotype.

A-10

Characterization and adaptation of plasmids in *Lactobacillus salivarius*

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Introduction: *Lactobacillus* is one of the most common organisms that has been used as a probiotic. About 38% of the species in this genus harbor plasmids. Characterization of endogenous plasmids from *Lactobacillus* is of great interest as they confer a number of useful traits upon the host. In addition, they can be developed into molecular tools to analyze genes that contribute to probiotic characteristics. Some *Lactobacillus* plasmids have been adapted as cloning and expression vectors. However, additional gene expression vectors, expression monitoring and gene mutagenesis systems are required to investigate and analyze biologically relevant characteristics of probiotic lactobacilli. **Methods:** PFGE (pulsed-field gel electrophoresis) and Southern blot were used to detect pSF118-20 and pSF118-44 related plasmids in 27 *L. salivarius* strains. Gene cloning and

transmissible cloning vectors for probiotic lactobacilli were constructed by cloning either a stable replication region or the mobilization region and the replication region from pSF118-20 into an *E. coli* cloning vector. **Results and conclusion:** Endogenous plasmids of various sizes were detected in *L. salivarius* strains. A gene cloning and expression vector (pLS203) and a transmissible cloning vector (pLS208) were constructed based on plasmid pSF118-20 from *L. salivarius* UCC118. pLS203 is quite stable in lactobacilli in the absence of antibiotic selection and may thus be used as a gene cloning and expression vector. pLS208 can be mobilized from *L. plantarum* to *L. fermentum* with the help of a conjugative plasmid. pSF118-20 derivatives showed broad host range, high segregational stability and ability for horizontal gene transfer. Therefore, vectors that were derived from lactobacillus plasmids have the advantage to be developed into various genetic tools to monitor and understand behavior of probiotic lactobacilli *in vivo*.

A-11

Genomic variability in *Lactobacillus salivarius*

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Introduction: Megaplasmids are a general feature of the species *Lactobacillus salivarius*, with a number of strains harboring multiple megaplasmids of varying sizes, some of which are linear. The intestinal isolate *L. salivarius* UCC118 harbors a theta-replicating megaplasmid pMP118 (242 kb) which confers probiotic properties. Genomic diversity studies of the species will be used to examine the contribution of megaplasmids to the biology of *L. salivarius*. **Methods:** Comparative hybridisation of genomic DNA and megaplasmid DNA of 20 *L. salivarius* isolates, from various sources, has been initiated using oligonucleotide microarrays based on the genome of strain *L. salivarius* UCC118. The genome of strain UCC122 is being sequenced, using a hybrid Sanger/pyrosequencing approach. **Results:** CGH revealed high levels of genome plasticity in the septicaemia and animal isolates UCC121 and UCC122. These include: phage associated genes, transposases, conjugation associated genes, carbohydrate metabolism genes and plasmid associated genes of pSF20 and pSF44. The human intestinal isolate UCC120 demonstrated a high level of similarity to strain UCC118, with the exception of the pSF44, which appears to be absent. 4.9x coverage of strain UCC122 was achieved using Sanger sequencing, with an initial total contig length of 1,912,145 bp. Comparisons of strain UCC122 with the sequenced strain UCC118 estimates the presence of 265 kb of unique DNA sequence, including restriction modification system proteins, transposase proteins and phage associated proteins of a number of related species. **Discussion:** CGH analysis to date indicates that human intestinal isolates may differ significantly from septicaemia and animal isolates. The regions showing diversity are those commonly associated with genome plasticity among strains of the same species. Sequencing of the linear megaplasmid of strain UCC122 offers an opportunity to study the replication mechanism of linear megaplasmids in the species and the biological contribution of this extra-chromosomal element to the strain.

A-12

Patho-biotechnology

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Introduction: Given the increasing commercial and clinical relevance of probiotic cultures, improving their stress tolerance profile and ability to overcome the physiological defences of the host is an important biological goal. **Methods and Results:** In order to reach the gastrointestinal tract in sufficient numbers to exert a therapeutic effect, probiotic bacteria must resist the deleterious actions of low pH, elevated osmolarity and bile salts. 'Patho-biotechnology' describes the exploitation of highly adapted pathogenic stress survival and host evasion strategies for the development of improved probiotic cultures. Cloning the listerial betaine uptake system, BetL, into a probiotic strain *Bifidobacterium breve* UCC2003, significantly improved probiotic tolerance to gastric juice and conditions of elevated osmolarity mimicking the gut environment. Furthermore, whilst stable colonisation of the murine intestine was achieved by oral administration of *B. breve* UCC2003, strains harbouring BetL were recovered at significantly higher levels in the faeces, intestines and caecum of inoculated animals. Finally, in addition to improved gastric transit and intestinal persistence, this approach dramatically improves the clinical efficacy of the probiotic culture; mice fed *B. breve* UCC2003 ($BetL^+$) exhibited significantly lower levels of systemic infection compared to the control strain following oral inoculation with *Listeria monocytogenes*. **Conclusions:** The 'Patho-biotechnology' concept promotes the exploitation of valuable stress survival traits from pathogenic strains for the design of more technologically robust and effective probiotic cultures with potentially improved biotechnological and clinical applications, as well as the development of novel vaccine and drug delivery platforms.

