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The Food, GI-tract Functionality and Human Health Cluster

PROEUHEALTH

Abstracts and posters

1st Workshop

Saariselkä, Finland

1–3 February 2002

Edited by

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Otamedia Oy, Espoo 2002

Preface

All of us carry in our intestinal tracts a complex ecosystem of microbes. These bacteria are highly important to our health, providing us with protection against intestinal infections, supplying us with additional nutritional value from the food we eat, and contributing to the development of our immune system. The flip-side is that disturbances in this ecosystem can leave us more vulnerable to exogenous and endogenous intestinal infections. Intestinal bacteria have also been implicated in some chronic and degenerative diseases the gut. Understanding the relationships between food, the intestinal microbiota, and health and disease will enable scientists to develop foods and therapies that can maintain or improve our health.

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 4 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".

The 1st PROEUHEALTH Workshop at Saariselkä wilderness will be the start of our influential interaction!

Welcome!

Tiina Mattila-Sandholm
PROEUHEALTH Cluster Coordinator

Programme

FRIDAY, February 1

Food, GI-tract functionality and human health cluster results and prospects
Chair: Prof. Willem de Vos

9.00 - 9.15	Opening remarks *Dr. Jürgen Lucas*
9.15 - 9.30	PROEUHEALTH cluster *Prof. Tiina Mattila-Sandholm*
9.30 - 10.00	Highlights of MICROBE DIAGNOSTICS: Development and application of high throughput molecular methods for studying the human gut microbiota in relation to diet and health *Prof. Michael Blaut*
10.00 - 10.30	Highlights of CROWNALIFE: Functional foods, gut microflora and healthy ageing *Dr. Joël Doré*
10.30 - 11.00	Coffee
11.00 - 11.30	Highlights of PROGID. Probiotics and gastrointestinal disorders – controlled trials of European Union patients *Prof. Fergus Shanahan*
11.30 - 12.00	Highlights of DEPROHEALTH: Probiotic strains with designed health properties *Dr. Annick Mercenier*
12.00 - 12.30	Highlights of PROTECH: Nutritional enhancement of probiotics and prebiotics: technology aspects on microbial viability, stability, functionality and on prebiotic function *Prof. Dietrich Knorr*
12.30 - 13.30	Lunch
13.30 - 14.00	Posters
14.00 - 14.45	Introduction to new projects in PROEUHEALTH: PROPATH, PROSAFE, EU & MICROFUNCTION

14.45 - Arranged and free interaction between and within projects

19.00 Evening in a reindeer farm

If you need a warm outfit, please go and fetch one during the afternoon or at least half an hour before the departure from the office of Lapin Eräsafarit (Wildernes Safaris of Lappland). The office is in the main building of Tunturihotel. Bus transportation to the farm leaves at 19.00 from Tunturihotel.

SATURDAY, February 2

Novel developments for GI-tract foods
Chair: Dr. Annick Mercenier & Prof. Glenn Gibson

9.00 - 9.40 Genomic approaches to biomarkers of the GI-tract
Prof. Willem de Vos

9.40 - 10.20 LABDEL: a European funded project on lactic acid bacteria as delivery vehicles
Dr. Jerry Wells

10.20 - 10.50 Coffee

10.50 - 11.25 GI-tract disease models
Prof. Kevin Collins

11.25 - 12.00 Emerging techniques for prebiotic products
Prof. Fons Voragen

12.00 - 13.00 Lunch

13.00 - 14.30 TECHNICAL WORKSHOP on methodologies of GI-tract diagnostics.
Chair: *Prof. Michael Blaut, Dr. Joël Doré, Prof. Willem de Vos*

14.30 - Arranged and free interaction between and within projects

18.45 Symposium dinner

Bus transportation to Restaurant Huippu on top of the Kaunispää fell leaves at 18.45 from Tunturihotel. You have a possibility for shopping in Kaunispää during the evening.

SUNDAY, February 3

Consumers and foods for health
Chair: Prof. Charlie Daly & Dr. Liisa Lähteenmäki

8.30 - 9.00	Progress on the PROEUHEALTH Consumer platform *Dr. Liisa Lähteenmäki*
9.00 - 9.30	Comments from Consumer organisations: (Finland, Italy and Ireland)
9.30 - 10.00	Food & Health: Future consumer trends and attitudes *Prof. Klaus Grunert*
10.00 - 10.45	Industrial developments of gut-health foods *Prof. Seppo Salminen*
10.45 - 11.30	Coffee and snacks
12.00	Bus to the airport

Contents

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.

Development and application of high throughput molecular methods for studying the human gut microbiota in relation to diet and health

M. Blaut

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

The human gastrointestinal tract, in particular the colon, harbors a large and diverse community of microorganisms. This bacterial community affects the host in a number of ways related to gastrointestinal function, health and well-being. The composition and the activity of the intestinal microbiota are governed by endogenous and exogenous factors. Diet is the most important exogenous factor that influences the gut microbial ecology. 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 the composition of the intestinal microbiota. In order to improve this situation it is necessary to identify the parameters that influence the composition and the activity of the intestinal flora. This information can only be gained with reliable methods. Therefore, seven European partners cooperate in a joint project aimed 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. This includes the establishment of a fully comprehensive comparative 16S rRNA database for the human gut microbiota. and the design of a battery of diagnostic probes for age, geographic, disease and dietary based studies. In situ hybridization and dot blot hybridization probing methodologies are to be further automated to allow the analysis of high numbers of samples on the basis of 16S rRNA. The work is directed towards rapid, high throughput analyses of fecal samples using DNA arrays hybridized with fluorescing 16S rRNA-directed oligonucleotide probes. 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.

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.

Highlights of CROWNALIFE - Functional foods, gut microflora and healthy ageing

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

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[2] DifE, Germany
[3] University of Reading, UK
[4] University of Coleraine, UK
[5] University of Camerino, Italy
[6] Karolinska Institute, 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 in Europe. Yet ageing is associated with increased susceptibility to degenerative or infectious diseases, which may be exacerbated by a poor nutritional status. 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" is an EU project within the PROEUHEATH cluster, which acronym refers to its 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.

At present the evolution of the intestinal ecosystem with ageing has not been investigated in details. Current knowledge on the composition and function of the human intestinal microflora is nonetheless improving with the use of better suited methodologies. There has been a few reports that putatively protective lactic acid bacteria in general and bifidobacteria in particular are numerically less represented in the elderly faecal microbiota.

Using classical culture techniques, we have isolated lactobacilli and bifidobacteria from the elderly faecal flora. 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 will be 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, we will characterise the intestinal flora of the elderly in four countries across Europe. 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.

Assessing the biotherapeutic potential of probiotic lactobacilli and bifidobacteria in European Crohn's disease and ulcerative colitis patients

F. Shanahan

Department of Medicine, National University of Ireland, Cork, Ireland

It appears inevitable that novel strategies will be directed, as a result of our emerging understanding of microbial activities and both eukaryotic and prokaryotic gene expression *in situ*, at combining complementary therapeutic traits in the form of microbial probiotic consortia or genetically-enhanced organisms capable of targeted *in situ* delivery of bioactive molecules. Such therapies (or biotherapies) may have particular relevance for the treatment of certain intestinal disorders such as Crohn's disease and ulcerative colitis. Animal experiments have evaluated the establishment, persistence and localization of specific biotherapeutic agents within the murine intestinal tract, in addition to their ability to influence the development of inflammatory disorders. Open-label studies in patients with mild to moderate Crohn's disease have begun to expand the scientific rationale upon which the concept of bacteriotherapy of inflammatory bowel disease is based. However, overcoming doubts relating to efficacy *in vivo* will require rigorously performed, controlled, double blinded trials capable of lending credence and impetus to the widespread application of these sophisticated developments.

