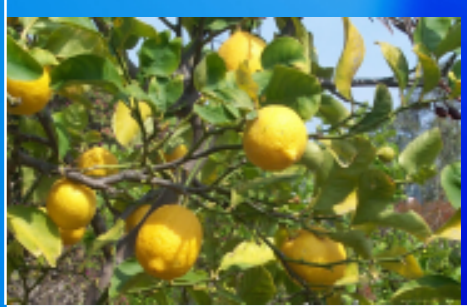
ESPOO 2003 VTT SYMPOSIUM 226

The Food, GI-tract Functionality and Human Health Cluster

PROEUHEALTH



Taormina, Italy

Workshop 2
March 3–5
2003

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The Food, GI-tract Functionality and Human Health Cluster

PROEUHEALTH

Abstracts and posters

2nd Workshop

Taormina, Italy

3–5 March 2003

Edited by

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Tiina Mattila-Sandholm

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Preface

Probiotics, prebiotics, and synbiotics aimed at improving intestinal health currently represent the largest segment of the functional foods market in Europe, Japan and Australia. Evidence continues to emerge demonstrating that these ingredients have the potential to improve human health in specific intestinal disorders. The European Commission, through its 5th Framework Programme, is presently investing a substantial research effort in the intestinal microbiota, their interaction with its host and methods of manipulating their composition and activity for the improvement of human health.

The Food, GI-tract Functionality and Human Health (PROEUHEALTH) Cluster (from 2001 to 2005) brings together eight complementary, multicentre inter-disciplinary research projects. All have the common aim of improving the health and quality of life of European consumers. The collaboration involves 64 different research groups from 16 different European countries. The research results from the cluster are disseminated through annual workshops the first of which was held in Saariselkä, Finland in February 2002. Our Taormina event is the second in the series of workshops, and involves the activities of three different platforms: a science, an industry and a consumer platform.

The 2nd PROEUHEALTH Workshop in beautiful Taormina will also serve as an arena for combining many other European research efforts on health foods. The EU Commission, in conjunction with the PROEUHEALTH Cluster, has established a series of other projects, which are ongoing in the health and food area and the audience has the priviledge of enjoying many prospects of future health foods research.

Wishing all attendants stimulating days within our networks!

Tiina Mattila-Sandholm
PROEUHEALTH Cluster Coordinator

Programme

SUNDAY, March 2

Closed meetings of the PROEUHEALTH projects 1 - 8.
Only PROEUHEALTH Cluster project partners can participate in the meetings.

MONDAY, March 3

8.00 - 9.00 Registration

9.00 - 9.15 Conference opening
Dr. Liam Breslin, European Commission, Belgium

Session 1. The PROEUHEALTH Cluster
Chair: *Dr. Jürgen Lucas, European Commission, Belgium*

9.15 - 9.30 Structure and function of the PROEUHEALTH cluster
Prof. Tiina Mattila-Sandholm, VTT, Finland

9.30 - 10.10 MICROBE DIAGNOSTICS: Who's who in the intestinal microbiota
Prof. Michael Blaut,
German Institute of Human Nutrition, Germany

10.10 - 10.50 DEPROHEALTH: Second generation probiotics
Dr. Annick Mercenier, Nestlé Research Center, Switzerland

10.50 - 11.10 Coffee and refreshments

11.10 - 11.50 PROGID: Probiotic clinical trials in IBD patients
Prof. Fergus Shanahan, University College Cork, Ireland

11.50 - 12.30 CROWNALIFE: Probiotics for the elderly
Dr. Joël Doré, INRA, France

12.30 - 13.10 PROTECH: Nutritional enhancement of probiotics and prebiotics
Prof. Dietrich Knorr, TU Berlin Food Technology, Germany

13.10 - 14.30 Lunch and posters

Chair: *Prof. Charlie Daly, Ireland*

14.30 - 15.10 PROPATH: Probiotics and prebiotics against pathogenic microbes in the gut
Prof. Luc de Vuyst, Vrije Universiteit Brussels, Belgium

15.10 - 15.50 EU & MICROFUNCTION: Interactions between gut microbiota and the host
Prof. Glenn Gibson, University of Reading, United Kingdom

15.50 - 16.30 PROSAFE: Biosafety issues
Prof. Herman Goossens, University of Antwerp, Belgium

16.30 - 17.00 Coffee

17.00 - 18.30 **Debate No. 1. Probiotics: dead vs. alive**
Moderators:
Prof. Willem de Vos, Wageningen University, the Netherlands,
Prof. Glenn Gibson, University of Reading, United Kingdom and
Prof. Seppo Salminen, University of Turku, Finland

TUESDAY, March 4

Session 2. Cancer and ageing: roles of food in cause and prevention
Chair: *Lorenzo Morelli, Istituto di Microbiologia UCSC, Italy*

9.00 - 9.45 Food and gut – the medical perspective
Prof. Philippe Marteau,
Hôpital Européen Georges Pompidou, France

9.45 - 10.30 Synbiotics and cancer prevention in humans
Dr. Jan van Loo, ORAFTI, Belgium

10.30 - 11.00 Functional foods against colon cancer
Dr. Ben van Ommen,
TNO Nutrition and Food Research, the Netherlands

11.00 - 11.45 Antioxidants and colon cancer
Prof. Gary Williamson,
Institute of Food Research, United Kingdom

11.45 - 12.30 Diet and colon cancer – a mechanistic approach with ApcMin mouse
Dr. Marja Mutanen, University of Helsinki, Finland

12.30 - 14.00 Lunch & posters

14.00 - 16.00 **Debate No. 2. Food and ageing population: What can we do?**
Moderators:
Prof. Kaisa Poutanen, VTT, Finland,
Prof. Ian Rowland, University of Ulster, Northern Ireland,
Dr. Sian Astley, Institute of Food Research, United Kingdom and
Dr. Edmond Rock, INRA, France

WEDNESDAY, March 5

Session 3. Consumer issues & Foods for specific population groups
Chair: *Dr. Maria Saarela, VTT, Finland*

8.30 - 9.00 Health as a quality factor in food products
Prof. Klaus Grunert,
MAPP Centre, The Aarhus School of Business, Denmark

9.00 - 9.35 Food and prevention of osteoporosis
Dr. Kevin Cashman, University College Cork, Ireland

9.35 - 10.10 Multicentre study of eating disorders and obesity
Dr. David Collier, University of London, United Kingdom

10.10 - 10.45 Coffee and refreshments

10.45 - 11.20 Consumers and health: getting the message across
Dr. Liisa Lähteenmäki, VTT, Finland

11.20 - 12.00 Factors influencing consumer choice of healthy foods
Prof. Patrick Morrissey, University College Cork, Ireland

12.00 - 13.30 Lunch and closing address

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ABSTRACTS

Food, GI-tract functionality and human health cluster, PROEUHEALTH

T. Mattila-Sandholm

VTT Biotechnology, Finland

The Food, GI-tract Functionality and Human Health Cluster brings together 64 research partners from 16 European countries in the quest to obtain greater knowledge of the role of the intestinal microbiota in human health and disease and to develop new functional foods and therapies. The reseach will run over 5 years starting February 2001 and is subsidised by the European Commission's 5th Framework, Quality of Life and Management of Living Resources Programme.

The cluster aims to provide:

- A clearer understanding of the relationships between food, intestinal bacteria and human health and disease.
- New molecular research tools for studying the composition and activity of the intestinal microbiota.
- New therapeutic and prophylactic treatments for intestinal infections, chronic intestinal diseases, and for healthy ageing.
- A molecular understanding of immune modulation by probiotic bacteria and testing of probiotics as vaccine delivery vehicles.
- Biosafety evaluation of probiotics for human consumption
- Commercial opportunities for food and pharmaceutical industries.

Eight complementary multicentre European projects are included in the cluster. They cover all aspects in the development of new probiotic foods, from designing molecular tools to study the ecology of the intestinal microbiota, to understanding mechanisms of bacterium-host interactions, providing solutions to food technology issues, and finally to conducting human clinical trials to assess efficacy in preventing or treating disease.

The research innovations produced by the cluster will be disseminated to target audiences through three platforms:

- The Science Platform will provide an internal dissemination and networking platform for the cluster.

- The Industry Platform will enable the cluster to disseminate its research innovations to probiotic industries throughout Europe and the world and maximise the potential for commercial exploitation of results from the cluster's research.
- The Consumer Platform will provide information to consumers about the cluster and its innovations in an appropriately tailored format, ensuring that the general public is kept informed and benefit from the research.

MICROBE DIAGNOSTICS: Who's who in the intestinal microbiota

M. Blaut

Department of Gastrointestinal Microbiology,
German Institute of Human Nutrition, Germany

The bacterial community in the human intestinal tract affects the host with respect to gastrointestinal function, health and well-being. In spite of the importance of the gut microbiota for human health, the targeted manipulation of the gut microbiota by dietary means is still in its infancy. This is partly due to the complexity of the interactions between gut microorganisms, host and diet, and partly to the variability between human individuals in respect of the composition of the intestinal microbiota. To improve this situation it is necessary to identify the key parameters that influence the composition and the activity of the intestinal microbiota. This information can only be gained with reliable methods.