A-13

Prebiotic metabolism by *Bifidobacteium. breve* UCC2003: Molecular Dissection of *apu*B

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Introduction: The gastrointestinal tract (GIT) is inhabited by a complex community of microorganisms which play an important role in both human health and disease. Bifidobacteria constitute one of the most important bacterial groups in the GIT where they perform a beneficial role in promoting a state of eubiosis while also having a favourable immunomodulatory effect. Despite increasing research and use of probiotic bifidobacteria in functional foods to fortify the gut microbiota of patients with chronic gastrointestinal disease, key aspects such as metabolic routes and the regulation of bifidobacterial physiology remain relatively unexplored. Relevant molecular studies have been hindered by the lack of efficient and reliable transformation procedures as well as a paucity of effective molecular tools for genetic manipulation, such as cloning vectors, overexpression systems and chromosomal gene inactivation systems. In order to study gene function in bifidobacteria a gene disruption strategy is required that is independent of transformation frequency and stably maintains the integrated plasmid. **Results and Conclusion:** In this work, the construction and characterisation of an insertion mutant of the amylopullulanase gene (*apu*B) of *B. breve* UCC2003 is described. *apu*B encodes a 1,708 amino acid bifunctional class II pullulanase which has both a-amylase and pullulanase activities, hydrolysing both a-1,4 and a-1,6 glucosidic linkages in starch and related polysaccharides. Although non–digestible dietary carbohydrates represent the principal nutrients for bacterial growth in the colon, starch that has evaded digestion in the small intestine, also referred to as resistant starch, can enter the large intestine where it can be used as carbohydrate source for colonic bacteria such as bifidobacteria. To our knowledge, this work represents the first targeted gene disruption described for the genus *Bifidobacterium* by a site directed homologous recombination event.

A-14

Administration of folate producing bifidobacteria improved folate status in rats

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Introduction: Folates are water-soluble vitamins that function as cofactors in 1-carbon transfer reactions occurring in purine and pyrimidine biosynthesis. Folates play a significant role in the reduction of cancer risk, are involved in regulation of rectal cell proliferation and their supplementation is recommended for patients with inflammatory bowel diseases. Three folate-overproducing *Bifidobacterium* strains (*B. adolescentis* MB 227, *B. adolescentis* MB 239, and *B. pseudocatenulatum* MB 116) grew in a folate-free medium and produced high concentration of vitamin in faecal cultures. Therefore, they could be used as probiotics in order to supply this vitamin *in vivo*. **Methods:** *B. adolescentis* MB 227, *B. adolescentis* MB 239, and *B. pseudocatenulatum* MB 116 were administered to rats with induced folate deficiency. For 14 days, 4 groups of animals were given solid folate-free diets with following additions: I) no functional supplements (control); II) folate-producing bifidobacteria (probiotic); III) oligofructose (prebiotic); IV) folate-producing bifidobacteria and oligofructose (symbiotic). Tissue folate levels were evaluated at the end of the treatment. **Results:** Plasma concentration was much higher in rats receiving diets containing folate-producing bifidobacteria as probiotics alone and as symbiotic components. As a consequence of pre-, pro-, and symbiotic administration, lactobacilli and bifidobacteria significantly increased, while coliforms and enterococci decreased. Moreover, diets containing bifidobacteria and/or oligofructose led to a significant acidification of faeces due to enhanced saccharolytic metabolism. **Conclusion:** These *in vivo* experiments describe encouraging effects of the administration of wild-type folate-producing bifidobacteria to enhance folate status. Folate-producing strains may exert both the beneficial effects of probiotics and continuously provide the host with a constant vitamin supply in case of inadequate folate intake.

A-15

Fermentation of galacto-oligosaccharides by *B. adolescentis* MB 239

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Introduction: In the last few decades GOS have been increasingly introduced as prebiotic ingredients for dietary or pharmaceutical applications since they cause bifidobacteria proliferation and SCFAs increase. The addition of fructo- and galacto-oligosaccharides mixtures to infant formulas stimulated bifidobacteria growth and metabolic activity in the colon, thus reproducing the bifido-dominant microflora of breast-fed infants. Nevertheless, few metabolic and kinetic information on GOS fermentation by bifidobacteria is available. **Methods**: The kinetics and the metabolism of *Bifidobacterium adolescentis* MB 239 growing on galacto-oligosaccharides (GOS), lactose, galactose, and glucose were investigated. An unstructured unsegregated model for growth in batch cultures was developed and kinetic parameters were calculated with a recursive

algorithm. Lactate, acetate and ethanol yields allowed the calculation of carbon fluxes toward fermentation products and ATP yields. **Results**: The growth rate and cellular yield were the highest on galactose, followed by lactose and GOS, and were the lowest on glucose. Similar distribution between 2- and 3-carbon products was observed on all the carbohydrates (55 and 45%, respectively), but ethanol yield was different on glucose, GOS, lactose, and galactose, in decreasing order of production. The highest ATP yield was obtained on galactose, while it was 5, 8, and 25% lower on lactose, GOS, and glucose, respectively. During the fermentation of the GOS mixture, lactose and the trisaccharide were first to be consumed, while a delay was observed until longer oligosaccharides were utilized. Moreover, throughout the growth on both lactose and GOS, galactose accumulated in the cultural broth. **Conclusions**: A correspondence among ethanol production, low ATP yields, and low biomass production was established, demonstrating that carbohydrate preferences may result from different distribution of carbon fluxes through the fermentative pathway. Both intracellular and extracellular hydrolysis are involved in the utilization of β-(1-4) galactosides. Moreover, stringent substrate selectivity based on the degree of polymerization was observed.

A-16

Modulation of natural killers by probiotics: a review of the evidence

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Natural killer (NK) cells are large lymphocytes of granular morphology which make up 5-20 % of peripheral lymphocytes. They are chiefly found within the blood circulation and in the spleen but also exist in lymph nodes and in gut-associated lymphoid tissues. Due to their innate capability to lyse virally infected or cancerous cells, they are of vital importance as a first line of defence against viral infections and to combat tumour development. Several human intervention trials, animal and *in vitro* studies have shown that NK cells and their cytolytic activity are a potential target of probiotic immunomodulation. Probiotic administration may be efficient to enhance NK cell activity in animal models of vaccination and tumour development, whereas our own studies in healthy rodents and pigs, which also included the gut-associated lymphoid tissue, did not show a modulation of the cytolytic activity of NK cells by probiotic treatment. Reports on the influence of probiotics on NK cell numbers vary. Results from human intervention trials suggest that elderly and individuals with a low NK cell activity, as it occurs in a lower percentage of healthy persons, in cancer patients and in immunocompromised people, could particularly benefit from the immune-stimulating properties of probiotics. Our recent intervention study in healthy subjects with low NK cell activity is underway to study this hypothesis. Mechanistic studies indicate that bacteria-to-cell-contact seems to be of vital importance for NK cell activation by probiotics which includes regulation of gene expression. Stimulation of NK cells by probiotics appears to be dependent on the presence of accessory cells which provide adequate co-stimulatory signals. Whether whole, vital bacterial cells are necessary for NK cell modulation or whether heat-inactivated bacteria or even bacterial fractions are sufficient, remains to be determined.