The PROGID project represents the continuation of long-term scientific collaborations funded through the biotechnology programmes and initiatives of the European Commission. Within this project, the PROGID partners have initiated two distinct long-term (one year), large-scale, multi-centred, randomised, double blind, placebo-controlled probiotic-based clinical trials of remission maintenance within a subset of the European Union population suffering from Inflammatory Bowel Disease (IBD). These studies, which comply with standards of good clinical practice (GCP) normally associated with the assessment of pharmaceutical compounds, have received approval from local ethics committees in Ireland, France, Finland and Spain. In addition, the Irish involvement in the studies has received a positive evaluation from the national authority responsible for implementation of the forthcoming EU Directive on clinical trials. Completion of the on-going trials will elucidate the role of enteric flora in initiating or promoting inflammatory events in the pathogenesis of inflammatory bowel disease and determine whether microbial components of the

dysfunctional gut may be influenced through consumption of probiotic micro-organisms.

Progress achieved in completing the PROGID trials, emerging results and the support of the European Commission have been the subjects of dissemination strategies involving television and newspaper interviews, publication of relevant scientific review articles, and dedicated internet web-sites.

Probiotic strains with designed health properties

A. Mercenier

Institut Pasteur de Lille, France

The Lactic Acid Bacteria (LAB) are well-known for their extensive use in the preparation of fermented food products. In addition, the potential health benefits they may exert in humans has been intensively investigated during the last century. However, the mechanisms underlying the health promoting traits attributed to LAB, especially lactobacilli, remain vastly unknown which has impaired the rational design of probiotic screening methods with accurate predictive value. As the probiotic area corresponds to a rapidly expanding market, the EU granted DEPROHEALTH (Probiotic strains with designed health properties) project first aims at establishing a correlation between *in vitro* tests and mouse models mimicking important human intestinal disorders such as Inflammatory Bowel Disease, *Helicobacter pylori* and Rotavirus infections. As these diseases correspond to major public health problems, second generation probiotic strains with enhanced prophylactic or therapeutic properties will also be designed during the project. These « designed » strains and the isogenic parental ones will be compared to unravel mechanisms involved in the immuno-modulation capacity of specific *Lactobacillus* strains.

The general purpose of the DEPROHEALTH project is to acquire knowledge about the molecular factors affecting the immunomodulation and/or immuno-genicity of selected probiotic lactobacilli so that, in the future, isolates with enhanced protective or therapeutic effect can be screened for or engineered.

The aim of this proposal is:
- on one hand, to unravel mechanisms and identify key components of the immunomodulation capacity of probiotic lactobacilli ;
- on the other hand, to design « second generation » probiotic strains with enhanced properties against gastro-intestinal disorders.

From a practical point of view, this research programme includes the construction and immunological evaluation of a collection of recombinant *Lactobacillus* strains which will produce a biological factor susceptible to modulate the immune response either directly (e.g. interleukins, antigens) or indirectly (e.g. adhesion or targeting molecules). The properties of the modified strains will be tested *in vitro* and *in vivo* (mouse models of intestinal inflammation or infection) and compared to those of the parental strain.

The DEPROHEALTH project belongs to an EU granted research cluster on probiotics (Title : Food, Gastrointestinal-tract Functionality and Human Health ; Acronym : PRO-EU-HEALTH). The overall objective of this collaboration is to foster scientific networking between complementary European research projects that have the common objective to enhance consumer health through the development and exploitation of efficacious probiotic functional foods.

HIGHLIGHTS OF PROTECH: Nutritional enhancement of probiotics and prebiotics: Technology aspects on microbial viability, stability, functionality and on prebiotic function

D. Knorr

Department of Food Biotechnology and Food Process Engineering
Berlin University of Technology, Germany

The summary of the Scientific Concepts of Functional Foods in Europe (EUR 18591, European Commission, Luxembourg, 2000) as a result of a Commity research project on 'Functional Food Science in Europe' (FUFOSE) listed various key technological challenges and research opportunities in functional food science.

Among those the following seemed especially relevant:

- to identify viable technologies for the use of new raw materials as sources,
- to generate new functional carbohydrates,
- to modify fermentation processes for microorganisms to retain their viability during food processing, storage or preparation,
- to develop probiotics with increased resistance to the environment within the human intestinal tract,
- to optimize the beneficial effects of new carbohydrates.

Based on such challenges and opportunities the PROTECH project has been developed with special emphasis on probiotic viability and stability, on probiotic-prebiotic interaction, and on prebiotic function and probiotic functionality. The ultimate goal of the project is to develop product concepts and process design that can be transformed into real food products and process technologies by the industrial partners involved.

Data will be presented in relation to the project milestones and deliverables achieved so far.

PROPATH: Molecular analysis and mechanistic elucidation of the functionality of probiotics and prebiotics in the inhibition of pathogenic microorganisms to combat gastrointestinal disorders and to improve human health

L. de Vuyst

Research Group of Industrial Microbiology, Fermentation Technology and Downstream Processing, Vrije Universiteit Brussel, Belgium

The PROPATH project brings together 7 research partners (3 universities: VUB, AUN, AUA; 2 research institutes: INSERM, IPH; 2 companies: Beldem S.A., Dexter Com srl) and 2 subcontractors (gastroenterology hospitals) from 5 European countries (Belgium, France, Norway, Greece, and Romania). It will be carried out in the frame of Key Action 1 (Food, Nutrition and Health) of the 5th Framework Programme (Quality of Life and Management of Living Resources).

The PROPATH project will contribute to the scientific approval of underlying health-promoting properties ascribed to probiotic lactic acid bacterium strains. It will try to understand the mechanism of the inhibition of pathogenic bacteria that cause gastrointestinal disorders. Two cases will be studied in detail:

- enterovirulent *Salmonella typhimurium* that cause diarrhoea; and
- *Helicobacter pylori*, a common strain causing gastritis and peptic ulcer disease.

The scientific and technological objectives of the PROPATH project are:

- to obtain a selection of probiotic or potentially probiotic lactic acid bacterium strains – in particular *Lactobacillus* spp. that belong to the *Lb. acidophilus* group, and *Bifidobacterium* spp. –, that display a clear inhibition of Gram-negative pathogenic bacteria, in particular towards pathogens (*S. tyhimurium*, *H. pylori*);
- to identify/purify and characterise the metabolite(s) and/or genes of selected strains (selected *Lactobacilli*, selected *Bifidobacteria*) responsible for the inhibition and/or killing of these Gram-negatives;
- to have the conditions unravelled and the kinetics modelled of the *in vitro* production of the antimicrobials of selected strains to predict their *in vivo* action;

- to establish *in vitro* (simulated gut fermentations, human cell lines) and *in vivo* (animal models) co-culture models to study the interaction between selected *Lactobacilli* and *Bifidobacteria*, inhibitory towards Gram-negative pathogens, and these Gram-negatives, and to have understood these interactions;
- to have the selected, approved probiotic lactic acid bacterium strains tested in clinical studies in certain gastrointestinal diseases, including *H. pylori*-positive dyspepsia, irritable bowel syndrome, and acute infants' diarrhoea.