This project aims to facilitate and improve human gut microflora monitoring with molecular methods, to understand antagonistic and synergistic interactions of the intestinal microbiota and to find links between major dysfunctions and the intestinal flora. This requires a more complete coverage of the microbial diversity and the establishment of a fully comprehensive comparative 16S rRNA database for the human gut microbiota. So far, 1536 rDNA clones derived from 5 healthy adults,1 baby and 2 patients suffering from inflammatory bowel disease have been analysed. These and previous data indicate that the vast majority of organisms in the gut have eluded scientific description. To know the role and the catalytic capability of as many of these undescribed bacteria, more than 100 bacteria have been isolated from human faeces. Twenty novel species and over 10 novel genera of gut bacteria have been discovered. Owing to the improved database of ribosomal RNA sequences, the design of diagnostic probes has become easier. So far, 16 new oligonucleotide probes targeting gut bacteria at different levels of the phylogenetic hierarchy have been designed in the project. To take full advantage of the developed probes the available methods of culture independent probing are being further improved. The work is directed towards the analysis of high numbers of samples on the basis of 16S rRNA. Flow cytometry has been successfully introduced as a rapid method for the detection of fluorescently labeled gut bacteria. In parallel it has been possible to provide proof of concept for the microbiota array chip.

Since the identification of bacteria is not sufficient to understand synergistic and antagonistic interactions of the intestinal microbiota, the development of molecular methods enabling the monitoring of functional gene expression is another major challenge within the project. Proof of principle for the detection of faecal bacteria catalysing the conversion of fluorogenic substrates has been accomplished. Moreover, the first step in demonstrating in-situ detection of gene expression has been successful.

The methods developed are to be applied to the analysis of human fecal samples in order to generate baseline information on the development of the bacterial community in the intestine and to identify components of the normal bacterial flora that may contribute to the onset of inflammatory bowel disease. Knowledge of these factors can be used to devise specific measures for disease prevention and treatment, such as the use of prebiotics and/or probiotics. Comparison of microfloras of healthy and diseased subjects with the molecular methods developed is expected to lead to the identification of microorganisms indicative of the disease or involved in its aetiology.

DEPROHEALTH: Second generation probiotics

A. Mercenier

Institut Pasteur de Lille, France
Present address: Department of Bioscience, Nestle Research Center, Switzerland

The DEPROHEALTH project aims at developing probiotic strains to be used as novel anti-inflammatory treatments or oral vaccines against *Helicobacter pylori* and rotavirus. It is also evaluating the predictive value of current probiotic screening methods.

During this first year, wild type strains of lactobacilli were analysed for their potential capacity to resist passage through the upper part of the intestinal tract and for their capacity to interact with the host immune system. While certain *Lactobacillus* species seem to be better suited for oral administration, the immunomodulation profile appears to be rather strain specific. This *in vitro* evaluation has been extended by studies conducted in mouse colitis models which confirmed that specific strains exert a beneficial effect on intestinal inflammation. Mutants of *L. plantarum* NCIMB8826 were obtained that synthesise altered lipoteïcoic acids. Four recombinant lactobacilli secreting fair levels of murine IL-10 are being evaluated for their protective effect *in vivo*. The first generation of recombinant strains producing a protective antigen from *Helicobacter pylori* or rotavirus have been obtained and immunisation experiments have started in mice. Two surface proteins of *Lactobacillus crispatus* 247 are subjected to detailed analysis in order to provide food-grade targeting signals for cell surface presentation of therapeutic molecules.

Testing the theory – Probiotics in practice (PROGID)

F. Shanahan

Dept. Medicine, National University of Ireland and Cork University Hospital, Ireland

Analyses of the potential role of probiotics in modern medicine have ranged from dubious, to reserved and enthusiastic. Descriptors such as 'conbiotics', 'snakeoil' and 'bugs for the new millennium' have appeared in the scientific literature. Probiotics are an attractive option because of their relative safety, but optimal probiotic usage remains limited by large gaps in understanding of the normal flora and by several potential clinical challenges and pitfalls. There is a strong rationale for probiotics in patients with inflammatory bowel disease (IBD) but controlled trials are required. At present, worldwide there are over one hundred and fifty trials underway testing new biologic theapies for patients with Crohn's disease. The range of options for patients with ulcerative colitis is more limited. Only one component of the pathogenesis – the host immune response – is targeted by most current therapeutic strategies for IBD.

Notwithstanding remarkable advances, the efficacy of immunomodulatory agents is limited to a subset of patients, requires increased vigilance for toxicity and is associated with considerable cost. A more comprehensive and sustained therapeutic response is likely to require that the environmental contribution to IBD be addressed.

Gastroenterologists have already learned a salutary lesson regarding the importance of resident bacteria and human disease. For decades, the management of peptic ulcer disease consisted of modifying the host response by suppression or neutralisation of gastric acid, but it was only when the contribution of *Helicobacter pylori* to the pathogenesis was discovered that lasting therapy was accomplished. In Crohn’s disease and ulcerative colitis, the relationship between the enteric flora and disease pathogenesis may be more complex and subtle. At present, there are two large trials of probiotic therapy underway in Europe for Crohn's disease and ulcerative colitis. These are required in order to bring the field of probiotics and IBD into the realm of evidence-based medicine. Details of trial design, criteria for strain selection, problems, pitfalls and progress of the trial will be presented.

CROWNALIFE – Functional foods for the elderly

J. Doré[1], M. Blaut[2], R. Rastall[3], I. Rowland[4], A. Cresci[5], E. Norin[6], J. Van Loo[7] and L. Diop[8]

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[2] DifE, Germany
[3] Univ. Reading, UK
[4] Univ. Coleraine, UK
[5] Univ. Camerino, Italy
[6] Karolinska Institutet, Sweden
[7] Orafti, Belgium
[8] Danone Vitapole, France

Advances in science and medicine as well as improved living standards have led to a steady increase in life expectancy throughout Europe such that one third of the population will be over 60 by year 2025. Yet ageing is associated with increased susceptibility to degenerative or infectious diseases. The intestinal microbiota will mediate crucial events towards the protection or alteration of health. It is hence essential and timely that strategies of preventive nutrition aimed at maintaining or improving the quality of life of the ageing population be developed.

CROWNALIFE (1) is a European research and Development project within the PROEUHEATH (2) cluster. Its acronym refers to emphasis on the preservation of the period of independence of the elderly, recognised as the “crown of life”. The project aims at assessing age-related alterations and exploring strategies to restore and maintain a balanced healthy intestinal environment.

Current knowledge on the composition and function of the human intestinal microflora is improving markedly with the use of better suited methodologies. Yet so far the evolution of the intestinal ecosystem with ageing has not been investigated in details. There has been a few reports that putatively protective lactic acid bacteria in general and bifidobacteria in particular may be numerically less represented in the elderly faecal microbiota (3).

Using classical culture techniques, we have isolated lactobacilli and bifidobacteria from the elderly faecal flora (4). These are currently being typed and will be compared with isolates from the adult and infant. Direct molecular assessments of dominant species composition have shown a huge species diversity within the elderly faecal microbiota, many species corresponding to yet non cultured micro-organisms. This constitutes the basis for the validation of

culture-independent, hybridisation-based strategies to assess the composition of the elderly gut microbiota. Combining the latter with conventional and molecular tools to assess functional traits of the elderly intestinal ecosystem related to degenerative diseases (5), we are characterising the intestinal flora of elderly subjects from four countries across Europe in comparison with young adults. Ensuing results will constitute a baseline for the validation of functional-food (prebiotic, probiotic, synbiotic) based strategies aimed at providing health benefits for the elderly.

(1) http://www.crownalife.be
(2) http://proeuhealth.vtt.fi
(3) K. Saunier & J. Doré. Digestive and Liver Disease 3 Suppl 2 (2002) S19–S24.
(4) S. Silvi, M.C. Verdenelli, C. Orpianesi & A. Cresci. Journal of Food Engineering 56 (2003) 195–200.
(5) C.I. R. Gill and I.R. Rowland. British Journal of Nutrition 88 Suppl 1 (2002) S73–S87.

PROTECH: Nutritional enhancement of probiotics and prebiotics

D. Knorr[1] and P. Ross[2]

[1] Berlin University of Technology, Germany
[2] Dairy Products Research Centre, Ireland

Main research emphasis ot the project for the enhancement of probiotics is directed towards improvement of microbial resistance during freezing or hot air drying for preservation of starter cultures. Further, better understanding of microbial stress responses, the identification of biomarkers using proteomic tools and the increase of stress resistance of probiotics during their fermentation, storage or consumption is sought.

Improvement of prebiotics is performed by physical and biochemical means and probiotic-prebiotic interactions and their performance is being evaluated in feeding trials.

Key emphasis of this presentation will be on the improvement of microbial viability and stability and on the generation of productive and functional probiotics for and during processing of probiotic foods.

Probiotics against pathogenic microbes in the gastrointestinal tract

L. De Vuyst, L. Avonts, E. Makras, H. Holo, A. Servin, P. Managkoudakis, E. Tsakalidou, G. Kalantzopoulos, D. Sgouras, A. Mentis, L. Savu, S. Cappelle and I. Nes

Department of Applied Biological Sciences, Vrije Universiteit Brussel, Belgium

Recently, a lot of attention has been paid to the health-promoting properties of certain lactic acid bacterium strains, some of which have been promoted as probiotics. Examples are strains of lactobacilli and bifidobacteria that are utilized in yoghurt production and milk fermentation, or that are supplied as pharmaceutical preparations. Of particular importance is the potential of probiotics to reinforce mucosal defence, especially at the gastric and small bowel levels, for instance to prevent or combat infections caused by *Helicobacter pylori* and *Salmonella enterica* serovar Typhimurium, respectively. Possible underlying mechanisms of inhibition are the interspecies competition for substrates, the prevention of pathogen adherence to epithelial cells, the production of antimicrobial substances by a resident or transient microflora, and/or an enhancement of the host immune response against pathogens. The PROPATH project aims to isolate and characterise the responsible antimicrobials to combat the Gram-negative pathogens mentioned above.