A-17

Butyrate differentially regulates macrophage inflammatory responses to bacterial stimulation: a cautionary tale for probiotic development

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Introduction: Mucosal tolerance is central to the efficient gastrointestinal tract (GIT) function, thus tolerating food and commensal bacteria. Macrophages resident in the GIT are critical in maintaining this tolerance, however, in chronic inflammatory conditions such as Crohn's disease, macrophage functions are dysregulated, hence tolerance broken. Whether this dysregulation is a direct effect on resident macrophages or infiltrating pro-inflammatory macrophages is not understood.

Gut immune defences can be fortified by probiotic and prebiotic approaches. Butyrate, a bacterial fermentation product, has been demonstrated to exhibit potent anti-inflammatory effects on monocytes, thus would appear to have potential for maintaining a healthy digestive tract and therapeutic treatment of chronic gut inflammation. We hypothesise that any beneficial anti-inflammatory effect of butyrate is dependent on the macrophage subset encountered.
Methods: The human monocyte cell line, THP-1, was used to investigate the anti-inflammatory potential of butyrate when stimulated by the bacterial patterns, endotoxin (LPS) and peptidoglycan (PGN). Anti-inflammatory and pro-inflammatory macrophages were generated by differentiation in the presence of vitamin D3 and PMA respectively and effector responses measured by ELISA of culture supernatants for TNFa, IL-1b and IL-10. **Results:** Data indicates that butyrate differentially regulates monocyte and macrophage cytokine production. TNFa is suppressed in all cell types; IL-1b is suppressed in monocytes and anti-inflammatory macrophages, whereas IL-10 is augmented in these cell types and suppressed in pro-inflammatory macrophages. **Conclusion:** This study demonstrates that butyrate differentially regulates monocyte and macrophage cytokines and that the exact anti-inflammatory effect is dependent on the route of macrophage differentiation. Beneficial effects of butyrate used in probiotic approaches will be determined by macrophage profiles present in the gut.

A-18

Macrophage phenotype, the key to the gut mucosal immune system NOD-ing off to commensal bacteria

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Introduction: NOD2 is an important intracellular pattern recognition receptor; a bacterial sensor recognising the muramyl dipeptide (MDP) motif derived from peptidoglycan. This molecule has received attention as a functional mutation associated with Crohn's disease (CD). Its function is a source of controversy however, and would appear to both augment and suppress inflammatory cytokine production dependent on the cells investigated. A major cellular source of these inflammatory cytokines in pathogenic situations such as CD is the macrophage. Development of a comprehensive understanding as to how MDP suppresses or augments inflammatory mediators in macrophage cells may facilitate the development of anti-inflammatory bacterial products being utilised as probiotics and/or therapeutic approaches to chronic gut inflammation.
Methods: Human peripheral blood-derived monocytes and the monocytic cell line, THP-1, was used to investigate the modulatory effects of MDP on cytokine production by monocytes and monocyte-derived macrophages using a range of differentiation factors (GM-CSF, IFNg, M-CSF, IL-4/IL-13, PMA and vitamin D3). Cytokine production was induced by the bacterial products lipopolysaccharide (LPS) and peptidoglycan (PGN) and effector responses measured by ELISA of culture supernatants for TNFa and IL-1b. **Results:** Data indicates that MDP differentially regulates cytokine production in monocytes, pro-inflammatory- and anti-inflammatory-macrophages. Specifically, MDP augments PGN- and LPS-induced TNFa and IL-1b□production by monocytes and pro-inflammatory macrophages, whereas MDP suppressed PGN-induced TNFa and IL-1b□in pro-inflammatory macrophages. **Conclusion:** MDP activation of NOD2 differentially regulates monocyte and macrophage inflammatory cytokines; an effect dependent on the route of macrophage differentiation. Beneficial effects of NOD2 activation will result from priming of anti-inflammatory macrophage phenotypes in chronic inflammatory settings. In the case of NOD2 mutated CD patients: the jury is still out!

A-19

Effects of two probiotics on serum immunoglobulins and cells of Peyer's patches and spleen in mice

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Introduction**: The aim of this study was to observe the effects of two probiotic bacteria on serum immunoglobulin isotypes and spleen and Peyer's patches cells.** Methods**: 20 BALB/c mice were fed with a basal diet, supplemented or not with two probiotic bacteria, *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis subsp. lactis* Bb12, respectively at 10^9 and 10^8 c.f.u./g of feeding during 6 weeks. Blood samples were collected individually to determine immunoglobulin isotype concentrations. Phagocytosis, natural killer (NK) activities and T-lymphocytes phenotypes from spleen and Peyer's patches (PP) were measured by flow cytometry. Data were analyzed using a one-way analysis of variance. Least significant differences were used to compare means at the 5% confidence level.** Results**: In probiotic feeding group compared with control group we found: (1) significant higher levels of IgG and IgA, and significant lower IgM level, (2) improved phagocytose activity in spleen, by increasing the number of monocytes and enhancing their effectiveness (on *E.coli* phagocytose), (3) no effect on NK activity, (4) splenocytes exhibited significantly more $CD3^+$ and $CD8^+$ (due to an increase of $CD3^-CD8^+$ subpopulation) and less $CD4^+$ (induced by the reduction of $CD3^-CD4^+$) cell subpopulations and (5) in PP, reduced levels of $CD3^+$ and $CD4^+$, due to reduction of $CD3^+CD4^+$, and unchanged level of $CD8^+$ accompanied with an increase of $CD3^-CD8^+$ and a decrease of $CD3^+CD8^+$ cell subpopulations.** Conclusion**: Probiotics feeding modified several immunological markers. Their ability to modulate the activities of immunological regulators (monocytes, $CD3^-$ dendritic cells and Th cells) and immunological effectors (Tc cells and immunoglobulins) could be different in spleen and PP.**