Biosafety evaluation of probiotic lactic acid bacteria used for human consumption (PROSAFE)

H. Goossens, B. Ooms, C. Lammens, S. Chapelle and V. Vankerckhoven

Department of Medical Microbiology, University of Antwerp, Belgium

In recent years, interest has renewed in health promotion and disease prevention by the incorporation of probiotic bacteria into foods to counteract harmful bacteria in the intestinal tract. Most of these bacteria are part of fermented dairy products, but some are sold in the form of powders, capsules or tablets as nutritional dietary supplements, or as biotherapeutic agents. The bacteria used as probiotics usually belong to the genera *Lactobacillus*, *Lactococcus, Bifido-bacterium* and *Enterococcus.* This group of genera is also called lactic acid bacteria (LAB) because of similar physiological and biochemical properties and the sharing of a common ecological niche: the gastro-intestinal tract. These probiotic LAB have a long history of safe use, but recent clinical reports associate LAB with human infection in healthy and immunocompromised subjects, and there is evidence that these organisms play an important role in the spread of antibiotic resistance genes.

The aim of the project is the safe use of probiotic LAB (e.g. lactobacilli, lactococci, enterococci, bifidobacteria) for human consumption, by proposing: (i) criteria, standards, guidelines, and regulations; (ii) procedures and standardised methodologies of pre-marketing biosafety testing and post-marketing surveillance.

The expected results of the project are: culture collection and database of probiotic and other LAB, standardised methodologies to detect antibiotic resistance in LAB, investigation of (potential) virulence properties in LAB, and their association with clinical disease and results obtained in rabbit and rat endocarditis models, potential immunological adverse effects of LAB, genetic stability and colonisation of probiotic LAB in the human gastro-intestinal gut and recommendations for biosafety evaluation of probiotic LAB.

The progressive increase in bacterial antibiotic resistance has stimulated scientists to look for other strategies to combat spread and effects of microbial resistance. Probiotics may provide an alternative to antibiotics, and the safe use of probiotics might reduce the use of antibiotics and thus the selective pressure.

The final result of the present project proposal will be a classification of potential probiotic strains with respect to safety. It will allow the discrimination of strains that may cause problems and strains that can safely be used as a food additive, even for people with a disease.

The end user groups of these results are: academia, general food industry and starter culture producers, consumers.

Overview of EU and MICROFUNCTION project

G. Gibson

Food Microbial Sciences Unit, The University of Reading, UK

The principal intention of the project will be 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.

Project partners are University of Reading, UK; University of Turku, Finland; Wageningen University, The Netherlands; Lund University, Sweden; University of Tartu, Estonia; Tiense Suikerraffinaderij NV, Belgium; Probi AB, Sweden and University New South Wales, Australia.

Genomic approaches for developing biomarkers for the gastrointestinal tract

W.M. de Vos

Wageningen Centre for Food Sciences and Laboratory of Microbiology,
Wageningen University, The Netherlands

The human gastro-intestinal (GI) tract harbors a vast array of microbial species that have only partly been cultured but interact in various ways with the host.in which they reside. A variety of molecular methods have been applied to disclose the diversity of these GI-tract microbes but novel approaches need to be developed to determine, predict and possibly control their functionality and analyze their effect on the host. We have initiated high-throughput rDNA, partial and complete genome-based approaches to quantify the relation between the microbial composition and the host, to determine the diversity of specific functional groups of GI tract microbes, and to follow the fate and monitor the gene expression of specific ingested microbes. Based on family studies and analysis of mono- and dizygotic twins it was found that the host genotype contributes to the dominant microbial diversity indicating specific interactions between microbes and man. Molecular avenues to provide insight into these interactions and find the functionality of GI tract microbes based on the global and specific gene expression of the microbes and their host will be discussed together with their potential for developing new molecular biomarkers.

LABDEL: a European funded project on lactic acid bacteria as delivery vehicles

J.M. Wells

Head Bacterial Infection & Immunity, Institute of Food Research, Norwich, UK

In the EU funded LABDEL project *Lactococcus lactis* and *Lactobacillus plantarum*, both members of the group of harmless commensal lactic acid bacteria will be used to orally deliver vaccines and therapeutic agents to the intestinal tract (LABDEL). As strains that are lytic in the intestinal tract (IT) are anticipated to be more effective at delivering therapeutic enzymes and allergens to the gut we will engineer LAB strains that are conditionally lytic in the IT (IT-lytic strains). Genetic systems will be developed to control dissemination of live recombinant LAB and to provide non-antibiotic selection systems for fermentation and cell engineering. Strains of LAB expressing protein antigens will be evaluated as a live and killed cell vaccine to protect against invasive disease and nasopharyngeal carriage with *S. pneumoniae*. The possibility of preventing allergy or inhibiting allergic responses using IT-lytic and laboratory strains of LAB expressing an allergen will be tested in an animal mode of human type I allergy. Similarly, the therapeutic and health related effects of LAB delivery of an antioxidant and an enzyme for the treatment of a nutritional disorder will be evaluated in animal models. Research is also aimed at improving the efficiency of existing fermentation technology for *L. lactis* and developing a fermentation method for *Lb. plantarum*.

GI tract disease models

J.K. Collins

Depts of Medicine & Microbiology, National University of Ireland Cork, Ireland

There is a consensus view developing that inflammatory bowel disease (Crohn's disease and ulcerative colitis) in humans results from a combination of genetic immunologic and environmental factors, in particular gut microbial flora. Much of the recent advances in understanding mucosal immunity, microbial flora and pathophysiological consequences have been derived from *in vitro* and *in vivo* animal models. These models provide information on the early events in the development of colitis, the chronic inflamed state and the induction of colon cancer.

We have used an *in vitro* transwell model to investigate the tricellular cross talk between the epithelium, the lamina propria and the microbial flora. This model can be challenged with proinflammatory stimuli and probiotics in order to screen for the most active anti-inflammatory strains. *In vivo* animal models using mice, rats, rabbits and primates include spontaneous, inducible, adoptive transfer, transgenic and gene knockout. Spontaneous models may closely mimic human disease.

In a germ -free state or under high dose broad spectrum antibiotic therapy inflammation in most models is either greatly reduced or absent indicating the activity and maintenance role of laminal microbial antigens or flora. These models provide an invaluable insight into pathological and protective immunoregulatory mechanisms. They also provide us with opportunities to test new and novel therapeutic strategies including probiotics. Results to date suggest that there are model specific responses to probiotics and hence, these models may help to screen probiotic strains for application in specific conditions. Studies with these models have led to the selection of strains currently being evaluated in human clinical trials.

Emerging techniques for prebiotic products

F. Voragen, G. Beldman and S. Hinz

Laboratory of Food Chemistry, Wageningen University, The Netherlands

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon (Gibson and Roberfroid, 1995). The most important group of prebiotics are formed by non-digestible oligosaccharides (NDO's), carbohydrates with a low degree of polymerisation (ranging from approximately 2–20 monosaccharide units) which are not digested by the host's digestive system. The chemical structures of oligosaccharides may vary widely and are determined by the identity of the monomeric sugar moieties, the degree of polymerisation, the type of linkage between the monomeric units, the complexity of the molecule (branched or linear) and possible linkage to non-carbohydrates.

NDO's are produced commercially using mainly enzymatic processes involving either the hydrolysis of polysaccharides or synthesis starting from smaller carbohydrates using predominantly a transglycosylation reaction. NDO's also occur naturally and can be obtained by extraction from suitable sources.