It is well known that probiotic lactic acid bacteria, in particular strains of species of the *L. acidophilus* and *L. casei* groups as well as bifidobacteria, contribute to a stable gut microflora. They may also prevent the colonisation of pathogenic bacteria. This in part may be ascribed to the production of organic acids, resulting in a decrease of the local pH. Anti-*H. pylori* activity most probably results from such a pH alteration. *In vitro* experiments indicate the killing of *H. pylori* cells by lactic and acetic acids produced by these strains. Moreover, undissociated acetic acid most probably plays a pivotal role in the anti-infectious activity of bifidobacteria. On the other hand, several *Lactobacillus* strains produce antibacterial, acidic, low-molecular-mass, non-proteinaceous heat-stable compounds with an inhibitory spectrum including both Gram-positive and Gram-negative bacteria. For instance, the widespread probiotic *L. johnsonii* La1 strain inhibits cell adhesion and cell invasion by enterovirulent bacteria both *in vitro* and *in vivo* (in mice), including both Gram-positive (e.g. *Listeria monocytogenes*) and Gram-negative (e.g. *Salmonella typhimurium*) bacteria. However, the nature of the antimicrobial component(s) secreted by *L. johnsonii* La1 remains to be determined. Recent experiments indicate that supernatant of *L. johnsonii* La1, as well as an extract of it, inhibits the growth of *H. pylori in vitro*.

This inhibitory activity was heat resistant, protease-sensitive, and independent of the presence of lactic acid. Therefore, the overall inhibition of gastrointestinal pathogens by probiotics may be due to a synergistic action of organic acids, proteinaceous substances, and other hitherto unknown inhibitory agents.

Update on EU and MICROFUNCTION project (Interactions between gut microbiota and the host)

G. Gibson[1], A. Ouwehand[2], W. de Vos[3], G. Molin[4], M. Mikelsaar[5], J. van Loo[6], D. Adawi[7] and P. Conway[8]

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[2] University of Turku, Finland
[3] Wageningen University, The Netherlands
[4] Lund University, Sweden
[5] University of Tartu, Estonia
[6] Tiense Suikerraffinaderij NV, Belgium
[7] Probi AB, Sweden
[8] University New South Wales, Australia

This project aims to determine, through mechanisms of effect, the influence that probiotics, prebiotics and synbiotics can exert on gastrointestinal health. The study will investigate host-microbe interactions and the effects of functional foods. The influence of probiotics, prebiotics and synbiotics on the gut microbiota and their interactions will be studied using quantitative and qualitative analytical methods and biomarkers. Specific physiological functions addressed in this project include microbial diversity, gut microbial fermentation and its modulation, bacterial translocation and the colonic mucosal barrier. The effects of exogenous microorganisms (probiotics and synbiotics) will be determined in each of these areas. Additional work will develop a safety protocol for probiotics and synbiotics to assess the acceptability of these functional foods.

The project consists of 6 scientific workpackages designed to investigate interactions between probiotic and prebiotic agents on the host (human) gastrointestinal ecosystem. Workpackages WP1 and WP2 deal with the fermentation of prebiotics and generation of synbiotics (which are combinations of pro- and prebiotics). As bacterial translocation is an important pathological phenomenon which may predispose to various clinical conditions, WP3 will investigate the positive, prophylactic, aspects that probiotic and prebiotic intake may provide. The premise behind WP4 is that cross communication occurs between gut microorganisms and the host, and will use molecular technology to elucidate this. We also propose to assess the safety aspects of probiotic, prebiotic and synbiotic intake through the development of model systems. In WP6 a human volunteer trial is proposed. Selected health indices will be assessed in response to synbiotic intake and will include the use of molecular probing strategies for diagnostic bacteriology. To ensure good dissemination activities

and close partner contact, a final workpackage will cover such issues as collaboration, website formation, reporting and other publicity.

To realise the full potential of gut microflora management through the diet, there is now a requirement to identify the realistic health outcomes associated with probiotic and prebiotic intake and, importantly, give rigorous attention towards determining their mechanisms of effect. The project will therefore produce information on:

- Markers and indicators of dietary exposure to probiotics, prebiotics and synbiotics
- Derive information leading towards improved nutritional and health status through dietary factors that affect the human gut flora
- Study the mucosal interactions with gut bacteria and bioactive molecules
- Exploit molecular approaches to reliably identify and track gut microbial species
- Use model systems to estimate microbial interactions at various sites in the gut
- Develop reliable quantitative tools to assess host-microbe interactions
- A human volunteer trial will also be conducted which will use data and technologies developed throughout the project to investigate the effects of synbiotics on selected health indices such as gut function and microbiology, blood lipids and toxins in urine.

The project will have ran for one year by the time of workshop 2 and an update on all workpackages will be given.

Biosafety evaluation of probiotic lactic acid bateria used for human consumption (PROSAFE): First Year's Results

H. Goossens[1,7], V. Vankerckhoven[1], C. Vael[1], T. Van Autgaerden[1], M. Vancanneyt[2], G. Huys[2], R. Temerman[2], J. Swings[2], I. Klare[3], C. Konstabel[3], G. Werner[3], W. Witte[3], M-B. Romond[4], M-F. Odou[4], C. Mullié[4], P. Moreillon[5], J. Knol[6], A. Mensink[6] and E. Wiertz[7]

[1] University of Antwerp, Belgium
[2] University of Ghent, Belgium
[3] Robert Koch Institute, Germany
[4] University of Lille, France
[5] University of Lausanne, Switzerland
[6] NUMICO research, the Netherlands
[7] Leiden University Medical Centre, the Netherlands

Probiotic bacteria, mainly lactic acid bacteria (LAB) have been considered safe for human consumption. However, recent reports of clinical infection and the spread of resistance genes have caused concern of safety. This 4-year project aims to assess the biosafety of LAB and the results of the first year will be presented.

A large strain collection (n = 600) was established in Work package 1 (WP) consisting of clinical (n = 230), faecal (n = 239) and probiotic (n = 131) strains. The clinical isolates originate from invasive infections, including sepsis, endocarditis, meningitis, abscesses and wound infections. They belong to the genera *Bifidobacterium* (n = 6), *Lactobacillus* (n = 125), *Enterococcus* (n = 81), *Lactococcus* (n = 4), *Pediococcus* (n = 13) or *Leuconostoc* (n = 1). The faecal strains were isolated from adults and children and comprise bifidobacteria (n = 76), lactobacilli (n = 108), enterococci (n = 34), pediococci (n = 20) and Weisella (n = 1). Thusfar 23 European companies are participating in the PROSAFE project by providing their strains, which has resulted in a probiotic collection of the following genera: bifidobacteria (n = 21), lactobacilli (n = 76), enterococci (n = 16), lactococci (n = 5), pediococci (n = 3) and propionibacteria, streptococci and yet unidentified strains (n = 10).

The main goal of WP2 is to obtain a reliable identification of all probiotic and human LAB strains collected in the course of WP1. For the speciation of lactobacilli and enterococci, a pre-existing reference database generated from genotypic AFLP (Amplified Fragment Length Polymorphism) fingerprints was

further extended and, since the start of the project, routinely applied on half of the lactobacilli. For the purpose of identifying bifidobacteria, a taxonomic reference framework was first constructed from BOX-PCR fingerprints and already applied on 65% of the isolates. Strains that remained unidentified (7% of total) are classified using the standardized SDS-PAGE of whole-cell proteins technique and/or 16S rDNA sequencing. At present, our data demonstrate that the identity of the strains as received was not confirmed for 9% and 6% of the human and probiotic isolates, respectively. AFLP and rep-PCR furthermore allow to search for intra-species genomic groups which might be correlated with source (clinical/non-clinical), geographic origin and safety aspects. PFGE of macrorestriction fragments will be used for detection of clonality among probiotic and human isolates. A project database containing all descriptive taxonomic and typing data is compiled using the specialized Bionumerics software.

In WP3 antibiotic susceptibility testing of LAB was performed by microbroth dilution test with cation-adjusted Mueller-Hinton broth (enterococci) and by a mixture of 10% deMan-Rogosa-Sharp (MRS) broth plus 90% Isosensitest broth (lactobacilli, lactococci, pediococci). Sixteen antibiotics were tested for activity against 118 enterococci (117 *E. faecium*, 7 *E. faecalis*; in the majority of clinical origin). Resistance was observed in *E. faecium* to penicillin and ampicillin (each 31.5%), gentamicin and streptomycin (high level; 5.4% and 37.8%, resp.), erythromycin (56.8%), quinupristin/dalfopristin (4.5%), oxytetracycline (46.8%), chloramphenicol (0.9%), vancomycin (15.3%), teicoplanin (11.7%), fusidic acid (20.7%), trimethoprim and trimethoprim/sulfamethoxazole (each 9.0%) but not in probiotic strains (only one probiotic strain with erythromycin resistance needs further investigations). The results were underlined by PCR for the responsible resistance genes. Additionally we standardised susceptibility testing of lactobacilli, lactococci, pediococci and started with testing these LAB.

In WP4 virulence properties of LAB were investigated, and a multiplex PCR system was developed for the rapid detection of four of the virulence factors known in *Enterococcus faecalis*: gelatinase (gelE), enterococcal surface protein (esp), cytolysine (cylA) and aggregation substance (asa1). Previously published primers were used for the detection of asa1. For gelE, cylA and esp new primer pairs were chosen. The esp primers detected the gene in both *E. faecalis* and *E. faecium*. An additional PCR was also developed to detect a new potential virulence gene, *hyaluronidase* (hyl), in *E. faecium*.

Food and gut: The medical perspective

P. Marteau

Gastroenterology unit, European hospital Georges Pompidou, France

Food plays a major role in the modulation of intestinal physiology and in many intestinal diseases including irritable bowel syndrome, inflammatory bowel disease and colon cancer. I will discuss the three following aspects:

1. What is known on the role of food components in IBS, IBD and colon cancer?

   In this part I will give an overview on the role of fibres, fruits and vegetables, calory intake, calcium, vitamins, and some specific food components in the three diseases.