A-20

A multiparametric comparative method to study the immune system and its changes

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Introduction: The immune system is a multicomponent dynamic entity, very sensitive to many exogenous and endogenous stimuli. Lymphocytes are the pivot cells of the system and are associated with the specificity of the immune response; they differentiate in many subsets expressing different sophisticated functions. Their amount and activities change sensitively with the age. Due to complexity and diversity of these components it is crucial the employment of multiparametric investigation technologies in order to acquire a systemic vision of the global immune activity both physiological and pathological. **Methods**: We routinely apply multiparametric analysis of the lymphocytes subpopulations by using flow cytometry and monoclonal antibodies of six different fluorochromes. The usual monoclonal panel employed is: CD45, CD3, CD4, CD8, CD16, CD56, CD57, CD19, CD23, CD27, CD5, HLA-DR. In order to establish standard references for the various subset profiles, we created a data bank composed by data from the afferent subjects to our laboratory. To evaluate the parameters significance we compare values of the single subject with a group of subjects (about 200) of the same age. The obtained series of values, corresponding to the percentile of all parameters, are plotted in a graphic representation which allows an immediate evaluation of the totality of parameters. **Results**: With this method we evaluated the data of about 4,500. We made synthetic graphic as a fingerprint of each individual and identified the shifts from the reference group. Conclusion: This system can identify a pattern for every subject and show the changes during time. Concerning probiotics, we could find groups of subjects with patterns associated to a more or less sensitivity to their immune modulation activity.

A-21

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

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Introduction: Reliable testing of intestinal bacteria for immuno-biological activities depends largely on the set up of the culture model. Adding bacteria directly to cells of the immune system inevitably leads to strong responses, representing artefacts rather than therapeutically relevant activities. **Methods:** To avoid such artificial findings, we developed a co-culture model (EDI-Co gut) which separates the bacteria to be tested from the immune cells (whole-blood cultures) by a highly differentiated, functional layer of gut epithelial cells. Thus, the change of immune cell activities in this cell culture model can occur only in two ways: a) immunologically active components access the immune cell compartment ("baso-lateral") via regulated absorption by the gut epithelial cells, or b) active substances cause epithelial cells ("luminal compartment") to secrete own metabolites or mediators that cause a secondary modulation of immune cell activities. **Results:** Different strains of *E.coli* as well as common bacterial metabolites, such as butyrate, iso-valerate, and ammonia were tested in the co-culture model described above. Despite the fact that there was no direct contact of the bacteria or the metabolites (incubated on the luminal side of the gut epithelium) and the leukocytes (in the baso-lateral compartment), clear-cut changes in the response of the immune cells towards subsequent experimental activation could be demonstrated.
Surprisingly, strong lot-to-lot differences in the activities of one of the *E.coli* strains elicits the question of standardisation of the manufacturing process for bacterial preparations used in microbial therapy. **Conclusions:** Reliable testing of probiotic bacteria and bacterial metabolites for meaningful results can be done in human organo-typic cell culture models. Yet this requires a minimum of prerequisites: a) an organo-typic culture set up, preventing the bacteria to access immune-cells directly, b) a tight and functional intestinal epithelium, c) a standardised performance of the culture model, d) a standardised manufacturing process of the samples to be tested.

A-22

Effect of *Lactobacillus casei* administration in a mouse model of gluten hypersensitivity

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Introduction: Celiac disease (CD), is the most common food-sensitive enteropathy in humans, caused by the lack of oral tolerance to wheat gluten (1). To study this disease we use a transgenic mouse model expressing the HLA-DQ8 molecule in the absence of endogenous class II genes (2). The adjuvant function *Lactobacillus casei* is well known (3). In this study we analyzed the effects of oral administration of *L. casei* in DQ8 transgenic mice following mucosal sensitization with gliadin along with cholera toxin. **Methods:** DQ8 transgenic mice were administered intragastrically with 500 mg of a chymotryptic digest of gliadin (ct-gliadin) along with 25 μg of cholera toxin (CT) on days 0, 7 and 14. In someexperiments *L. casei* ($1x10^{10}$/dose, four doses/week) was co-administered per os. On day 21 mice were sacrificed. Spleen and MLN were isolated for *in vitro* assessment of gliadin-specific immunity. Small intestine fragments were collected for RNA analysis and immuno-morphometric measurements. **Results:** Co-administration of *L.casei* in gliadin-sensitive DQ8 transgenic mice caused a strong increase of the intestinal gliadin-specific cell mediated immune response (SI: 2.75 ± 0.1 vs 4.4 ± 0.03; control vs *L. casei*; $p < 0.05$) as well as the IFNg□response (pg/ml: 3976 ± 212 vs 9891 ± 2649; control vs *L. casei*; $p < 0.05$). Probiotic administration was also associated to a Th1 polarization of the small intestinal mucosa. However, the strong increase in the IFN-g production did not provoke a gliadin-specific enteropathy. **Conclusion:** Exacerbation of the gliadin-specific Th1-like immune response by *L. casei* is not associated to induction of enteropathy in our model of CD. Other parameters are then required to induce the mucosal damage. From this point of view administration of *L. casei* can be considered safe also in an ongoing intestinal hypersensitivity to food antigens. **References**: (1) Maki, M., K. Mustalahti, J. Kokkonen, P. Kulmala, M. Haapalahti, T. Karttunen, J. Ilonen, K. Laurila, I. Dahlbom, T. Hansson, P. Hopfl, and M. Knip. 2003. Prevalence of Celiac disease among children in Finland. *N. Engl. J. Med*. 348: 2517-2524; (2) Cheng, S., J. Baisch, C. Krco, S. Savarirayan, J. Hanson, K. Hodgson, M. Smart, and C. David. 1996. Expression and function of HLA-DQ8 (DQA1*0301/DQB1*0302) genes in

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A-23

Identification of commensal bacterial strains modulating *Yersinia* and DSS-induced inflammatory responses: implications for the development of probiotics