A number of potential benefits of prebiotics (NDO's) are mentioned in the literature: Low plasma glucose and insulin excursions, plasma lipid modification, low oral acidification (dental caries prevention), low energy value, value as laxatives (constipation), dietary bulking, stool bulking/dilution, generation of butyrate and acidification of the colon (Livesey, 2001), enhance a more saccharolytic rather than a proteolytic fermentation (putrification), decrease in the production of mutagenic or carcinoginic intermediates from specific food components, enhance production of bacteriocins, influence attachment of other micro-organisms (pathogens), may be anti-microbial, increase mineral absorption. Also potential risks of prebiotics are mentioned (Livesey, 2001): diarrhoea, abdominal discomfort, impaired nutrient intake (infants and young children). Prebiotics may also effect other characteristics of a food product e.g. viscosity, chemical reactions with other food components (Maillard reactions), bulking, sweetness and mimic fat characteristics.

Prebiotics can also be used in combination with probiotics. Such formulations are called Synbiotics and have the ability to beneficially affect the host by improving the survival and the implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating

the metabolism of one or a limited number of health-promoting bacteria (Roberfroid, 1998). Essentially the interaction between pre-and probiotics takes place in the colon, after its consumption. They can both contribute to the health promoting processes taking place in the gut. However, it is envisioned that a positive interaction between pre- and probiotics can already occur in an earlier stage of the production of a synbiotic. For instance the viability of a spray-dried probiotic preparation could be increased by a prebiotic (for instance NDO's). Besides this processing effect it is also expected that survival of probiotic bacteria in the first part of the digestion track (low pH, inactivation by bile acids) will be improved by a protecting effect of prebiotics.

Once 'survived' the processing step and the first part of the digestion track, we foresee that the vitality of probiotic bacteria will be better, due to the direct availability of a potential substrate.

In our presentation we will discuss these aspects in the context of current developments.

Progress on the PROEUHEALTH consumer platform

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The aim of the consumer platform is to deliver information about the ongoing research in the ProEuHealth-cluster in a form that is comprehensible for the consumers. The tools for doing this are one page leaflets, website and press releases that tell about the results gained in projects.

One page leaflets contain an overall description of the cluster and a short description of the aims of the five cluster projects. The basic text was written in English and then translated into nine (French, German, Italian, Spanish, Greek, Dutch, Portugese, Swedish, Finnish) European languages. The text was checked by experts before and after translations. The leaflets are freely available for all those interested and by the end of October 2001, 1850 leaflets have been distributed to European consumers. The leaflets have the address for the ProEuHealth website as a reference for further information.

Website forms the core action for the consumer platform. In addition to the short leaflet texts, the website has a longer description of all projects in English. The website also contains a direct mail link that enables sending comments and questions to the consumer platform. The questions will be answered by the platform directly or they will be referred to the appropriate person within the cluster projects.

From each project a short press release of the first year results will be written by March 2002. These can directly be used by newspapers and magasines in different parts of Europe. The English press releases will be located in the ProEuHealth cluster's own website and also FLAIR FLOW -network is asked to distribute them to their own contacts.

Cluster will have new projects added to it in the beginning of 2002. The leaflets and websites will be renewed by adding these components into them. Contact address for the website is http://proeuhealth.vtt.fi and questions and requests for consumer leaflets can be sent directly by e-mail to proeuhealth@vtt.fi.

Health in consumer food choice

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Health is one of the major motives in consumer food choice. However, health competes with other basic motives like sensory pleasure and convenience, healthy aspects of food products may be misperceived or ignored, and positive health effects of food products are largely invisible and abstract to the consumer. For these reasons, potentially positive health effects of food products may actually not materialise. In this presentation, the importance of health in consumer food choice will be compared over time in a number of EU countries, examples of the way consumers associate food characteristics with health will be presented, and a number of conditions for positive acceptance of functional foods will be discussed. We will also address a number of unresolved issues concerning health in consumer food choice.

Industrial developments in probiotic functional foods: the health-promoting potential of current and future probiotics

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A probiotic has been defined by the ILSI Europe working group as "*a viable microbial food supplement which beneficially influences the health of the host*" (1998). This definition implicitly takes into account the fact that safety and efficacy of probiotics and industrial probiotic products have to be scientifically demonstrated for each strain and product. Demonstration of health effects requires several different aspects of scientific studies, which have been conducted with several products. The same ILSI Europe working group also defined probiotic foods to be functional if they have been satisfactorily demonstrated to beneficially affect one or more target functions in the body beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease (1999). These definitions set the basis for both developing and assessing the health promoting potential of probiotics.

It is important to understand that all probiotic strains are different and their identification and characteristics should be well-defined. Thus, studies on even closely related strains cannot be extrapolated without great caution. It is important to clearly identify them using modern methodology and also to make all study strains available for all research groups participating in assessment work. At the end, the studies should be conducted using the final product in all human studies. Examples of this procedure can be found in t he Probdemo working group reports.

The assessment of the health promoting potential of probiotics and probiotic functional foods has to be based on valid scientific hypothesis and studies supporting the hypothesis. Knowledge of the mechanisms is an important factor, complemented with target functions and biomarkers that are accepted as relevant to the state of health and well-being or reduction of risk of disease. The hypothesis can be supported by studies carried out *in vitro* using cell culture models or *in vivo* using animal models. However, the most important studies are carefully monitored clinical studies in human subjects. All data has to be assessed by studies in human subjects, conducted preferably by at least two independent research groups in different locations. Multi-center studies offer a

good solution for this assessment area. In conclusion, well designed human studies with requirements similar to those for pharmaceutical studies, are required to demonstrate health benefits. Additionally, epidemiological studies or post-marketing surveillance are recommended to further assess both safety and efficacy of probiotics. Using these criteria, several health promoting effects can be considered scientifically proven for specific strains and products. Understanding the scientific basis for probiotic action has provided us with new selection procedures for future probiotics and set a basis for developing new probiotic functional foods. The future appears to offer opportunities for general probiotics as well as disease specific probiotic foods.

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POSTERS

First studies on the effect of pulsed electric field treatment on the stability and productivity of probiotics

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Pulsed electric field treatment is one of the emerging processing technologies which is categorized into non thermal processing. By the exposure of biological cells to an external electrical field for a few microseconds a rapid electrical breakdown and local structure changes of the cell membrane could occur. This effect results in a drastic increase in permeability of the cell membrane. Based on the irreversible permeabilization of cell membrane, several practical applications were developed, such as inactivation of microorganisms and enhancement of mass transfer during extraction of intracellular material.

On the other side, exposure of biological cells, such as plant tissue or microorganisms towards a sub-lethal treatment conditions can induce stress reaction, mainly based on transient electrical polarisation along the cell membrane. This phenomenon of stress reaction in microorganisms using other physical agents was observed. Incubation of microorganisms in sub-optimal growth temperature led to the accumulation of stress metabolites, which protect them against severe conditions (e.g. exposure to heat and freezing). In our ongoing work the effect of pulsed electric field treatment to induce stress in probiotics as a processing strategy to increase their stability is investigated. Furthermore it will be evaluated how the productivity of probiotics could be positively affected with regard to milk acidifying capacity. Reversible pore formation during exposure to external electric field also offers an interesting approach in active insertion of cryoprotectants into microbial cells to facilitate intracellular accumulation.