2. What has been tried to influence the risk of diseases through modification of diet in the last 20 years?

   I will summarise the results of the randomised controlled trials using fibres, prebiotics, vitamins, probiotics or other food components in the three diseases and discuss what we learned from the positive but also from the negative trials (for example on fibres to prevent colon adenoma recurrence).

3. What are the new ideas to modify diet and prevent or alleviate the symptoms of these diseases?

   I will discuss:
   - the need to use new surrogate markers or research strategies and the ideas of what should be the end points of the new studies
   - the place for probiotics and prebiotics.

On the nutritional properties of prebiotics, with a focus on their anticancer properties

J. Van Loo

Orafti, Belgium

Present paper will focus on prebiotics, what they are, what they mean from a nutritional point of view and what the possible mechanisms are. Prebiotics is a term which was reviewed in '99 by the ENDO team (AIRII CT94 1095). They are food ingredients which are not degraded by digestive enzymes, and as such become available for selective gut fermentation : some bacteria grow well on it (saccharolysis), and others are suppressed (proteolysis). Bifidobacteria and LAB are considered indicators of a well functioning flora. Also other of the many unknown types of gut bacteria can be affected (1). The more distal in the colon where this effect can be induced, the better. The fermentation results in the production of SCFA and lactate. The stimulation of fermentation increases biomass in the lumen. This bulking effect improves bowel habit. Increased production of SCFA improves bowel motility. Butyrate is preferred energy source of epithelial cells in the mucosa. It is also involved as a signalling molecule in a.o. the apoptotic cascade. These properties are related to anticancer properties of prebiotics (in colon, as well as systemic-experimental models). Propionate, migrates through the portal vein to the liver, where it is thought to interact with lipid metabolism (reduced lipogenesis), by affecting regulation of expression of digestive hormones (GIP, GLP1, insulin). SCFA decrease the intestinal pH, making minerals more soluble. Along with the increased mass of the absorptive gut tissue, mineral absorption (calcium, magnesium) is increased. Stimulation of the metabolism of the gut flora increases the interaction with GALT. Along with possible release of signalling molecules, effects on the immune system have been observed. Prebiotics are active at daily intake doses of 5-10g per day. They are present in many foodplants, especially in the roots and fruits, which are thought to have been a staple food of mankind during evolution. Supplementation of the nowadays diet with prebiotics can offer a means to improve general health status (or : improve resistance against disease, or : reduce risk for disease or discomfort) of the consumer.

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Functional food against colon cancer – Development of a genomics and proteomics based screening assay

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A screening assay is developed for the selection of (functional) food ingredients with a specific preventive or inhibitory effect on the development of colorectal cancer. The assay is based upon evaluation of the expression of a large number of genes and proteins involved in colorectal carcinogenesis and their assumed preventive or protective mechanisms. Through a variety of methods, genes relevant for the process of colorectal cancer development are identified for the human and rat model:

- Subtractive hybridisation in rat after induction of colon tumours with PhiP (a heterocyclic amine) and also after combination of PhiP with resveratrol, a flavonoid with possible anti-carcinogenic activity.
- Subtractive hybridisation of a human colon adenocarcinoma against human normal colonic epithelium.
- DNA-array based expression analysis of human colorectal tumour samples with different (characterised) pathologies and their corresponding "normal" tissues.
- Proteomics analysis of the same samples is produces a proteome map of colorectal carcinogenesis.
- Mechanistic knowledge of the process of colorectal carcinogenesis and protective mechanisms involved.

As screening tool, a set of 14 human and rat colorectal cancer cell lines have been assessed in terms of their transcriptome and proteome. Subsequently, changes in gene and protein expression resulting from exposure to bioactive food constituents butyrate, resveratrol, quercetin and curcumin were measured in four human cell lines representing different stages of colon carcinogenesis (Caco-2, HT-29, T84 and NCM 460). Thesechanges were related to changes observed in different stages of the colorectal molecular pathogenesis. Exposure of these cells to colon cancer preventive agents (functional food ingredients) results in genomic changes resembling in vivo observed changes. Model phytochemicals to validate the in vitro observations used were quetcetin, rutin, resveratrol, curcumin and benzyl isothiocyanate. It is envisaged that this panel of cell lines will allow for medium throughput screening of potentially anti-carcinogenic food constituents,

based on transcripomic changes, either interpretated on a mechanistic level or using pattern recognition tools.

* As representative for the FW5-FFACC project team consisting of 16 collaborators of 6 European Institutions, who gratefully acknowledge the EU funding (QLRT-1999-00706).

Antioxidants and colon cancer

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The presentation will focus on the effect of dietary polyphenolic antioxidants on the reduction of risk of colon carcinogenesis. A high consumption of vegetables and fruit protects against cancer of the mouth, pharynx, oesophagus, lung, stomach, colon and rectum, and possibly against cancer of the larynx, pancreas, breast and bladder; part of this protection may arise from the high polyphenolic content of these foods. Carcinogenesis, however, is an extremely complex multiple stage process. Mechanistically from *in vitro* studies, polyphenols are predicted to be good inhibitors of early stages of carcinogenesis, since they inhibit free radical-mediated DNA damage, enhance the ability of target tissues to intercept and metabolise mutagens, induce apoptosis, suppress mitosis, inhibit angiogenesis and inhibit COX-2. However, the effects of polyphenols on colon cancer *in vivo* are strong for animal studies but less good for humans. Animal studies show good protection at high doses of polyphenols against induction of experimental tumours by suppression of either the induction of preneoplastic lesions such as aberrant crypt foci or suppression of fully developed tumours. Human studies on modulation of biomarkers of carcinogenesis by polyphenols are less common and show mixed results. Furthermore, epidemiological evidence for the action of polyphenols against carcinogenesis is also mixed. Several factors contribute to the apparent disrepancies between results from *in vivo*, *in vitro* and epidemiological studies. The major factors are proposed to be: (1) Foods contain a broad range of polyphenols each with different biological activities; (2) Polyphenols are metabolised by the body and only some reach target tissues; (3) There is extensive metabolism of most polyphenols that reach the colon by the microflora; (4) Many biomarkers of carcinogenesis are not fully developed or validated. These issues will be discussed during the presentation.

Diet and colon cancer – a mechanistic approach with *Apc*Min/+ mouse

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The incidence of colorectal cancer is increasing in all industrialized countries. Epidemiological evidence suggests that diet is clearly involved, but the mechanisms through which food, nutrients or non-nutritive compounds either promote or inhibit colonic carcinogenesis are still far from clear.

Colon tumorigenesis involves activating mutations in proto-oncogenes as well as genetic inactivating mutations in tumor suppressor genes, including the adenomatous polyposis coli (*APC*) gene. These genetic events, in turn, lead to epigenetic changes in signal transduction pathways, which regulate apoptosis, cell differentiation and proliferation. Dietary factors may affect cancer development either at the level of mutations or, more likely, at the different steps of cell signaling pathways. Several signaling pathways are related to colon tumorigenesis such as APC-β-catenin, NF-κB, epidermal growth-factor (EGF), and cyclo-oxygenase (COX-2).

Two main experimental approaches have been used in studies on diet and colon carcinogenesis: 1) rats treated with colon specific chemical carcinogens in order to initiate tumor development, 2) genetically modified mouse, e.g. *Apc*Min /+ mouse, with the mutation in their *Apc* tumor suppressor gene. Since most human colorectal tumors are initiated by inactivation of the *APC* tumor suppressor gene, this model offers a promising way forward for mechanistic studies between diet and colon cancer. Mutations of the *APC* gene cause aberrant accumulation of cytosolic β-catenin, which is assumed to alter the expression of several genes regulating apoptosis, cell differentiation and proliferation. It is currently assumed that over-expression of β-catenin, which is caused by lack of *APC* gene function, results in abnormal gene transcription, which promotes tumor development. *Apc*Min/+ mouse develop numerous intestinal polyps along the entire intestine within a few weeks and several dietary factors have already been shown to affect polyp incidence and size.

The focus of our studies with *Apc*Min/+ mice is to understand how the above-mentioned signaling pathways are regulated by diet and furthermore how changes in these pathways are related to polyp formation in this animal model.

Health as a quality factor in food products

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Health is one of the major elements in consumers' food quality perception. Along with sensory pleasure it is one of the two major purchase motives in buying food products. However, the role that the health quality dimension will play in any purchase will depend on three main classes of factors: trade-offs with other purchase motives, availability and interpretation of health cues before the purchase, and availability and interpretation of reinforcement cues after the purchase.

In any concrete purchase decision, the health motive may be traded off against sensory pleasure and supplementary quality dimensions like convenience and desired process characteristics like organic production or animal welfare. The result of this trade-off process will depend not only on the profile of the consumer and his/her health-consciousness, but also on situational factors like the type of meal, taking care of others' preferences, stage in family life cycle etc. Even when the health motive plays a major role in the purchase decision, the fact that health is a credence quality, i.e., an invisible quality, plays a major role. Before the purchase, consumers have to infer the healthiness of a product based on the cues available in the purchase situation. Consumers will interpret these cues based on their own subjective theories of healthiness, and they will weigh information available before purchase by the credibility of the information source. In this before-purchase situation, *informational cues* will have most importance.

When evaluating product quality after purchase, the weighting of the different quality dimensions often changes, with more weight given to those quality dimensions that can actually be experienced after the purchase, like sensory pleasure. This may impede repeat purchases of products positioned as healthy, and the repurchase of such products will therefore depend largely on reinforcement cues. These may also be informational, but in addition sensory cues can also reinforce the impression of healthiness.