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Introduction: An increasing body of evidence suggests that probiotic bacteria are effective in the treatment of enteric infections although the molecular basis of this activity remains elusive. To identify putative probiotics we tested commensal bacteria in terms of toxicity, invasiveness, inhibition of *Yersinia* induced inflammation *in vitro* and *in vivo* and modulation of dextran-sodium-sulfate (DSS) induced colitis in mice. **Methods:** Commensal *E. coli*, *Bifidobacterium adolescentis*, *Bacteroides vulgatus*, *Bacteroides distasonis* and *Streptococcus salivarius* were screened for adhesion to, invasion into as well as toxicity for host epithelial cells (EC) and the strains were tested for their ability to inhibit *Y. enterocolitica* induced NF-□B activation. Additionally *B. adolescentis* was administered to mice, orally infected with *Y. enterocolitica* and to mice with a mucosa impaired by DSS treatment. **Results:** None of the commensal bacteria tested was toxic for or invaded into EC. *B. adolescentis*, *B. distasonis, B. vulgatus and S. salivarius* inhibited *Y. enterocolitica* induced NF-□B activation and IL-8 production in EC. In line with these findings *B. adolescentis* fed mice had significantly lower mean pathogen burden in visceral organs, intestinal TNF-□□ mRNA expression and loss of bodyweight upon oral infection with *Y. enterocolitica*. In addition, administration of *B. adolescentis* decelerated inflammation upon DSS treatment in mice.
Conclusion: We suggest that our approach might help to identify new probiotics to be used for treatment of inflammatory and infectious gastrointestinal disorders.

A-24

Influence of gut microbiota on liver metabolism and drug-metabolizing capabilities in mice

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Introduction: The composition of mammalian gut microbiota (GMB) has recently been associated with obesity and insulin resistance status (1). Furthermore, it has been shown that acclimatization of germ-free (GF) rats induces a number of metabolic perturbations reflected in urine metabolic profiles (2). In a previous study, the investigation of the metabolic phenotype of GF mice revealed that GMB globally influence the host metabolism, inducing a number of metabolic perturbations, not only in gut but also in more spatially remote organs from the GMB such as the liver and kidney. **Methods:** The aim of this study was to investigate the liver metabolic perturbations induced by the acclimatization process in GF mice for 20 days. For this purpose, intact liver metabolic profiles were assessed every 5 days by high resolution magic-angle-spinning ^{1}H NMR technique coupled with pattern recognition methods. We also monitored cytochrome P450 (CYP) activity along the acclimatization. **Results:** Results showed that testosterone 6β-hydroxylase activity is highly reduced in liver of GF animals and do not recover after 3 weeks of colonization. In liver profiles, phosphocholine was higher in the GF group while uridine was highly associated with the conventional group. It was also observed that major metabolic changes in liver occurred the first week of colonization. In particular, colonization by GMB rapidly induced an increase in glucose level while phosphocholine and glycerophosphocholine remained higher in GF animals. **Conclusion :** Thus, this original chronological work shows that GBM play an essential role on liver homeostasis influencing the energy metabolism. It also demonstrates that GBM influence liver drug-metabolizing capabilities reflected by the CYP enzymes activities. **References: (1)** Bäckhed, F., Manchester, J. K.,

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A-25

Effect of oral administration *Lactobacillus casei* Shirota on experimental autoimmune encephalomyelitis in rat

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Introduction: *Lactobacillus casei* Shirota (LcS) modulates the innate and acquired immune response in experimental animals and humans. In the animals or human subjects whose natural killer (NK) cell activities are lower, administration of LcS recovers their NK cell activities. Moreover, administration of LcS augments delayed-type hypersensitivity response against parasite antigens in normal rats. From the standpoint of safety, it is of great importance to investigate whether LcS may excessively activate the immune response in the allergy or autoimmune diseases. So far, we have confirmed in animal models that LcS alleviates the disease symptom of diabetes (Matsuzaki T et al., 1997), improves the incidence of collagen-induced arthritis (Kato I et al., 1998), and elongates the life-span of systemic lupus erythematosus (Mike A et al., 1999). Here, to evaluate the effect of LcS in other autoimmune disease model, we examined the effect of LcS on experimental autoimmune encephalomyelitis (EAE). **Method:** Lewis rats were induced to develop EAE by sensitizing with spinal cord extract or myelin basic protein from guinea pig. They were randomly divided into two groups. Control and administration groups were orally given vehicle alone or live LcS by 1~2 x 10^9 CFU/day, respectively, from 7 days before the sensitization to 28 days after the sensitization. **Results:** After the sensitization, body weight decreased in association of appearance of neurological symptoms. However, the change of body weight and neurological scores were not significantly different between groups during the experimental period, in two different immunization protocols. **Conclusion:** Although much remains to be learned until we will obtain the solid conclusion about the effect of LcS on EAE, our results support that administration of LcS may not activate the immune response excessively in various autoimmune disease models.

A-26

Effect of probiotics on intestinal gene expression pattern of healthy individuals

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Background: Some bacteria have been found to play a role in the carcinogenesis of the GI tract, while probiotics have been proposed to play a protective role. Single genes or a small group of them have been described to play a role in this process but a full analysis of the complex bacteria–host interplay in the intestinal lumen has never been performed. **Aims:** To identify the gene expression pattern induced by *Bacillus clausii* in the intestinal mucosa of healthy individuals. **Methods:** Ten male patients (mean age 40±5 years) affected by endoscopically confirmed mild oesophagitis were treated for one month with esomeprazole, and were randomly selected to receive or not *B. clausii* (groups I and II, respectively). Duodenal biopsies were taken pre and post-treatment to identify the modification of gene expression, using the GeneChip Human U133A array. To validate the microarray analysis, real-time Syber-Green reverse transcriptase–polymerase chain reaction (PCR) of five target genes was performed in all patients. **Results:** After *B. clausii* administration, a total of 158 and 265 genes were up and down-regulated, respectively. Quantitative PCR confirmed the microarray data. *B. clausii* mainly affected the expression of genes involved in immune response and inflammation, apoptosis and cell growth, cell differentiation, cell–cell signalling, cell adhesion, signal transcription and transduction. **Conclusions:** Our data represent the first global analysis of *B. clausii* effects on

the gene expression profile in normal intestine. Several genes are up or down-regulated by *B. clausii* administration, including those involved in the carcinogenic and immunologic processes of the GI tract. Future studies, involving a larger number of patients are now needed to verify those findings in GI cancer.

