The Food, GI-tract Functionality and Human Health Cluster

PROEUHEALTH

Brussels, Belgium

Workshop 4
March 10-11
2005

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

Programme ***Abstract, page***

THURSDAY March 10, 2005

9.30 – 10.00 Arrivals and Welcome Coffee

The PROEUHEALTH Cluster: Scientific Highlights
Morning Chair: Willem M. de Vos, Wageningen University, Wageningen, NL

12.30 – 14.00 Lunch

The PROEUHEALTH Cluster: Scientific Highlights
Afternoon Chair: Maria Saarela, VTT, Espoo, FI

15.00 – 15.30 Coffee & Tea Break

19.00 Dinner at LA QUINCAILLERIE, Rue du Page 45, 1050 Bruxelles, www.quincaillerie.be

FRIDAY March 11, 2005

8.30 – 9.00 Welcome Coffee

9.00 – 9.10 PROEUHEALTH Benefits
Morning Chair: Charles Daly, UCC, Cork, IE

9.10 – 9.30 Industry Benefits - Lactic Acid Bacteria Industrial Platform (LABIP)
Aat Ledeboer, Unilever, Vlaardingen, NL *16*

9.30 – 10.30 Industry Benefits - Short Statements from Industrial Partners

Danone – Jean-Michel Antoine, Paris, FR
Orafti – Jan van Loo, Tienen, BE *17*
Danisco – Nina Rautonen, Helsinki, FI
Microscreen – Tjwan Tan, Groningen, NL *18*
Numico - Jan Knol, Wageningen, NL *19*
Valio – Tiina Mattila-Sandholm, Helsinki, FI *20*

10.30 – 10.45 Coffee break

10.45 - 11.05 Consumer Benefits *21*
Liisa Lähteenmäki, VTT, Espoo, FI

11.05 –11.25 Science Benefits *22*
Willem M. de Vos, Wageningen University, Wageningen, NL

11.25 - 11.45 Commission Benefits & Future of GutHealth Research *23*
Christian Patermann, DG Research, Brussels, BE

11.45 – 12.30 Press Conference

12.30 – 13.30 Lunch and Departures

PROEUHEALTH CLUSTER: CONCEPT & ORGANISATION

Tiina Mattila-Sandholm, Valio, and Maria Saarela, VTT Biotechnology, Finland

Probiotics have become an important and rapidly expanding segment of the food market as processed food manufacturers seek to improve market share by promoting the health benefits provided by functional ingredients in their products. Scientific progress has been expanding the knowledge on how foods influence consumers in relation to specific health parameters. Probiotic foods targeted towards improving the balance and activity of the intestinal microbiota currently provides the largest segment of the functional food market in Europe, Japan and Australia.

The human gastro-intestinal (GI)-tract is the metabolically most active body organ and is the prime target of present and future functional foods. It is the primary site of food conversion and uptake but also the location where the mucosal immune system is primed and various local and systemic disorders become manifest. The GI-tract is colonized by a myriad of microbes that contribute towards food conversion, digestion, communication with the host, and induction of specific biological responses contributing to many critical physiological functions.

Various sequential steps are essential in the development of efficacious functional foods. A prerequisite for mechanistic studies of action is an understanding of the composition and activity of the intestinal microbiota as well as interactions with the host in both healthy and diseased individuals. High-throughput molecular methods are required to examine the intestinal microbiota and to track the location and activity of probiotic strains in the intestinal tract. An understanding of the mechanisms by which probiotics exert beneficial effects allows the selection of strains of lactic acid bacteria (LAB) that can be tested in human clinical studies following demonstration of their safety. An important area of research includes the technologies to maximise the stability of functional traits of probiotics during manufacture, formulation, storage and in the intestinal tract. Finally, an understanding of the most appropriate approaches to communicate the benefits of the probiotic foods to consumers and the influence of health messages on consumer choice is essential to ensure that products are appropriately applied and targeted to benefit specific populations.

The Food, GI-tract Functionality and Human Health Cluster has brought 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 started in February 2001 and will run over 5 years ending in 2005 and it is subsidised by the European Commission's 5th Framework, Quality of Life and Management of Living Resources Programme. Eight complementary multicenter European projects have been included in the cluster. They have covered 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 following research areas demonstrate the impact of the cluster:

- New molecular research tools for studying the composition and activity of the intestinal microbiota have been developed. These tools consist of high throughput microarrays, use of cell sorting, monitoring in situ functional gene expression, development of functional probes and probes for clinical trials.
- A molecular understanding of immune modulation by probiotic bacteria and testing of probiotics as vaccine delivery vehicles have been under investigation. Results have shown protective effects of lactic acid bacteria in a colitis model, improved delivery of antigens, and delivery of anti-inflammatory molecules (IL-10) in humans.
- Biosafety evaluation of probiotic and other LAB for human consumption (900 strains) will result in the creation of database on safety parameters, genetic stability, and resistance genes of these LAB.
- New therapeutic and prophylactic treatments for intestinal infections and chronic intestinal diseases such as IBD, *Helicobacter* and rotavirus are being investigated.
- Several clinical trials have been performed or are still ongoing within European community which gives a comparison between North-Mid-South citizens. The trials are dealing with Intestinal Bowel Disease (IBD) and *Helicobacter* infections using either probiotics, prebiotics or their combinations. Elderly population has been given special attention in finding answers to possible linkages between age, gut microbiota, functionality and diet.
- Probiotic and prebiotic technology has created a database on stability and viability and stress tolerance of probiotic strains. In addition, new prebiotic modifications have been produced.
- Host-microbe balance combines the effects of probiotics and prebiotics and utilises novel model systems to study the functionality and crosstalk between microbes and host.
- A clearer understanding of the relationships between food, intestinal bacteria and human health and disease will be obtained by providing an extensive list of references and scientific publications.

The research innovations produced by the cluster have been disseminated to target audiences through three platforms:

- The Science Platform has provided the foundation for internal dissemination and networking, including exchange of data, biomaterials and personnel, and fostered external and international exposure.
- The Industry Platform has enabled the cluster to disseminate its research innovations to probiotic industries throughout Europe and the world while maximising the potential for commercial exploitation of results from the cluster's research.
- The Consumer Platform has provided 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 by their local language.

MICROBIAL DIAGNOSTICS OF THE GI-TRACT

Michael Blaut, Willem de Vos, Lionel Rigottier-Gois, Matthew David Collins, Kim Holmstrom

The project aimed to develop, refine, validate and automate the most advanced molecular methods for monitoring the human gut microbiota composition and bacterial gene expression in selected human populations in response to diet and life style. Considerable progress has been made in improving the knowledge of the microbial diversity of the human intestinal tract. This increase in knowledge is based on the isolation and characterisation of a considerable number of previously undescribed bacterial species and on the retrieval of a large number of novel 16S rRNA sequences in a culture independent approach. These sequences in conjunction with the already existing sequences have been used to complement the already existing set of oligonucleotide probes for the culture-independent detection of intestinal bacteria. The panel of available probes has been extended considerably and covers the majority of the gut microbiota at the sub-group level.

Three approaches have been used to develop high throughput methods for the detection of faecal bacteria. The three approaches include automated image analysis, flow cytometry and DNA array detection. All three methods have been demonstrated to be suitable for the rapid detection of fluorescent bacteria, each having certain advantages and disadvantages.

To demonstrate the accuracy and applicability of the probe technology, faecal samples from 91 individual were collected at five different locations in Europe and subsequently analysed using a panel of 18 phylogenetic groups of intestinal bacteria. Attempts were made to correlate the microbial data of the study participants with their nutrition habits.

The monitoring of catalytic activity at the cellular level could be successfully established and demonstrated to work in a rat model. In contrast, the monitoring of gene expression at the cellular level could not be realised.

CROWNALIFE: FUNCTIONAL FOODS, GUT MICROBIOTA AND HEALTHY AGEING

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The complex microflora colonizing the human gut changes with age and may play a role in the observed increase in both infectious and chronic disease in the elderly. Within Europe, there appears to be regional differences in incidence of disease as well as differences in life span and degree of independence in later years. The gut microflora is amenable to modulation using probiotics, prebiotics and synbiotics, and the elderly represent a population group for which such strategies may be particularly suitable. CROWNALIFE has confirmed that the gut microflora within elderly people differs from younger adults, showing a much greater degree of species diversity. Through generation of 16S rRNA based phylogenetic inventories of the faecal microflora of healthy elderly individuals (>65 years) the elderly 80% of cloned 16S rDNA sequences corresponded to previously uncultured bacteria while 1/3 of these represented novel phylogenetic lineages. In a pilot study examining microflora composition within different age groups in two different countries, the increased microflora diversity has been confirmed quantitatively using a set of 16 phylogenetic probes. To affect beneficial modulation within the gut microflora CROWNALIFE has employed a synbiotic formulation, and is determining the suitability and efficacy of this approach by monitoring changes within relative bacterial numbers and microbial biomarkers important in gastrointestinal health and disease. Using a holistic approach, microflora modulation has been analysed with respect to regional dietary differences within Europe. In parallel, novel synbiotic products, employing probiotic strains selected for anti-pathogenic activity from the elderly gut and efficacious prebiotics (both existing and novel) have been formulated to constitute second generation functional foods specifically designed to affect beneficial modulation of the elderly gut microflora towards improved gut health.