The presentation closes with a framework for analysing the interplay between the health purchase motive, health cues before purchase, and reinforcement cues.

The role of diet and food ingredients in the prevention of osteoporosis

K. Cashman

University College Cork, Ireland

Age-related osteoporosis is a major, and increasing, public health problem in Europe which has a considerable impact in terms of increased mortality, morbidity and reduced quality of life, as well as placing an increasing burden on health care systems. In a recent press release, the World Health Organisation has commented that unless health organisations as well as the general public act now osteoporosis may reach epidemic levels in the not so distant future. The lack of effective treatments for degenerative disease such as osteoporosis places increased emphasis on a preventative approach and offers considerable opportunities for the development of functional foods. These opportunities are now being realized with the increasing awareness among consumers of the links between diet and health and a rapidly growing market for functional foods. However, the development of such products must be based on a detailed understanding of the influence of dietary constituents on health and must be supported by independent and appropriate scientific evidence to demonstrate efficacy with respect to the claimed health benefits. Therefore, it is extremely important that more research is carried out to fully investigate the role of nutrients (and bioactive food components) in bone health. This presentation will outline some of the findings of a European Union's 5th Framework Programme shared-cost project *"Optimal Nutrition towards Osteoporosis Prevention: Impact of Diet and Gene-Nutrient Interaction on Calcium and Bone Metabolism (OSTEODIET)"*. The main objectives of this multidisciplinary research project were to investigate the influence of diet, and its interaction with individual genetic variation, on the metabolism of calcium and bone in humans. The project applied state of the art techniques in human studies to investigate how diet (sodium, calcium, protein, vitamin K) and individual genetic variation (genes which have been linked to osteoporosis) influence metabolic processes of calcium and bone. Four dietary intervention studies will be carried out in postmenopausal women. The presentation will also highlight other recent and important findings in the area of diet and bone health.

Multicentre study of eating disorders and obesity

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Eating disorders are complex gene-environment disorders, caused by interaction between individual specific experiences such as abuse and neglect, and vulnerability genes in about equal measure. Using multidisciplinary research, our aim is to identify psychosocial and genetic risk factors for anorexia, bulimia and obesity, and correlate these with neuroimaging and neuropsychology. We have examined environment by measuring psychosocial and endogenous risk factors such as childhood obesity, sexual abuse, personality and psychopathology, and in addition to confirming known risk factors, we have identified have also examined the human genome for genetic risk factors in large, well characterised European samples, including examination of the 5-HT2A, 5-HT2C and BDNF genes. We failed to confirm association between BDNF and anorexia nervosa but report that BDNF appears to be a risk factor for anorexia nervosa. Finally we have examined the brain by performing fMRI and PET neuroimaging to explore neuroanatomical and neurochemical correlates, and neuropsychology to reveal cognitive factors that trigger and prolong these disorders. By fMRI, we have identified specific neural correlates: In response to food stimuli, medial orbitofrontal and anterior cingulate cortices were more activated whereas the lateral prefrontal cortex, inferior parietal lobule, left precuneus and posterior cerebellum were less active in patients than in the comparison group. In our neuropsychological sstudies, we have found that people with anorexia nervosa are significantly less flexible and slower on a battery of neuropsychological tests, which include cognitive, perceptual and motor set shifting tasks. These abnormalities are not explained by weight loss and they persist after weight gain and after full recovery from the illness. Finally we plan to model interactions between psychopathology, genes, culture, gender and psychosocial risk.

Consumers and health: Getting the message across

L. Lähteenmäki

VTT Biotechnology, Finland

Health is one of the most commonly mentioned reasons behind food choices, but it may have diverse meanings for people. The possible health effects of foods cannot be directly experienced, instead consumers have to rely on the information they receive about the food products. As consumers give different connotations to healthiness also health-related messages are interpreted in several ways depending on the existing beliefs of individuals.

So called functional foods had brought a new kind of health messages into food domain. The traditional nutritional advice has concentrated in making the right choices for a wholesome diet without putting emphasis on single food products. Functional food products claim that eating a single product will have a targeted positive effect in the body, if it is consumed in sufficient quantity as part of a normal diet. These positive effects can be verified by instrumental measures but not usually sensed directly.

The content or strength of the health-related message or claim does not guarantee how it will be comprehended. If a claim contains information about a component that is connected in consumers' minds with positive health effects, adding the information about these consequences does not increase the benefit value of the claim. Consumers use all the information they have available when making judgements. This means that, in addition to actual content of the message, all beliefs evoked by a piece of information are utilised.

One hurdle in effective communication of beneficial heath messages is the difference in the nature of scientific and everyday thinking. Health benefits tend to be based on scientifically proved probabilities whereas consumers tend to think in dichotomic terms: something either is good or bad for them. Consumers easily make associations between two things that occur at the same time regardless of the reason behind this simultaneity. Furthermore, consumers can generalise connections from individual cases, whereas scientific thinking requires controlled evidence on number of incidences. In communicating about food-related health issues to consumers these differences in thinking create great challenges, as messages need to be simple enough for consumers to comprehend, and on the other hand, they need to include the probabilistic nature of the health effects.

The health-related messages included in functional foods may be more easily understood than advice based on nutritional guidelines for healthy eating, since these messages tell about one effect caused by a single product. Yet, consumers need to improve their abilities to assess the food-related health messages. Citizens should be trained to handle the information that is based on likelihood better than before. Furthermore, there should also be more research on tools that help to manage health-related information in a manner that is salient to individual consumers with varying interests.

Sensory factors influencing the healthy food choices of older consumers

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The percent older population of many developed countries is increasing rapidly. For this population group a nutritious diet is fundamental to good health, longevity and independent living. However, food has no nutritional value until it is chosen, accepted and consumed. Therefore, understanding the factors that determine choice, acceptability, and sustained consumption is an essential part of healthy eating policy directed towards the elderly.

Food Choice is a complex behaviour, determined by economic, social and personal factors. An individual draws upon their experience and their resources, and in the context of concerns about health, well-being, planned eating context and convenience, make their choices. It is the role of the food providers and marketers to ensure that acceptable healthy options, in convenient formats, at the right price, are targeted at consumer needs.

The “taste” of food is most often cited as the most important factor determining sustained consumption. Our sensory systems act as a “gatekeeper”, evaluating and distinguishing the foods that to us as individuals are acceptable for consumption. As ageing progresses persons lose sensory function, in parallel with the loss to other biologic functions. This loss results in food tasting different, and often bland, for older consumers, leading to loss of eating pleasure and compromising intake.

This knowledge led to the EU funded HealthSense project (Healthy Ageing: How Changes in Sensory Physiology, Sensory Psychology and Socio-Cognitive Factors Influence Food Choice) as a concerted effort to re-evaluate the food sensory requirements of older consumers and provide guidelines for tasty and acceptable product development. HealthSense is a pan-European collaborative effort involving 24 research partners from 10 different countries.

POSTERS

Assessment and evaluation of the effects of probiotics on faecal microbiota of patients with Inflammatory Bowel Disease

K. Ben-Amor, I. Heikamp-de jong, A. Akkermans, W. de Vos and E. Vaughan

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Probiotics have the potential to improve human health in specific intestinal disorders: lactose intolerance, acute gastro-enteritis, colorectal cancer, constipation and inflammatory bowel disease (IBD) (1). However, very few data are available on the role of probiotics in IBD due to either the inadequacy of the methods used or the complexity of the colonic ecosystem (2, 3). In response, the PROGID project will evaluate the efficiency of two selected probiotic strains (*Lactobacillus.salivarius* subsp.*salivarius* UCC118 and *Bifidobacterium.infantis 35624)* on IBD patients during two distinct long term, large-scale, multi-center, randomized, double blind, placebo-controlled feeding trials on maintenance of remission. Therefore the influences exerted by these probiotics on colonic microbiota will be assessed by: (i) monitoring the faecal microbial changes using DGGE, (ii) identifying specific bacterial groups by cloning/sequencing and (iii) enumerating the major intestinal bacteria in faecal samples using Fluorescent *in Situ* Hybridisation (FISH) and Flow Cytometry (FCM). Since we deal with large number of samples, a rapid DNA-extraction method from faeces is needed. Therefore, four different extraction methods were compared: i) a WU-method based on phenol extraction and bead beating, ii) FastDNA kit, iii) QIAmp stool kit and, iv) Combination of the 2 kits. Different set of primers was used to amplify the 16S rDNA of total bacteria, Lactobacilli and Bifidobacteria (4, 5). The DGGE profiles show that for total bacteria, the WU- method gave better results in terms of total bacterial diversity and was comparable to the combination of the 2 kits. For *Lactobacillus* and bifidobacteria the stool kit and/or the combination of the two kits gave higher number of bands. Using the combination of the 2 kits, PCR-DGGE fingerprinting of faecal microbiota from patients at different time points of the trial was compared. For a more quantitative and as a high throughput approach, FISH is used in combination with flow cytometry to enumerate faecal bacteria from IBD patients. Group specific probes Bif164, Lab158, Bac303, Erec482, Ecoli1531 and Ato291 are used to count *Bifidobacterium* sp, *Lactobacillus/Enterococcus* group, *Bacteroides*/*Prevotella* cluster, *Eubacterium rectale/Clostridium coccoides* group, *Enterobacterecae* and *Atopobium* group receptively (6). The FISH probes were revalidated for flow cytometry application and signal to noise ratio was optimised.