A-27

The effect of 14 days supplementation with Yakult probiotic on circulating hormonal, leukocyte, and cytokine responses to prolonged cycling in man

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Introduction: Prolonged intense exercise has been associated with a transient fall in immune function that is associated with elevated levels of anti-inflammatory stress hormones and cytokines. We investigated the effects of 2 weeks of daily oral supplementation of Yakult on immunoendocrine responses to a bout of prolonged cycling. **Methods:** 5 trained male cyclists participated in a randomised, double blind, placebo-controlled, crossover design study (with 2 weeks washout) of Yakult (PRO) or Placebo (PLA), 130 ml/day, for 2 weeks followed by a 2.5-hour cycle ergometer exercise trial at 60% of aerobic capacity. Saliva (for IgA) and venous blood samples were taken before exercise, immediately post-exercise, and 1 h post-exercise for blood leukocytes and lymphocyte subsets (FACScan, CELLQUEST software), neutrophil function and plasma hormones and cytokines. Data were analysed using 2-way ANOVA. **Results:** Total leukocytes, lymphocytes, CD3+CD8+ and CD3-CD16+CD56+ cells and plasma hGH, IL-6 and IL-10 all increased post-exercise to a similar extent on the PRO and PLA trials. Post-exercise blood neutrophil counts and plasma ACTH were higher in the PRO trial compared with PLA (both $P<0.05$). CD3+CD4+ cells and the CD4+/CD8+ ratio were higher on the PRO trial (main effect of Trial, both $P=0.01$). No interaction effects were observed for neutrophil function measures. Saliva IgA concentration tended to be higher on PRO than PLA ($P=0.06$). **Conclusion:** 2 weeks supplementation with Yakult had little to no effect on the immunoendocrine response to prolonged exercise and did not attenuate the post-exercise depression of neutrophil function. However, potential benefits included an increased circulating CD4+ cells and CD4+/CD8+ ratio which was evident both at rest and post-exercise. This could be interpreted as a potentially beneficial effect of Yakult supplementation for physically active individuals as CD4+ cells fall transiently following acute bouts of exercise and the CD4+/CD8+ ratio falls during prolonged periods of heavy training. A large scale trial is needed to confirm the indication from the salivary IgA data, that Yakult supplementation may boost mucosal IgA.

A-28

Determination of the genotoxic activity in faecal human water: A study using the alkaline comet assay

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Introduction: Epidemiological studies have shown an inverse association between dietary intake of whole grains and the risk of cancer. The interest in the effects of food ingredients in the aetiology of colon cancer has increased in recent years and two ways to influence carcinogenesis have been hypothesised. Certain dietary constituents or their metabolic products by colonic bacteria may provoke neoplastic changes. Other food ingredients, however, exert chemopreventive properties and one of the proposed mechanisms is that by shifting the balance in the gut microbiota genotoxic species from colonic bacteria metabolism decrease. Faecal water genotoxicity is thought to reflect the carcinogenic burden in the gut and can be used to monitor changes due to dietary intervention. In the present study the effect of consumption of whole grain wheat compared to wheat bran on faecal water genotoxicity was assessed. **Methods:** A placebo-controlled, double-blinded human feeding study was carried out in 31 healthy volunteers. The potential of human faecal water to induce DNA strand breaks in HT29 was screened by the single-cell gel electrophoresis (alkaline version of the "Comet assay"). The fluorescence intensity of the tail of the comet images was measured. **Results:** Faecal water genotoxicity varied greatly between volunteers at the beginning of the study ranging from 6.3% tail intensity to 66.1% tail intensity (CV: 74.4%). Neither WG nor WB significantly influenced the potential of faecal water to induce strand breaks. However, for the quartile of volunteers who had the highest genotoxic activity at baseline, there was a reduction in the faecal water genotoxicity after intervention with both cereals. **Conclusion:** The decrease of genotoxic activity in volunteers with high baseline values might be due either to the observed changes in faecal microbiota or to an increase of antioxidants such as ferulic acid after cereal intake.

A-29

Winter illness and probiotic use – a feasibility study in an occupational setting

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Introduction: There is some evidence for an effect of regular use of *Lactobacillus* probiotic products in reducing sickness absence in an occupational setting (Tubelius *et al* 2005). In preparation for a large-scale placebo-controlled trial, this pilot feasibility study was run from February to April 2007 in a postal industry setting. **Methods:** Employees were invited to participate in a non-blind, non-placebo controlled trial with a single portion of Yakult (choosing either standard or sugar-reduced product) taken daily for 12 weeks. Following provision of written consent, the subjects completed a questionnaire about health, bowel function and winter illnesses (frequency and duration), then commenced drinking a daily bottle of Yakult, containing 6.5 x 10^9 *Lactobacillus casei* Shirota. After 4 and 12 weeks the questionnaire was completed again. The organisation's human resources department provided sickness absence data in coded form, for participating subjects and a control group of non participating employees, for the study period and for the same period in the previous year (2006). Information about cause of absence was also provided. The study was approved by the research ethics committee of King's College London. **Results:** Eighty employees enrolled but only 21 were fully compliant throughout the 12 week period and completed all questionnaire responses. Responses to the questionnaire showed that the average number of respiratory ailments reported in the fully compliant group was 5.1 at the beginning, 2.7 at week 4 and 3 at week 12 ($p < 0.022$, ANOVA). Reported duration of winter illnesses in the fully compliant group fell from 3.5 days at the beginning to 3 days at week 4, to 2 days at week 12. There were fewer numbers of days absent during the 2007 probiotic treatment period (91 days absent) compared to the number of days absent in the same period in the previous year, 2006, for the same subjects (262 days absent). Among the non-participating employees the number of days absent in the same periods was similar (2007: 137 days of absence, 2006: 126 days of absence). However, limiting these to absences to winter illness and infections among the fully compliant sub-group gave an insufficient number of episodes for statistical comparison and no clear trend. **Conclusion:** There was a clear indication of improvement in gut function of the participants over the course of the trial: the number of complaints relating to constipation was reduced from 12 at the beginning to 3 after 12 weeks; an IBS score was reduced by half during the study. The historical reporting of winter illness episodes suggested a potential improvement during treatment but comparison with nationally reported data for disease frequency during the same period showed that the trial followed the expected seasonal trend. The incidence of cold episodes, however, was lower that than reported for the UK. The relatively low number of