Bacteriocin production by probiotic *Lactobacillus* strains may cause the antibacterial activity against *Helicobacter pylori*

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There are several reports on the inhibition of the gastric pathogen Helicobacter pylori through probiotic Lactobacillus strains. However, the underlying mechanism is not clearly understood. This study aims at the isolation of bacteriocins from probiotic *Lactobacillus* strains that may be responsible for growth inhibition of *H. pylori*.

Eight *Lactobacillus* strains belonging to the *Lactobacillus acidophilus* and *Lactobacillus casei* groups, encompassing several probiotic strains, were examined for the production of bacteriocins. Bacteriocinogenic material was isolated from a 12-h culture in MRS broth using a protocol developed for the isolation of such small and hydrophobic peptides. It was tested against a variety of closely related, Gram-positive bacteria. Subsequently, active samples were characterised with respect to heat stability, pH stability and sensitivity towards several enzymes. The molecular mass was estimated by tricine-SDS-PAGE. Finally, the active material was examined for its ability to inhibit the *H. pylori* reference strain ATCC 43504 by supplementation of a buffer solution of *H. pylori* cells with purified bacteriocin samples.

Six strains were shown to produce bacteriocins, namely *Lb. johnsonii* LA1, *Lb. casei* YIT 9029, *Lb. casei* Imunitas, *Lb. gasseri* K7, *Lb. amylovorus* DCE 471 and *Lb. acidophilus* IBB 801. The antibacterial molecules were characterised as heat-stable and stable in a pH-range from 2.2 to 8.0. Their proteinaceous nature was shown by the loss of bacteriocin activity after treatment with several proteinases. The molecular masses were determined at 3.4, 4.8, 6.3 and < 6.5 kDa for the bacteriocins from *Lb. johnsonii* LA1, *Lb. amylovorus* DCE 471, *Lb. gasseri* K7, and *Lb. acidophilus* IBB 801, respectively. Four bacteriocins were tested against the *H. pylori* reference strain ATCC 43504. Already after 2 h of

incubation, a decrease in CFU titre of *H. pylori* of almost one log unit was observed for the bacteriocins from *Lb. johnsonii* LA1, *Lb. casei* YIT 9029 and *Lb. amylovorus* DCE 471, but not for that of *Lb. acidophilus* IBB 801.

Several probiotic *Lactobacillus* strains produce bacteriocins. The present study indicates that this production of bacteriocins may play an important role in the growth inhibition of the common gastric pathogen *H. pylori*.

Improved survival of *Lactobacillus paracasei* NFBC 338 in spray dried powders containing gum acacia

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Viability and stability of probiotic bacteria in spray dried milk powders are requirements for their application in the development of functional dairy foods. We have previously demonstrated that the stability of Lactobacillus paracasei NFBC 338 following spray drying was inversely related to storage temperature (Gardiner et al., 2000). In this study, we examined the potential of encapsulation of the probiotic Lactobacillus during spray drying in gum acacia for enhanced stability during powder storage. Lactobacillus paracasei NFBC 338 was grown in a mixture of reconstituted skim milk (RSM, 10%) and gum acacia (10%) to mid log phase and spray dried at outlet temperatures between 95 and 105 °C. The resulting probiotic powders were stored at 4, 15 and 30 °C for up to 2 months and the survival of the gum-treated lactobacilli compared with non-encapsulated (free) cells. The encapsulated lactobacilli survived up to 5-fold better than the non-encapsulated free cells in powders stored for 2 months at 4 °C and 30 °C, while the protection afforded by encapsulation was higher in powders stored at 15 °C for 2 months, with 200-fold higher survival of the Lactobacillus paracasei NFBC 338 culture obtained. The survival of the probiotic spray dried culture was also enhanced by encapsulation, when subsequently exposed to porcine gastric juice, in an effort to investigate the performance of the encapsulated probiotic strain during gastric transit. Survival of encapsulated Lactobacillus paracasei NFBC 338 declined by up to 4 logs following 2 hours exposure to porcine gastric juice, in which the pH was maintained constant at 3.0, while there was a 6-log decline in the non-encapsulated spray dried culture. The data indicate that encapsulation of probiotic Lactobacillus paracasei NFBC 338 in gum acacia prior to spray drying enhances survival during subsequent powder storage and during gastric transit.

Evaluation of the effect of atmospheric conditions and broth media on the growth of *Bifidobacterium* species

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The growth of four strains of bifidobacteria isolated from human sources was investigated under a variety of atmospheric conditions and in a range of different commercial broth media. Bifidobacterium infantis UCC 35624 reached numbers of 2 x 10^9 cfu/ml in MRS-cysteine when grown either in an anaerobic jar or under a layer of mineral oil. Levels of 3 x 10^8 cfu/ml were obtained when this strain was grown as a static culture or in an nitrogen filled chamber. MRS-cysteine was the optimum growth media for this particular strain; the numbers obtained in other media were 3.5 x 10^7 cfu/ml for RCM, 7 x 10^8 cfu/ml for BHI and 1 x 10^8 cfu/ml in TPY. In the case of B. breve NCIMB 8807, highest cell numbers (2–3 x 10^9 cfu/ml) were attained under mineral oil or in an anaerobic jar in MRS-cysteine or TPY. For the other Bifidobacterium strains examined the highest numbers obtained were between 4–7 x 10^8 cfu/ml in RCM and anaerobic conditions generated using mineral oil were as effective as those generated by other methods.

Molecular diversity of *Lactobacillus* spp. in the human intestine evaluated by specific amplification of 16S rRNA and DGGE

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Current molecular approaches for rapid evaluation of the gastrointestinal tract (GIT) microbiota focus on numerically dominant bacteria and do not address the diversity of other species present in low numbers such as the lactobacilli, that are less than 1% of the total community. A Lactobacillus group-specific PCR primer was developed to selectively amplify 16S ribosomal DNA (rDNA) from lactobacilli and related lactic acid bacteria, including members of the genera Leuconostoc, Pediococcus, and Weissella. Amplicons generated by PCR from a variety of GIT samples, including those originating from feces and cecum, resulted predominantly in Lactobacillus-like sequences. Approximately 28% were most similar to the 16S rDNA of Lactobacillus ruminis, and also Leuconostoc species were retrieved that, so far, have only been detected in environments other than the GIT, such as fermented food products. Changes in time of the GIT bacterial community in different age groups were studied using Lactobacillus-specific PCR and denaturing gradient gel electrophoresis (DGGE) of the 16S rDNA amplicons. The Lactobacillus community in adults over a two year period showed variation in composition and stability depending on the individual, while successional change of the Lactobacillus community was observed during the first five months of an infant's life. Furthermore, the specific PCR and DGGE approach was used to study the retention in faecal samples of a Lactobacillus strain administered during a clinical trial. In conclusion, the combination of specific PCR and DGGE analysis of 16S rDNA amplicons, allows the diversity of important groups of bacteria that are present in low numbers in specific ecosystems to be characterised, such as the lactobacilli in the human GI-tract.

Inulin, fermented milk and brussel sprouts modulate the genotoxicity of IQ in rats associated with a human fecal flora

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To evaluate whether a diet which modulates the colic flora (fermented milk, inulin) may alter the genotoxicity of a heterocyclic amine (IQ) and the endogenous system of detoxication. Heterocyclic amines are pyrolysis products present in food and related to carcinogenesis.