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(2) Micro.Ecol.Health. Dis. 2002. 14: 65–74
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Identification and characterisation of mucin-degrading bacteria from the human gastrointestinal tract

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The dynamics of the human gastrointestinal tract (GI tract) ecosystem is a result of a delicate interplay between host, diet and microbes. It has been proposed that these interactions are facilitated by communication between gut micro-organisms and the host. Intestinal mucus forms a crucial intermediate layer between host and microbe since it provides protection to microbial invasion on one hand and constitutes an important energy source on the other. Several microbes, including potential probiotics, have been isolated that proliferate on, or bind to, mucus or its components. The aim of the study is to identify and isolate new mucus associated bacteria from the GI tract and to characterise them.

The phylogenetic diversity of enrichments of human fecal samples (adult and baby) and human colonic biopsies cultured in a medium containing mucin as sole carbon source was monitored by molecular methods included DGGE (Denaturing Gradient Gel Electrophoresis) as well as construction of 16S ribosomal DNA (rDNA) clone libraries and comparative 16S rDNA sequence analysis.

Molecular analysis allowed the identification of mucus-degrading bacteria such as *Ruminococcus torques, Bacteroides sp, Streptococcus anginosus*, *Bifido-bacteria* and a previously undescribed bacteria species that was also isolated by anaerobic culturing techniques and characterised for its physiological properties and 16Sr DNA sequence.

A strategy to isolate novel bacterial species in human faecal samples

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Molecular taxonomic inventories obtained directly from human faeces have shown that the vast majority (60–80%) of generated rDNA sequences do not correspond to known organisms and clearly derive from hitherto unknown species within the human gut. However, it is not known if this 'hidden' flora are non-culturable or if they represent organisms which have so far eluded identification using traditional phenotypic taxonomic methods. A novel scheme for isolating strictly anaerobic bacteria from the human intestinal microflora was devised to try and unravel some of these hidden species. Using traditionally based techniques we chose to use a range of 'nutritionally rich' media in the initial screening for new species. One special medium was elected for its ability to sustain growth of a high number of bacteria with a high level of diversity (evaluated by a visual assessment of phenotype and morphology). A representative number of isolates were then chosen based on differences in colony-morphology, colony-size and colour. Similarly looking colonies were also included. All colonies were streaked onto new plates and eventually subjected to a molecular typing based on generating restriction enzyme digests of 16S rDNA PCR-products using *AluI*. Using this approach we were able to reduce the number of isolates to study further assuming that identical profiles represented identical species. A total of 85 unique profiles were identified and subsequently subjected to further phenotypic characterisation and phylogenetic analyses using 16S rDNA sequencing. Results will be presented on the progress of identifying the isolated species and correlating phenotypic and genotypic (16S sequence data) characterisation.

Developing a method for assessment of specific microbial activity in individual *Bifidobacterium* sp. using a mRNA-targeting approach

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One of the workpackages in the Microbe Diagnostics project of the PROEUHEALTH cluster is specifically dedicated to the development of a method for monitoring specific microbial activity (*i.e.* gene expression) in members of the human intestinal microflora targeting mRNA´s in single cells. Two bifidobacterial species *Bifidobacterium bifidum* (DSM20215) and *B. infantis* (DSM20088) were selected as model strains potentially representing probiotic organisms having a beneficial effect on human health and welfare. In order to address this challenge we are working on two fronts: first, the method developing front and, second, the biological front trying to identify relevant *e.g.* enzymatic activities to monitor in *Bifidobacteria* sp. As a starting point the method rely on the ability to conduct an intracellular mRNA-targeting RT-PCR. The most critical point in this context is to achieve permeabilisation conditions that will allow for constituents of the PCR-mixture to enter the interior of the cells without leaving the cells so porous that the intracellularly generated PCR-products will diffuse out once formed. To assess such conditions we are employing a FISH assay. The progress in this will be presented. The biological front is aimed at studying and revealing any regulatory network in the expression of β-galactosidase genes in the model strains under investigation. In *B. bifidum* at least three different β-galactosidase genes have been identified[1], and in *B. infantis* at least two (1,2). These studies are based on traditional physiological growth experiments using different carbon sources and Northern analyses and precede the use of these genes (or transcripts) as potential targets in the single-cell based mRNA targeting procedure that will be developed. Data and progress on this part of the project will also be presented.

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Antagonistic activity and organic acid production of probiotic strains

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Probiotic strains affect beneficially the host's health, having an antagonistic activity against different pathogenic bacteria and decreasing the risk of infection.

The aim was to determine *in vitro* antagonistic activity against pathogens and organic acid production of seven probiotic strains.

We studied seven probiotic strains for production of organic acid and evaluated their antagonistic activity. Five of these strains were lactobacilli: *L. rhamnosus* LGG, *L. fermentum* ME-3, *L. acidophilus* La5, *L. plantarum* 299v and *L. casei* 8700:2; and two bifidobacteria: *B. lactis* Bb12, *B. longum* B46. The selected target bacteria were *E. coli* ATCC 700336, *E. coli* ATCC 700414, *S. enteritidis* ATCC 13076, *S. sonnei* ATCC 25931. Antagonistic activity was investigated by two different methods: i) antagonistic activity on agar plate (streak line method); ii) antagonistic activity in liquid medium (MRS medium without triammonium-citrate and sodium-acetate). Acetic, lactic and succinic acid production was determined in 7 probiotic strains by gas liquid chromatography.

Good antagonistic activities were estimated for *L. fermentum* ME-3, *L. rhamnosus* LGG and *L. plantarum* 299v by both investigated methods against all tested target bacteria. These three probiotic strains showed the highest production of organic acids. *L. casei* 8700:2 showed remarkable antagonistic activity in liquid medium. Bifidobacteria showed better antagonistic activity in liquid medium.

Obligately homofermentative (OHEL) and facultatively heterofermentative (FHEL) strains of *Lactobacillus* spp., which produce a lot of amount of organic acids, had better antagonistic activity than obligately homofermentative lactobacilli (OHOL). The production of organic acids is clearly involved in antagonistic activity of probiotic bacteria *in vitro*.

Design and validation of 16S rRNA probes to enumerate members of the subgroup *Clostridium leptum* and of cluster XVIII (*Clostridium ramosum* assemblage) in human fecal microbiota

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The design of signature regions was based on alignment of 16S rRNA sequences isolated from feces of 5 healthy adults. We showed that 22% of the sequences were related to *C. leptum* subgroup, but only 61% of these sequences were targeted with the current set of probes: Fprau 0645 (42%), Rbro 0730 (12%) and Rfla 0729 (7%). To enlarge the detection of the *C. leptum* subgroup, Clep 0866, Cvir 1414, Edes 0635 and Rcal 0733 were developed. In silico, these new probes targeted 80% of *C. leptum* related sequences. They were validated using FISH combined with flow cytometry on a collection of 30 target and non target strains. The Relative Probe Fluorescence (RPF) was determined to assess the accessibility of the probe to their target site. To optimise specificity Clep 0866, Cvir 1414, Rcal 0733, Rbro 0730 and Rfla 0729 were associated with competitors. All the probes presented RPF superior to 34%, which showed that they easily accessed to their targeted site. Cra 0757 for the *C. ramosum* assemblage was also validated. These new probes will be added to the panel of 19 probes to analyse 100 human fecal samples collected from five European sites within Microbe Diagnostics project (QLK1-2000-00108).

Screening of probiotic strains isolated from the elderly for antimicrobial activity against gastrointestinal pathogens

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The human gut microflora changes in old age both in species diversity and relative bacterial numbers. This may lead to a reduction in the colonization resistance afforded by the gut microflora towards ingested exogenous microorganisms and may account for the epidemiologically important increase in gastrointestinal infections seen in the elderly. Important gastrointestinal pathogens of the elderly in terms of incidence and severity include verocytotoxic *Escherichia coli* (VTEC), enteropathogenic *E. coli* (EPEC), *Campylobacter jejuni* and *Clostridium difficile*.109 lactobacilli and *Bifidobacterium* strains, isolated from the faecal microflora of healthy elderly individuals were screened for their ability to inhibit these pathogens. A deferred diffusion assay was employed to screen for anti-pathogen activity. Briefly, a 1cm x 2cm probiotic streak grown to confluency anaerobically, was removed with a glass slide and any remaining cells inactivated over chloroform vapour. The surface of the nutrient agar plate was then spread with $10^{5–6}$ CFU of the pathogenic strain. Zones of clearance in the lawn of pathogen were measured after 24 h growth. Fifteen probiotic strains showed significant and reproducible anti-pathogenic activity and were chosen for further characterization of their anti-microbial capabilities. Importantly, certain strains showed a broad spectrum of anti-pathogen activity, inhibiting all four pathogens. Conversely, different strains of the same probiotic species showed different spectra of anti-pathogenicity.

Molecular taxonomy of probiotic strains isolated from the elderly gut microflora

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The incidence and often severity of gastrointestinal infections increases in old age. One contributory factor towards this increased disease susceptibility may be the documented reduction in numbers of gastrointestinal bacteria seen as beneficial. As a population group the elderly may thus be particularly amenable towards dietary fortification with probiotic foodstuffs. 109 putative lactobacilli and bifidobacteria were isolated from the faecal microflora of healthy Italian elderly subjects and characterised using traditional microbiological techniques. Here we report the taxonomic positioning of these putative probiotic strains based on partial sequencing of the 16S rRNA gene. Of the 109 strains, 43% showed greater than 98% identity to species belonging to the genera lactobacilli; 38% to the bifidobacteria and 19% to other bacterial genera. In all 39% of strains characterised were most closely aligned to previously uncultured bacteria. The majority of these novel isolates belonged to the bifidobacteria. Accurate molecular taxonomy is important in the development of probiotic foods both in terms of maintaining product integrity and monitoring beneficial health outcomes upon product consumption.

rep-PCR fingerprinting for identification of *Bifidobacterium* species

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Bifidobacteria are the dominant genus within the gastro-intestinal microbiota of infants and remain the third most common genus as humans age. Their role in public health has long been recognized. Currently over 30 species of *Bifidobacterium* have been validly described and the taxonomic position for some of these taxa has been debated. Although various methods have been reported for the identification of bifidobacteria, many of them turn out to be time-consuming, laborious and do not permit the differentiation of closely related species. In the present study, the applicability of rep-PCR fingerprinting was assessed for identification of a wide range of *Bifidobacterium* species. For this purpose, several primersets (GTG_5, BOX, ERIC and REP) targeting repetitive DNA elements were evaluated on a subset of representative strains. The BOX primer was found to be the optimal choice for the establishment of a reference framework comprising a broad taxonomic range of bifidobacteria. We were able to differentiate 26 of the 31 included species. The remaining taxonomic inconsistencies within the framework were subsequently analysed with 16S rDNA sequence analysis. We are currently testing the method on a collection of bifidobacterial isolates from probiotic dairy products. In conclusion, rep-PCR fingerprinting using BOX primers is a promising tool for differentiation of a wide range of bifidobacteria at the (sub)species level.