subjects resulted in a lack of power to demonstrate any true difference and seasonal trends in winter illness emphasise the need for a placebo-controlled trial with much larger subject numbers

A-30

Probiotic milk drink – Benefit for periodontal health?

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Introduction: Gingivitis is an inflammatory periodontal disease and can initiate irreversible destruction of periodontal structures. Experimental gingivitis is a well-established model for studying different products and procedures. The aim of this study was to verify the beneficial effect of a probiotic drink on experimental gingivitis in an immunocompetent host.
Methods: 50 male and female volunteers (mean age 24.4 years) were introduced in the study with a 4 days plaque accumulation to induce an experimental gingivitis. 8 weeks before starting the experimental gingivitis, the test group (n=25) drunk 65 ml probiotic drink (Yakult®, Honsha Co. Tokyo, Japan) daily, the control group (n=25) did not change alimentation. Plaque index (PI, Turesky) and papilla bleeding index (PBI) were recorded at baseline (1), before (2) and after (3) experimental gingivitis. At the same times crevicular fluid samples were collected for determination of activities of myeloperoxidase (MPO), neutrophil elastase (NE) and of the concentration of MMP3. Friedman/Wilcoxon tests and U-test were used for statistical analysis. **Results:** The results were as follows: PI Y: 1-0.76, 2-0.98, 3-2.52 ($p<0.001$), C: 1-0.68, 2-0.82, 3-2.14 ($p<0.001$); PBI Y:1-0.67, 2-0.99, 3-1.17 ($p<0.001$), C:1-0.80, 2-0.89, 3-1.12 ($p<0.001$); MPO Y:1-2.66 μU, 2-4.43, 3-3.58 (n.s.), C: 1-3.83, 2-4.92, 3-8.50 ($p=0.024$); NE Y:1-0.0043 μU, 2-0.0011, 3-0.0199 ($p<0.001$), C: 1-0.0054, 2-0.0144, 3-0.0430 ($p<0.001$); MMP3 Y: 1-4.366 ng, 2-0.327, 3-0.540 ($p<0.001$), C: 1-1.655, 2-0.417, 3-0.604 (n.s.). After experimental gingivitis PI was significantly higher in the test group ($p=0.001$). MPO was significantly lower in the test group ($p=0.014$), elastase had a tendency ($p=0.064$). MMP3 was significantly different between the groups at (1) and (2) ($p=0.001$, 0.016) but not at (3). **Conclusions:** Within the limits of the study it can be concluded, that even at higher plaque challenge the inflammatory response seems to be lower in the test group. It might be that the probiotic product diminishes experimentally induced gingivitis.

A-31

Effect of a probiotic mix on the microbiota and their metabolic activity in healthy volunteers and patients with atopic dermatitis

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Introduction: Effects of probiotics are mainly based on their ability to modulate the composition and/or the metabolic activity of the intestinal microbiota. Differences in the composition of the microbiota between patients with atopic dermatitis (AD) and healthy subjects have been reported. An investigation was performed to study the influence of a probiotic mix consisting of *Lactobacillus paracasei* LPC37, *Lactobacillus acidophilus* 74-2 and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 on the microbiota of healthy subjects and patients with AD. The concentrations of short-chain fatty acids and the faecal pH were determined as biomarkers for the metabolic activity. **Study design:** A placebo-controlled and cross-over study was conducted. The 15 healthy adults and the 15 patients with AD consumed 2 x 100 ml/d of a probiotic or a placebo drink for 8 weeks. A wash-out period of 2 weeks was interconnected before the intervention was crossed. Stool samples were collected at the end of each period. **Methods:** The total number of bacteria and the faecal levels of the administered species were determined by quantitative real-time PCR. The concentrations of short-chain fatty acids were measured by gas chromatography. **Results:** The total number of bacteria remained stable during the study. Faecal proportions of *L. paracasei* and *B. lactis* increased significantly ($P < 0.05$) after probiotic intervention, while the proportion of *L. acidophilus* was just marginally affected. Relative proportions of the three species did not differ significantly between healthy subjects and patients with AD. No significant changes were observed for the faecal concentrations of short-chain fatty acids. However, in patients with AD faecal pH decreased significantly after probiotic intervention. **Conclusion:** The results suggest that *Lb. paracasei* LPC 37 and *Bf. lactis* 420 are able to colonise transiently the intestine of healthy adults and patients with AD. Despite the probiotic mix has no effect on the concentrations of short-chain fatty acids, the decreased faecal pH indicates that other metabolic end products might be affected in patients with AD.