42 germfree rats were inoculated with the fecal flora of a human donor with a high β-glucuronidase activity. They were thereafter divided into 5 groups. They received a human-type diet enriched or not with either one of four candidate protective ingredients: inulin (short and long chains), fermented milk and brussel sprouts. After 4 weeks, DNA-damage induced by IQ was measured by the comet assay in the colon of 4 rats per group. In the remaining rats, phase I (cytochrome P450 1A2) and phase II (GST, UGT, NAD-QR) enzymes were assayed in the liver and the bacterial enzymes and metabolites in the caecal contents.

The genotoxic effects of IQ were decreased by the consumption of Brussel sprouts, the probiotic and inulin. Brussels sprouts increased cytochrome P450 1A2, glutathion-S-tansferase, NAD-quinone reductase and UDP-glucuronosyl-transferase activities. Short-chain inulin decreased the activity of ß-glucuronidase. Inulins increased short-chain fatty acids in the caecum and decreased the D-lactate. All diets decreased ammonia.

Pre and probiotics modified the metabolic properties of the gut microflora while brussel sprouts modulated the endogenous detoxication pathwayto inhibits the colic genotoxicity of IQ by.

A large expertise to understand the mechanisms of protection of pre- and probiotics on the colon: experimental carcinogenesis and gnotobiotic rats

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Although the role of nutrition in carcinogenesis is largely investigated, fewer studies take into account the diversity of the human flora in the beneficial effects of prebiotics and probiotics.

Several bacterial enzymes are involved in the metabolism of xenobiotics. They also interfere with the enterohepatic recycling of carcinogens and may potentiate their toxicity. Indeed, several colon carcinogens are (not)less potent in germ free than in gnotobiotic rats.

We are developing a research group focused on the relationship between the colon carcinogenesis and the gut microflora. We have a large expertise (physiology, nutrition, bacteriology, biochemistry and toxicology) and a unique experimental model of gnotoxenic rats and mice. We can: 1. examine the flora and its dynamics by molecular means and computer-assisted data analysis, 2. analyze and identify the bacterial metabolites of dietary substances, 3. follow the detoxication of carcinogens and anti-carcinogens both by the endogenous and bacterial enzymes (with a special attention to β-glucuronidases in vivo), 4. study a large panel of biological markers of carcinogenesis such as cell proliferation and apoptosis, genotoxicity, and preneoplastic lesions.

We are currently evaluating the effects of some pre and probiotics on the colon carcinogenesis in rats associate with a human flora (see the poster from humblot et al in this session). Further developments of this model are considered. Any proposition of partnership can be discussed.

In vitro evaluation of the probiotic potential of *Lactobacillus* strains and their application in yoghurt

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The aim of this study was to examine the probiotic potential of *Lactobacillus* spp. and their performance in yoghurt. Thirty-four *Lactobacillus* strains isolated from traditional Greek dairy products or obtained from international culture collections were examined for properties relevant to their survival in human gastrointestinal tract. Strains were first identified by sugar fermentation patterns, type of lactic acid produced and by SDS-PAGE analysis of whole cell proteins. Strains were then tested with respect to resistance to low pH conditions, as well as resistance to bile salts and proteolytic enzymes. In view of their applicability in fermented dairy products, their ability to acidify milk as well as their antagonistic activity against yoghurt starters was also examined. Seven strains were found to possess properties allowing them to survive in the human gastrointestinal tract. Three of these strains were used as adjunct starters in probiotic yoghurt production. Among them, strain *L. paracasei* subsp. *tolerans* ACA-DC 4037, isolated from Kasseri cheese, has been identified as the best strain contributing to a high microbiological, physicochemical and sensory quality yoghurt.

Expression of the rotaviral antigen VP8* in *Lactobacillus casei* and *Lactococcus lactis*

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We have chosen the VP8* fraction of the rotavirus capsid VP4 protein to improve Lactobacillus casei immuno-protective properties against rotavirus, because purified VP8* is a highly immunogenic polypeptide in mice, inducing effective homotypic protection against disease in pups born to dams immunized with this antigen.

The nisin inducible system (NICE) have been used to control the expression of VP8* in Lactococcus lactis and Lactobacillus casei.. A very efficient expression of VP8* was achieved using Lc. lactis as a host. The nisRK regulatory genes were integrated in monocopy into L. casei chromosome, which proved to drive constitutive expression of VP8* from the nis promoter in this host. A strong VP8*-specific IgG response was obtained in mice after IP administration of cell extracts from VP8*-expressing L. casei and Lc. lactis strains.

The cDNA coding for rotaviral VP8* protein was also cloned under the control of the lactose inducible promoter from L. casei which permits the controlled expression in this host. Several constructions have been engineered which allow the secretion of the antigen and its anchoring to the cell wall be means of the secretion signal and cell wall anchoring domain of the endogenous L. casei surface protease (PrtP). Experiments on immune response after oral administration in mice of different expressing strains are under way.

Starch based controlled release systems for probiotics

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The main carbohydrate in all nutrition, consumed mostly in cereal and root crop based foods is starch. Starch granular properties can be modified by using physical, enzymatic or chemical treatments. Native starch polymers offer many functional properties, and especially linear polymers are easily forming structures which are rather stable against acids and amylases.

This work has focused on development of starch based technology for improving stability of probiotics mainly against ambient and high humidity and in the human digestive tract. The main idea of the technology is to try to apply a batch process, in which bacteria are first cultivated and bound into starch granules, and then the whole mixture is coated with amylose-rich starch. Native potato starch granules are used as bacteria carriers, and in order to make granules more porous they are slightly hydrolysed by amylase during the growing period. Before the coating step, amylose-rich starch is dissolved in water. The coating is performed by addition of the amylose solution, which will precipitate on the bacteria slurry. The product powder is produced by spray- or freeze-drying of the slurry.

As a test strain *Lactobacillus rhamnosus* (VTTE800, human origin) has been used. The product is white and smooth and the viability of the bacteria in the powder is stable at least 6 month when stored at room temperature and humidity. The powder has been also tested in *in-vitro* small intestine conditions, and showed to be stable against low pH and pancreatic amylase. Furthemore it was observed that in *in-vitro* fermentation the starch granule carriers were degraded relatively slowly by the colon bacteria. The research is at moment focused on of use of spesific hydrophobic materials for coating in order to improve the stability against high humidity, and on scaling up and optimisation of the process.

"Microbial Viability Technology" - team in the novel VTT research programme "Tailored Technologies for Future Foods"

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Tailored Technologies for Future Foods (TTFF) is a four-year food research programme, which emphasizes the development of specific technologies for tailored food quality attributes. The research is conducted by seven teams, including "Microbial Viability Technology". The research work within this team is executed in seven projects, both national and international, ongoing at VTT Biotechnology. Microbial viability technology -projects develope and apply molecular and technological tools to control the viability, activity and stability of yeasts and probiotic bacteria. The developed tools are applied to isolation and characterization of microbes, selection of growth media and growth conditions, and improvement of down stream processes and food products. The special emphasis of the team is placed on controlling stress effects of probiotics in production and storage, ensuring probiotic functionality, and protection of probiotic cells by starch encapsulation. Microbial activity and viability is evaluated by conventional methods as well as non-culture techniques, such as RT-PCR (reverse transcriptase PCR) and fluorescence techniques.