Keywords: *Bifidobacterium*, Identification, Rep-PCR

Investigation of bifidobacterial substrate preferences towards modifying the intestinal microflora of formula-fed infants

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Breast-feeding is associated with an improved health status during infancy and childhood. Additionally, research has shown microbiological differences in the predominant faecal flora of breast- and formula-fed infants, both with respect to bacterial population levels and species composition. The overall aim of our research is to identify dietary modulations to enhance a breast-fed type microflora in formula-fed infants. The objective of this study was to investigate the substrate preference of bifidobacterial strains isolated from breast- and formula-fed infants' faecal samples. To this end, growth was monitored during pure culture experiments using 13 substrate mixtures; and pH, cell numbers and morphology were examined at the end of the growth experiments. The infant bifidobacterial isolates were able to ferment all of the substrate mixtures used (with a drop in pH of 2–4 units observed). Overall, similar mixtures were identified as the top 4 'preferred' substrates for each of the bifidobacterial isolates studied. Subsequent mixed culture fermentation studies have begun using these substrate combinations to investigate the effects of such mixtures on formula-fed infant faecal flora *in vitro* using batch fermentations and infant gut model systems.

Composition and stability of faecal microbiota in irritable bowel syndrome as compared to healthy controls

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Irritable bowel syndrome (IBS) is a functional disorder, which is characterised as regular intestinal pain and discomfort. An altered indigenous microbiota has been suggested to be related to IBS, although specific target microbial groups or species are still not known. The aim of the present study was to compare the faecal microbiota of IBS subjects (fulfilling the Rome criteria) healthy volunteers (without intestinal symptoms, age and sex matched with IBS group) by conventional culture and PCR-DGGE methods. Fresh faecal samples (26 IBS subjecst, 23 controls) obtained on two occations (3 months apart) were cultured on several selective and non-selective media for enumeration of total anaerobes, total aerobes, bacteroides, bifidobacteria, spore-forming bacteria, lactobacilli, coliforms, enterococci and yeasts. Faecal samples of 20 IBS and 18 control subjects (three samples from each subject obtained at 0, 3 and 6 months time points) were analysed by PCR-DGGE to study stability of the dominant bacterial population. No differences were detected in the prevalence or mean numbers (cfu/g wet weight) of the target microbial groups. However, PCR-DGGE revealed more instability in the dominant population in the samples of the same individual in IBS group than in the control group. Identification of the dominant species detected by culture and PCR-DGGE is in progress to reveal if specific species can be associated with the detected instability.

Modulation of the effect of Dextran Sulphate Sodium (DSS)-induced acute colitis by the administration of different probiotic strains of *Lactobacillus* and *Bifidobacteria*

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The effect of colonic inflammation on intestinal microflora specifically lactobacilli and *Bifidobacterium* are not clear although bacteriotherapy with lactobacilli has been reported to be effective both in ulcerative and pseudomembranous colitis. The present study was performed to investigate the effect of different *Lactobacillus* and *Bifidobacterial* strains, in DSS-induced colitis and the associated bacterial translocation and intstinal mucosal microflora changes.

Sprague-Dawley rats were used and divided into six groups, Colitis control and five groups of colitis with *Lactobacillus* and *Bifidobacterial* strains administered orally for 7 days before the induction of colitis and continued for another 7 days with the DSS. Colitis was induced by DSS 5 % (w/v) dissolved in drinking water for 7 days. Severity of colitis assessed daily using a Disease Activity Index (DAI). Samples were collected after 7 days from the induction of colitis, for bacterial microflora and bacterial translocation.

DAI decreased significantly on day 3, 4, 5, 6 and 7 in *Lb. Plantarum* 299v, *Bifidobacterium* B1, *Bifidobacterium* B2 groups compared to colitis control group. It decreased significantly on day 5, 6 and 7 in *Bifidobacterium* B2 group compared to *Lactobacillus paracaesi* 8700:2 and *Lactobacillus gasseri* LG1 groups. Bacterial translocation to the mesenteric lymph nodes decreased significantly in *Lactobacillus paracaesi* 8700:2, *Lactobacillus gasseri* GL1, *Bifidobacterium* B1, *Bifidobacterium infantis* B2 compared to colitis control group. The *Enterobacteriaceae* bacterial translocation to the liver decreased in all the treatment groups compared to colitis control.

Administration of certain strains of *Lactobacillus* and *Bifidobacteria (Lactobacillus Plantarum* 299v, *Bifidobacterium* B1, and *Bifidobacterium* B2) improved significantly the DAI in the DSS-induced colitis model. It also reduces bacterial translocation.

Genetic study of the role of D-alanine substitutions of teichoic acids in *Lactobacillus plantarum*

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The dlt operon (dltABCD) responsible for teichoic acid (TA) D-alanylation in *L. plantarum* was cloned. A pbpX gene encoding an homologous to LMW-penicillin-binding proteins was found to adjoin the cluster.

A dltB$^-$ strain unable to incorporate D-alanine in TA was constructed. dltB mutant reaches a lower OD that decreases in stationary phase which might result from autolytic activity. However, the relation between autolysis and non alanylated-TAs is not well understood. It might be a consequence of the more negative character of TAs due to the D-alanine absence, or be an indirect effect of modifying the cell wall ionic environment of autolysins. Demonstration of an increased anionicity of the mutant cell wall was gained from the observation of an enhanced binding of cytochrome c and increased sensitivity to nisin (both cationic compounds). XPS analysis showed a decrease of protonated nitrogen at the cell surface, in agreement with the loss of D-alanyl-ester substituents. Microscopy of the mutant suggest a septation problem (filaments and longer cells).

To study the role of autolysins in the OD decrease at stationary phase, we inactivated an AcmA-like autolysin (lpa) in the dlt mutant. The dlt-lpa double mutant doesn't show any late OD decrease. In addition, morphological phenotype of dlt-lpa seemed to be a combination of both single phenotypes (lpa$^-$ and dlt$^-$): chains containing filaments and longer cells.

Development of a high throughput diversity DNA array for the human intestinal microbiota

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A diversity macroarray for the detection of the numerically dominant bacteria present in human gastrointestinal tract has been developed. The array is made by spotting 16S ribosomal DNA from over 100 bacterial species of the gut intestinal tract microbiota on a nylon membrane. Validation was performed by hybridizing the array with hyper-variable regions of 16S rDNA from various bacterial clones present on the array. In order to prevent cross-hybridization, universal bacterial primers for amplification of the V6 region were fitted with PTO bonds, after which removal of the primer sites was achieved by using two enzymatic reactions with T7 Gene 6 Endonuclease and Mung Bean Nuclease. Hyper-variable regions of 16S rDNA of approximately 50-100 bp in length were labelled by incorporation of radioactive [α-^{32}P] dATP and hybridised to the array. The results indicated specific hybridisation demonstrating the proof of principle and suggesting the method has a strong potential for the development of a microbiota microarray. The array will be further tested by hybridisation with DNA or RNA isolated from fecal or mucosal samples to investigate the diversity and activity of the microbiota within the different niches in the human intestinal ecosystem.

Improvement of viability and stability of probiotics by acid- and heat shock treatments

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Bacterial strains have to survive in unfavourable conditions during food processing and storage and in the gastrointestinal tract. In stressful conditions bacterial cells try to survive by activating stress response mechanisms to protect cellular components and thereby helping the cells to survive. The aim was to investigate whether a sublethal stress treatment enhances the survival of probiotic strains in a subsequent otherwise lethal stress treatment (adaptive and cross-protective response). This theme for research is important when aiming at diversified application of probiotics. Four probiotic strains, *L. rhamnosus* E800, *L. brevis* E1877, *B. animalis* E2010 and *B. longum* E1884, were studied. The bacterial growths were carried out in a food-grade Edible Medium (EM) which is suitable for industrial applications. The strains were grown into stationary (18 h) growth phase and treated with suitable sublethal stress treatments (pH and heat shock) followed by second lethal stress treatment (acid, bile and temperature). The viability of the cells was assessed by culturing on MRS agar (*B. longum* additionally on TPY). For *B. longum* also viability staining (LIVE/DEAD BacLight) combined with epifluorescence microscopy was used to visualise and differentiate the viable, non-viable, and viable but non-culturable *B. longum* cells.