A-32

Hydrogen-breath test evaluation of the alterations in the intestinal anaerobic saccharolytic fermentation after the use of a synbiotic

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Aim: To monitor the intestinal anaerobic saccharolytic fermentation using hydrogen-breath test (HBT) before and after the application of the synbiotic Actiflora, containing *Lactobacillus bulgaricus*, *Streptococcus thermophillus*, pectin, inulin, etc. in the healthy Bulgarian population. In humans the intestinal bacterial anaerobic saccharolytic fermentation is the only source of hydrogen in the expired air. **Methods**: Fifty-four Bulgarians without severe organic disease (26 m / 28 f; mean age 32.9 ± 10.18) were tested. Thirty-seven individuals had no complaints. Seventeen persons had some long-term functional abdominal complaints, slightly impairing their daily activity: 6 persons had obstipation, 5 some food intolerance, 5 functional dyspepsia, 1 had both functional dyspepsia and obstipation. All persons received orally synbiotic 3 times daily at a single dosage of 90 billion CFU for 7 days. A clinical assessment and a HBT were followed-up.
Results: Initially 18 persons had increased HBT values (9 with abdominal complaints), 11 decreased (1 with complaints) and 25 (7 with complaints) normal. After 7-day course 12/18 with higher HBT showed normal values; 4/11 with decreased HBT had an increase; 12/25 with normal HBT showed an increase and only 1/25 had a decrease. A clinical improvement was established in 16/17 persons with complaints. The adverse effects (transient 1-day diarrhea in 4, flatus in 3) were negligible. **Conclusions**: The 7-day course with Actiflora was well tolerated and contributed to the regulation of the intestinal bacterial ecosystem, measured by HBT; the slight functional abdominal complaints were improved. The intake of Actiflora was helpful both in low and high levels of expired hydrogen.

A-33

A four-week course of *Lactobacillus casei* Shirota (Yakult™) supplementation significantly improves phagocytic capacity of neutrophil granulocytes in patients with alcoholic cirrhosis

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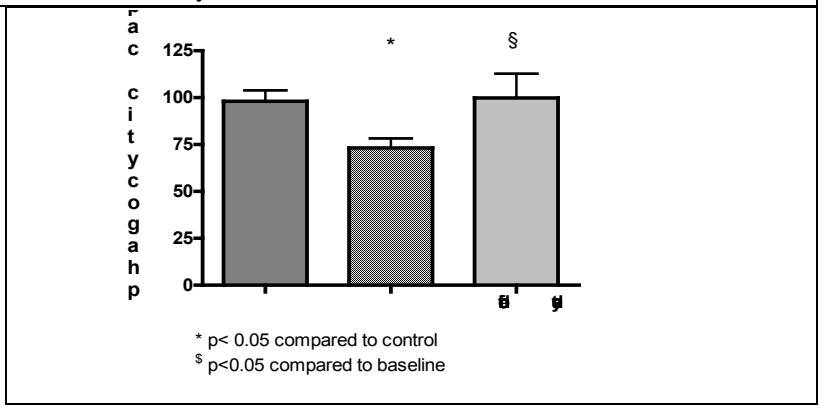
Introduction: Patients with alcoholic liver disease have an increased susceptibility to infections which is associated with a dysfunction of neutrophil granulocytes. We have previously shown that this neutrophil dysfunction (decreased phagocytic capacity and increased resting oxidative burst) is likely to be caused by endotoxin. Endotoxaemia in patients with alcoholic liver disease is likely to be due to increased gut permeability and an altered gut flora. Probiotics are known to be able to alter the composition of the gut flora and decreasing gram-negative organisms. Therefore we hypothesized that probiotic treatment in patients with alcoholic liver disease might decrease endotoxaemia and restore neutrophil function. **Patients and Methods:** Patients with alcoholic cirrhosis without any evidence of infection were included in this study. They received food supplementation with Yakult (65 ml containing *Lactobacillus casei* Shirota at a concentration of 10^8/ml) 3 times a day for 4 weeks. Neutrophil function (oxidative burst and phagocytosis) was determined by FACS-analysis; endotoxin was measured by a Limulus Amoebocyte Lysate assay; cytokines were analysed by ELISA and routine biochemistry and clinical data were recorded at the beginning and after 28 days of treatment with Yakult. Baseline data were compared to a control group of age and sex-matched healthy volunteers. **Results:** Twelve patients (8 male, mean age 53.9±3.4) were included; 10 completed the study. Baseline neutrophil function showed a significant decrease in phagocytic capacity in patients (73% versus 98%, $p<0.05$,

Figure 1). During treatment with Yakult no adverse events were noted. Overall compliance to the study medication was 80%. No changes in clinical and biochemical markers of inflammation and liver disease were found during the study. Neutrophil phagocytic capacity at the end of the study was significantly higher when compared to baseline (73% versus 100%, $p<0.05$, Figure 1). Endotoxin levels were under the limit of detection throughout the study. **Conclusion:** Treatment with 3 doses of Yakult a day for four weeks is safe in patients with alcoholic cirrhosis. Compliance to the study drug is excellent. Yakult improves the phagocytic capacity of neutrophils in these patients, possibly by altering gut flora and decreasing endotoxaemia. Our results justify a randomized, sufficiently powered, controlled study of Yakult in alcoholic liver disease.



A-34

Metabolic phenotype and metagenomic (gut microbial) analysis of the zucker rat model of type II diabetes

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Introduction: Metabolic diseases are increasing in prevalence and need to be investigated further to acquire a deeper understanding. Insulin resistance is one such condition and here was studied in relation to the gut microflora as the microbiome has a huge effect on the state of health in mammals. To achieve this, an animal model was used. The *fa/fa* strain of Zucker rat is characterized by its leptin deficiency resulting in early onset obesity and is a good animal model for study of insulin resistance and the relationship with gut microflora. **Methods:** Three strains of 10 week old male Zucker rats; *fa/fa* (obese) n=8, *fa*/- (lean) n=8 and -/- (lean) n=8, were metabolically phenotyped using high resolution NMR spectroscopy of urine samples. In addition, a metagenomic analysis of the gut microbiota was performed on faecal samples using DGGE. After the initial screening of bacterial bands in the DGGE profile, an O-PLS analysis was completed using NMR data as the X matrix

and an important bacterial band as the Y matrix. For further identification of interactions between metabolites and bacteria, O2 PLS analysis was undertaken. **Results:** This gives an impressive look into which bacteria interact or are involved in metabolic pathways. Each strain of rat has very different gut microfloral profiles and investigating the interactions with metabolites has proved interesting. Initial results have indicated that hippurate and citrate have links to several microfloral species. **Conclusion:** This new insight gives the opportunity to perform informed probiotic interventions for optimization of gut health.