Development of probiotic ice cream for use as a delivery system for strains of *Lactobacillus* and *Bifidobacterium*

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Ice cream was manufactured containing high numbers of Lactobacillus salivarius subsp. salivarius UCC118 or Bifidobacteria infantis UCC 35624 or both. These cultures were originally isolated at UCC from the human small intestine and have demonstrated significant probiotic potential. The cultures were allowed to grow, either on their own or in co-culture, in a number of different ice cream mixes prior to freezing. Cell numbers > 10^8 cfu/ml were achieved in both commercial and 'home made ice' cream mixes. Anaerobic conditions did not have any effect final cell numbers. Addition of 1% malt extract to the 'home made' mix did not result in elevated cell numbers, even though addition of malt extract did stimulate growth of these cultures in milk. Both species survived the freezing process resulting in products with cell numbers between 10^7 and 10^8 cfu/ml. After 3 months storage at –16ºC viability remained > 10^7 cfu/ml. Lactobacillus salivarius subsp. salivarius UCC118 produces the bacteriocin AB118. This bacteriocin was produced during growth in the ice cream mixes and was detectable in the frozen ice cream for at least 3 months.

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

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Lipoteichoic acids (LTA) and cell wall teichoic acids (WTA) represent a major constituent of the Gram-positive cell wall. They consist of polyglycerolphosphate or polyribitolphosphate substituted with D-alanine residues whose amino groups partially compensate for the negative charges of the phosphates. As a first step towards elucidating the function of D-alanine substitutions, the dlt genes responsible for teichoic acid D-alanylation in L. plantarum were cloned. The 4 dlt genes (dltA, dltB, dltC and dltD) were found to be clustered, as already described in other Gram-positive species. In addition, a pbpX gene encoding a protein homologous to low molecular weight penicillin-binding proteins (PBP) was found to adjoin the L. plantarum dlt cluster. Northern blotting analyses using dltA and pbpX probes demonstrated that the 5-gene cluster was transcribed as a single polycistronic mRNA. A DltB minus strain unable to incorporate D-alanine in teichoic acids was constructed. It displayed an autolytic phenotype in stationary phase. This might be explained by an activation of autolysins as a consequence of the more negative character of teichoic acids due to the absence of D-alanine, as the cationic autolysins might bind more tightly to D-alanine deficient teichoic acids. Demonstration of an increased anionicity of the mutant cell wall was gained from the observation of an enhanced binding of the cationic protein cytochrome c. Further evidence for acidification of TA was sought by assaying the sensitivity of the Dlt^- mutant to the cationic antimicrobial compound nisin. The $DltB^-$ strain turned out to be 10 times more sensitive to nisin as compared to the control strain. We also characterized the cell surface by two physicochemical approaches. X-ray photoelectron spectroscopy (XPS) analysis showed a decrease of protonated nitrogen at the cell surface, in agreement with the loss of D-alanyl ester substituents. However, no significant modification of the global surface charge could be detected by microelectrophoresis. This can be explained by the small contribution of protonated amino groups to the overall charge of the cell surface relative to carbonyl and phosphate functions.

Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut

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Although not without exception, results from animal and human studies have suggested a moderate cholesterol-lowering action of dairy products fermented with appropriate strain(s) of lactic acid bacteria and bifidobacteria. Mechanistically, probiotic bacteria ferment food-derived non-digestible carbohydrates to produce short-chain fatty acids in the gut, which can then cause a decrease in systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver. Furthermore, some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts and therefore affecting the metabolism of cholesterol, or by directly assimilating cholesterol.

The objective of this study was to evaluate the effects of certain human-gut indigenous bacterial species on cholesterol concentrations in vitro. The initial approach used was to investigate whether the bacteria could utilise cholesterol and if so to isolate/identify which species were involved. This was accomplished using continuous enrichment cultures in chemostats inoculated with faecal material from healthy human volunteers using basal media supplemented with cholesterol and bile acids (data not shown). Potential probiotic strains among those isolated at steady-state were identified by partial sequencing of the bacterial 16S rRNA gene. Seven potential probiotic strains were found: Lactobacillus fermentum strains F53 and KC5B, Bifidobacterium infantis ATCC15697, Streptococcus bovis ATCC43143, Enterococcus durans DSM20633, Enterococcus gallinarum, and Enterococcus faecalis. A comparative evaluation regarding the cholesterol assimilation ability of these strains was undertaken along with commercial probiotic strains. The degree of acid and bile tolerance of strains was evaluated. Lactobacillus fermentum KC5B was regarded as a candidate probiotic to be used in humans due to its high acid and bile tolerance and its ability to assimilate cholesterol in vitro. This strain was further tested for growth and cholesterol assimilation with a range of commercial prebiotic substances in order to determine the most effective symbiotic mixture.

Keywords: cholesterol assimilation, bifidobacteria, lactic acid bacteria, Lactobacillus, probiotics

Biodiversity during sicilian cheese manufacture assessed by PCR and denaturing gradient gel electrophoresis

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Ragusano cheese is an artisanal Sicilian cheese with unique organoleptic characteristics created from traditional manufacturing practices and environmental conditions in the Hyblean region of Sicily. In the present study the microbial ecology of Ragusano and related cheese types during manufacture, which are produced from raw milk without the addition of starter cultures, was investigated by a combination of molecular and classical techniques. Molecular methods based on ribosomal DNA (rDNA) or rRNA allowed cultivation steps to be bypassed, and population dynamics were rapidly assessed using denaturing gradient gel electrophoresis (DGGE). The dominant microbiota and 'Lactobacillus' community were studied by PCR using bacterial primers to 16S rDNA or rRNA isolated from samples including milk, milk with added rennet, fresh cheese, and ripened cheese. The PCR amplicons were resolved by DGGE that revealed the changing biodiversity during cheese manufacture. Clone banks of 16S rDNA sequences from DNA of curd or cheese samples was constructed. Sequence analysis of the clones and 16S rDNA genes of pure cultures obtained by cultivation on selective medium revealed that members of the Streptococcus and Lactobacillus genera comprised the dominant microbiota. Cultivation-independent methods may be used to evaluate and improve the microbiological quality and safety of artisan fermented foods.

Does *Kluyveromyces lactis*, a yeast from cheese, mend the health of hiv + patients?

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Kluyveromyces lactis is a yeast used in the dairy industry. K. lactis has been isolated many times in the Mycology Laboratory of the Besançon University Hospital between 1991–1994. About 85% of the K. lactis strains came from the oral cavity of HIV-infected patients. Since 1999, the colonisation by K. lactis did not change in spite of new highly active antiretroviral therapy. However K. lactis remains still rare in daily work (0.5% from strains isolated from buccal swabs, whose 87.5% coming from HIV+ patients).

Our clinical study confirmed that no pathogenic effect could be attributed to this yeast. As electrophoretic karyotype analyses of strains isolated from patients and from cheese show the same species, it was supposed that the strains isolated in the oral cavity had come from food.

Some reference strains of K. lactis harbor two plasmids, pGKL1 (8.9 kb) and pGKL2 (13.4 kb). A gene located on pGKL1 codes for a killer toxin. This toxin is able to inhibit in vitro other yeasts, in particular, Candida albicans. A plasmid similar in size to pGKL1 was detected by agarose gel electrophoresis and ethidium bromide staining for 6 K. lactis strains isolated from patients and 3 strains isolated from cheese. The inhibition effect to the toxin was observed for 2 strains isolated from patients. These results would suggest the hypothesis of an antagonism between the colonisation by K. lactis, without any pathogenic effect, and the colonisation by C. albicans, that could lead to oropharyngeal candidosis.