All of the strains showed good growth in EM. *L. rhamnosus* E800 culture treated with low pH (pH 4) showed better survival in otherwise lethal pH 2,5 in EM than the control culture. *L. brevis* E1877 treated in pH 3,5 or with heat (+47 C) tolerated better low pH (EM pH 2,5) than the control culture. Neither adaptive nor cross-protective stress responses were observed in *B. animalis* E2010 strain, whereas both stress responses were observed in *B. longum* E1884. *B. longum* E1884 cells treated with heat (+47 °C) tolerated better higher temperatures than the untreated culture. Tentative studies also showed that cells treated in pH 3,5 or heat (+47 °C) tolerated better bile (1,5 %) than the control culture. Evaluation of the effects of sublethal pretreatments on stability and viability of bacterial stains in food matrices (juice and yoghurt products) are ongoing. The viability and stability of bacterial strains during storage test will be assessed by culture techniques and by using fluorescent live/dead dyes.

Effects of oligosaccharide on the fecal microflora and on non-specific immune system in elderly

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The bifidogenic effect of fructooligosaccharides (FOS) is well accepted. The aim of this study was to document on elderly subjects other benefits linked to FOS intake, such as on non-specific immune defense parameters (phagocytosis, changes in lymphocyte subpopulations). The study was a pretest/posttest study of 19 elderly nursing home patients. Four g of FOS were given twice a day, through a period of 3 weeks. Fecal bacteria composition, lymphocyte subpopulation and relative expression of interleukin-6 (IL-6) were investigated.

Bacterial counts for bifidobacteria increased by a mean of 2.8 $\log_{10}$CFU/g ($p<0.001$). Phagocytic activity expressed as median fluorescent intensity changed for granulocytes from 130±10 to 52±2 ($p<0.001$) and for monocytes from 75±5 to 26±2 ($p<0.001$). A decreased expression of interleukin-6 mRNA in peripheral blood monocytes was also observed. These results confirm the bifidogenic effect of FOS in the frail elderly subjects and suggest a concomittant diminution in the inflammatory process. Further studies are needed to confirm these observations.

Evaluation of the survival of *Bifidobacterium animalis* DN-173 010 in faecal samples from healthy adults

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Within the EU Project CROWNALIFE, twelve adults ingested 10^{11} *B. animalis* DN-173 010 per day during 7 days either in fermented milk or in lyophilised form. Faecal samples were collected prior to-, at the end and 10 days after the end of supplementation. The faecal microbiota was analysed by FISH coupled with flow cytometry using rRNA targeted probes. A species-specific probe for *B. animalis* was developed and applied to quantify the probiotic strain. Faecal DNA was also subjected to PCR-TTGE to assess species dynamics of bifidobacteria.

A global stability using FISH was observed for the predominant groups of the gut microbiota. In 5 subjects, *Lactobacillus* or *Bifidobacterium* groups increased, and in 4 subjects the increase persisted 10 days after the end of supplementation. *B. animalis* DN-173 010 was consistently detected by PCR-TTGE in 11 subjects after 7 days of supplementation (including all those receiving the lyophilised form) and still detectable in 7 subjects 10 days after the end of supplementation.

This study demonstrates the global stability of predominant groups of the human faecal microbiota during the intake of *B. animalis* DN-173 010 in a fermented milk or in lyophilised form. Moreover FISH and PCR-TTGE allowed the specific detection of the probiotic *B. animalis* DN-173 010 indicating its transit in dominance.

Effect of consumption of fermented milk containing the probiotic *Lactobacillus casei* DN-114 001 on the intestinal microbiota of healthy humans

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The effect of consumption of fermented milk containing Lactobacillus casei *DN-114 001 on the gut microbiota composition in healthy humans was analysed*. Twelve adults ingested during 10 days 10exp10 probiotic bacteria per day in a fermented milk. Faecal samples were collected prior to-, during and after supplementation. Faecal microbiota composition was analysed by fluorescent *in situ* hybridization (FISH) coupled with flow cytometry using rRNA targeted probes. Faecal DNA was subjected to PCR-TTGE based analysis of species dynamics of lactobacilli and bifidobacteria.

FISH showed a global stability in the gut microbiota composition. In some subjects, *Lactobacillus* and *Bifidobacterium* groups increased transiently during the trial. Five subjects exhibited a 2 to 15% increase of the *Bifidobacterium* group which persisted 10 days after the end of intake for 4 subjects. The species distribution of autochtonous bifidobacteria and lactobacilli was specific for each individual by PCR-TTGE. *Lactobacillus casei* DN-114 001 was consistently detectable after 10 days of consumption but never evidenced 10 days after the end of the intake.

This work shows that dominant groups of the human faecal microbiota remain fairly stable during the intake of fermented milk containing *Lactobacillus casei* DN-114 001 although the probiotic reaches detectable levels of 10exp6 to 10exp8 per gram faeces.

Culture independent molecular analysis of the elderly faecal microflora reveals an extreme complexity

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Understanding age-related alterations in the gastrointestinal tract is important in the design of preventive nutrition strategies. The elderly fraction of the population is currently rising in Western societies, and yet specificities of its gut microbiota, involved in health, remain largely unknown.

The present study was conducted to evaluate the bacterial diversity of the dominant faecal microbiota of elderly.

We used comparative sequencing of 1584 cloned 16S rDNA from faecal DNA of 10 healthy elderly persons (6 women- 4 men, aged 70-87, mean 78).

By BLAST, we identified the 6 phylogenetic groups commonly found as dominant in adults: *C.leptum* (34,5%-10/10), *C. coccoides* (23,4%-10/10), *Bacteroides* (22,1%-10/10), enteric (2,7%-7/10), lactobacilli-streptococci (2,5%-7/10) and *Bifidobacterium* (1,8%-9/10). We further observed other dominant groups: *Sporomusa* (2,7%-9/10), *Acholeplasma-Anaeroplasma* (1,8%-10/10), uncultivable bacteria from clusters B (2,1%-8/10) and E (2,1%-7/10) and *Atopobium* (1,1%-9/10). Other remaining groups were less represented with a weaker prevalence. Among the 1584 sequences analysed, 79,2% corresponded to uncultured bacteria. Finally, the number of novel sequences (<2,5% identity with GenBank sequences) was 32,5% on average.

These results reveal an extremely high complexity of the elderly microbiota. Hence, to better appreciate the composition and dynamics of the elderly gut microbiota, new probes should be defined for hybridisation-based analysis.

Functional assessment of interactions between the human gut microbiota and the host: WP1 and 2 (prebiotics and synbiotics)

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Activities of the microflora in the human large intestine play a major role in host nutrition and health. Thus, dietary modulation of its composition can be very beneficial to health. The main objective of this aspect of the project was to determine prebiotic and synbiotic efficacy in an *in vitro* gut model. Initial anaerobic batch culture fermentations were performed with each of 11 prebiotics (1% w/v) in basal medium with faecal inoculum. Fluorescent In-Situ Hybridisation (FISH) quantified changes in the gut flora. FOS, Inulin, GOS and XOS were all effective prebiotics. In addition, an *in vitro* comparison of the candidate prebiotic(s) to resist digestion in the upper gastrointestinal tract isl also bein determined.

For the development of sybiotics, five species of bifidobacteria were grown anaerobically in basal medium containing one of 13 prebiotics (1% w/v). Late log phase starter cultures inoculate further cultures that were then monitored spectrophotometrically. Growth curves were constructed and gradients of the linear phase were calculated for comparison. Preliminary results suggested that FOS, GOS, and IMO demonstrated the best growth across the species tested. The preferred combinations will be further assessed in batch culture fermentations and subsequently in the gut model.

Effect of fermentation time and freeze-drying medium on viability and characteristics of probiotics

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Processing conditions may affect the probiotic cell viability and stability during culture production as well as within food matrices. In the present study the influence of freeze-drying medium and fermentation time on viability, storage stability and *in vitro* tolerance to acid and bile of probiotics were investigated. *Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* VTT E-97800 (E800) and *Bifidobacterium animalis* Bb12 strains were grown in an edible General Medium for 16, 19.5or 23-25 hours (for E800 up to 30 hours). Cells were harvested, resuspended in water or culture medium (neutralised vs. non-neutralised) supplemented with a cryoprotectant (saccharose or betaine) and freeze-dried. Freeze-dried cultures were assessed for viability by plate count, and for acid tolerance (pH 2.5 for 2 h) and bile tolerance (1.5% bile extract for 3 h). *L. rhamnosus* GG survived well in freeze-drying (over 95% survival) when saccharose was used as a cryoprotectant. Neutralisation of the cell mass prior to freeze-drying led to better survival rates of *L. rhamnosus* GG but not for *L. rhamnosus* E800, whereas fermentation time did not have dramatic effect on freeze-drying survival of either of the *L. rhamnosus* strains. *B. animalis* Bb12 did not survive well in freeze-drying (highest survival about 60%), especially when betaine was used as a cryoprotectant, and neutralisation and prolonged fermentation time decreased the survival rate. Freeze-dried *L. rhamnosus* GG and *L. rhamnosus* E800 cells had good acid tolerance, and no differences were observed between cultures produced under different conditions. Saccharose-protected *L. rhamnosus* GG cells tolerated bile better than betaine protected cells and prolonged fermentation time increased slightly the bile tolerance. Saccharose-protected *B. animalis* Bb12 cells tolerated acid and bile better than betaine-protected cells. Neutralisation of the cells prior to freeze-drying did not influence the storage stability of the freeze-dried cultures. In conclusion, processing parameters such as choice of the cryoprotectant have a significant but strain-dependent effect on the viability and characteristics of probiotic strains.

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The Food, GI-tract Functionality and Human Health Cluster, PROEUHEALTH

2nd Workshop

Abstract

The Food, GI-tract Functionality and Human Health Cluster PROEUHEALTH brings together 64 research partners from 16 European countries in the quest to obtain greater knowledge of the role of the intestinal microbiota in human health and disease and to develop new functional foods and therapies. The research will run for four years starting February 2001 and is subsidised by the European Commission's 5th Framework Programme, Quality of Life and Management of Living Resources Key Action 1, "Food, Nutrition and Health".

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