Keywords: Kluyveromyces lactis, Cheese, Oral cavity, Killer toxin.

Inhibition of *H. pylori* colonization and associated gastritis in the HpSS1 C57BL/6 mouse model via administration of the probiotic *Lactobacillus paracasei* subsp. *paracasei* ACA-DC 6002

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This study examined the effect of prolonged administration of the probiotic *Lactobacillus paracasei* subsp. *paracasei* ACA-DC 6002 of the Agricultural University of Athens Dairy Cultures (ACA-DC) collection, on the HpSS1-induced gastritis in mice. The particular strain had been selected after *in vitro H. pylori* inhibition screening. Six week-old C57BL/6 female mice were infected with *H. pylori* by triple intragastric administration of the human HpSS1 strain. After 1 month the probiotic strain was administered in the infected study group once by single intragastric administration and then every day in the water supply at a dose of $5x10^8$cfu. Control groups of Hp-infected mice received MRS medium in the water supply. HpSS1 colonization and associated gastritis were assessed over a period of 9 months with the use of culture, urease test, PCR and histology of the gastric biopsies. The kinetics of probiotic colonization was followed by viable counts and RAPD-PCR typing. In the probiotic study group the grade of associated gastritis, whenever diagnosed, was significantly milder compared to the control group. HpSS1 strain was detected by PCR in 16 and by culture counts in 8 out of the 18 cases in the probiotic study group possibly due to the low number of colonizing bacteria. RAPD-PCR-typing confirmed the clonal relationship between the administered probiotic and the lactobacillus strain isolated in the feces. To conclude, prolonged high dose-administration of the probiotic ACA-DC6002 significantly reduced the *Hp* colonization and associated gastritis in the probiotic study group. Prevention of *Hp* infection experiments in mice pre-colonized by ACA-DC6002 are under way to support the *Hp* inhibition of colonization data.

Bifidobacterium and *Lactobacillus* strains from faecal samples of elderly subjects for a possible probiotic use in functional foods

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A new EU-funded project called "Crownalife" – to emphasise the preservation of the period of independence recognized as the "crown of life" – has been set up both to assess structural and functional alterations of the intestinal flora with ageing in Europe, and to validate functional foods that promote health by improving the function of the intestinal microflora in the elderly.

Species of bifidobacteria and lattobacilli have been isolated from faecal samples of 12 elderly subjects (74 years average) recruited from the "Sassatelli" boarding home in Fermo-Italy. This has been a preliminary phase to provide other partners with Lactic Acid producing Bacteria (LAB) for testing their anti-microbial activities against gastro-intestinal pathogens to develop synbiotic used as functional food ingredients, and for assessing the phylogenetic specificities of the gastro-intestinal microflora in the elderly.

Both habitat-simulating and selective media have been used to isolate LAB strains. The isolated colonies with different morphologies were identified using Rapid ID32A system combined with API 50 CH for Bifidobacterium species and API 50 CHL for *Lactobacillus* species. Total anaerobic and aerobic, *Lactobacillus* and *Bifidobacterium* counts were also performed on the same faecal samples.

The distribution of the identified species of the two genera points out a wide microbial diversity among subjects and even in a single person. Some of the identification results have been validated by using culture independent methods.

Development of a fermentation medium for probiotic lactobacilli and bifidobacteria

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The lack of firm knowledge on the primary factors responsible for probiotic viability, stability, and performance often hinders the exploitation of potentially probiotic strains in various functional foods. Also only limited information is available on the impact of processing and storage on probiotic viability, stability and functionality. The EU-project "Nutritional enhancement of probiotics and prebiotics: Technology aspects on microbial viability, stability, functionality and on prebiotic function" aims to increase this knowledge. A part of this project is to develop suitable media for selected probiotic strains (including strains representing *L. rhamnosus, L. salivarius, B. longum* and *B. animalis*) and to study the effects of the medium ingredients on probiotic viability, stability and functionality. In this paper we present data for fermentation media of probiotic bacteria.

The medium development was performed in shake flask cultivations and the Box-Wilson experimental design was used. The carbon source concentration was 20 g/l. The other components for the medium were glucose, lactose, maltose, and malt extract as carbon source; enzyme digested soy peptone, acid hydrolysed casein as nitrogen source and yeast extract as vitamin source. The criterion for medium was highest biomass measured as colony forming units (CFU).

From the results a universal medium for all the used probiotic strains was obtained: glucose 20 g/l, soy peptone 30 g/l, yeast extract 7 g/l, $MgSO_4$ $7H_2O$ 1 g/l, KH_2PO_4 1 g/l, K_2HPO_4 0.4 g/l. The CFUs obtained were in most cases better or as good as in the MRS-medium. Only *Bifidobacterium animalis* Bb12 had a lower CFU-number in this medium than in MRS.

The growth and carbon yield in the universal medium.

Strain	logCFU	logCFU in MRS	Yield [CFU/g carbohydrate]
Lactobacillus rhamnosus E800	9.2	9.5	1.8E+08
Lactobacillus salivarius UCC500	9.4	9.5	2.1E+08
Lactobacillus rhamnosus GG	9.2	9.1	1.6E+08
Bifidobacterium longum NCC490	8.7	5.9	8.8E+07
Bifidobacterium animalis Bb12	7.9	8.7	2.7E+07

Development of molecular approaches to study survival and activity of *Lactobacillus plantarum* WCFS1 in the human GI tract

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The human GI tract represents a dynamic ecosystem harbouring a great variety of micro-organisms. To study the functionality of these micro-organisms and their interaction with the host we are aiming to determine their in situ activity based on culture-independent approaches. We have chosen as a model micro-organism, *Lactobacillus plantarum* WCFS1 that is of human origin, shows considerable survival following oral consumption, and whose complete genome sequence has been determined. Comparison of the 5 rDNA operons on the chromosome of *L. plantarum* WCFS1 revealed highly similar rRNA genes, but showed that the number and kind of tRNA's present differed considerably. Also the rDNA promoters showed major differences. The expression of the operons under different conditions is presently been studied. The feasibility of using in situ hybridisation with fluorescent labelled 16S rRNA targeted probes (FISH) to measure the metabolic activity of WCFS1 is being evaluated and optimised. The influence of different growth conditions on FISH and the total cellular RNA content will be demonstrated and quantified.

Molecular analysis of bacterial communities in feces and colonic biopsies

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The human gastrointestinal (GI) tract harbors a diverse microbial community which mainly consists of obligate and facultative anaerobic bacteria. These bacteria are found in the mucosa, lumen, and feces. Since most attention has been focused on bacteria present in feces, knowledge about the mucosa-associated bacterial community at different parts in the colon is limited. In this study, a 16S ribosomal RNA (rRNA) approach was performed to unravel the community structure of bacteria in feces and biopsies from the ascending, transverse, and descending colon. To visualize the diversity of the predominant and the Lactobacillus group community, denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA gene amplicons was performed. DGGE analysis revealed that the profiles reflecting the predominant community were more complex than those reflecting the lactobacilli and related lactic acid bacteria. The predominant mucosa-associated bacterial community appeared to be host-specific, uniformly distributed along the colon but significantly different from that recovered in feces.

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Title

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

1st 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 4 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".

Keywords
Food, microbes, bacteria, functional food, probiotics, human health, analysis, safety, nutrition, PROEUHEALTH

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