UPDATE ON EU AND MICROFUNCTION PROJECT (INTERACTIONS BETWEEN GUT MICROBIOTA AND THE HOST)

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Pirje Hutt, Leena Stsepetova, Marika Mikelsaar, University of Tartu, Estonia
Douwina Bosscher, Jan van Loo, Tiense Suikerraffinaderij NV, Belgium
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Sarah Amansec, Patricia Conway, 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 investigates host-microbe interactions and the effects of functional foods. The influence of probiotics, prebiotics and synbiotics on the gut microbiota and their interactions is being determined 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. Selected health indices will be assessed in response to synbiotic intake, in humans, and will include the use of molecular probing strategies for diagnostic bacteriology.

The project has ran for 3 years with the following aspects completed:

- Prebiotic comparisons in 3 stage continuous culture gut model studies
- *L. fermentum* VRI 002 ameliorated murine colitis
- Wild type and rifampicin variant strains of synbiotics were seen to behave similarly (fermentation studies) in both pure and mixed culture studies
- Various synbiotics were seen to increase populations of bifidobacteria in batch and continuous (3 stage) culture model systems of the gut
- Prebiotic and probiotic test products have been manufactured for the project human trial
- Probiotics, prebiotics and synbiotics were seen to confer varying levels of protection against translocation in 2 different animal models
- A human trial to reduce microbial translocation is ongoing
- *In vitro* model human intestinal cell lines, Caco-2 have been used to determine the influence of *A. muciniphila* upon the host. The organism could induce a response after 2 hours by inducing *IL-8* and *MUC 3* genes
- Based on the results of in vitro safety assays, five *Lactobacillus rhamnosus* strains have been selected for *testing in* healthy rats, rats with naturally infected *Helicobacter* spp., and rats with experimentally-induced colitis
- A pilot human study on probiotic intervention has been completed (including organoleptic aspects) to help define dose, intervention type and marker outcomes for a fuller trial

LACTIC ACID BACTERIA WITH DESIGNED HEALTH PROPERTIES

Annick Mercenier*, Institut Pasteur de Lille, France
On behalf of the DEPROHEALTH Network

In the scope of the DEPROHEALTH project, a range of *Lactobacillus* strains have been analysed for their ability to interact with the host immune system and for additional properties considered as important for probiotic applications. The results of these strain comparisons have been used to build a “probiotic data bank”. The *in vitro* studies led to identify strains with different immune profiles and these purported properties were further tested in mouse models mimicking the human intestinal disorders studied in DEPROHEALTH. Experiments conducted in mouse colitis models confirmed that specific strains possess higher intrinsic “anti-inflammatory” properties. Two of them were engineered to produce murine IL10 and are presently evaluated *in vivo* for their protective effect. Cell wall mutants of *L. plantarum* NCIMB8826 were analysed for their phenotypic traits, immune modulation ability and capacity to act as live carriers. This led to identify mutants with either increased anti-inflammatory or antigen delivery properties. Prototype recombinant lactobacilli producing *Helicobacter* or rotavirus antigens were shown to induce at least a partial protection against these intestinal pathogens in murine infectious models. In the course of these experiments, a new method to evaluate the *Helicobacter* load in tissues was developed. Novel targeting signals for cell surface presentation of therapeutic molecules were identified. Remarkably, a safe biologically contained lactococcal strain secreting human IL10 was constructed, thus opening the way to a small trial in patients suffering from IBD.

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PROGID – PROBIOTICS IN GASTROINTESTINAL DISEASE

Barry Kiely PhD, on behalf of the PROGID study group

Probiotics may be operationally defined as commensal organisms that can be harnessed for health benefits, usually in the form of an orally consumed food ingredient. Probiotics are beginning to enter the arena of evidence-based medicine but rigorous proof of efficacy in several conditions remains to be demonstrated. The best evidence for efficacy in any human disorder is the role of probiotics in antibiotic-associated diarrhoea with confirmatory metanalyses. There has also been a favourable Cochrane report on the use of probiotics in childhood and adult enteric infections. In irritable bowel syndrome, there is unexpected but persuasive evidence for therapeutic efficacy of some but not all probiotic strains. The efficacy of probiotics in different animal models of inflammatory bowel disease has been confirmed by several research groups but efficacy in humans with inflammatory bowel disease is less clear. Although, efficacy for probiotics has been shown in preventing pouchitis in patients undergoing colectomy with ileo-anal pouch anastomosis, their role in most patients with ulcerative colitis or Crohn's disease is uncertain. Indeed, there have been some small studies that failed to demonstrate any benefit. It may be that the clinical course of many patients with inflammatory bowel disease is too aggressive for probiotics to have a realistic chance of maintaining intestinal homeostasis. The PROGID study undertook a double blind placebo-controlled prospective clinical trial of two different probitics (a lactobacillus and a bifidobacterium) in the maintenance of remission in patients with ulcerative colitis. A similar trial was also undertaken in Crohn's disease. In both conditions, the trial involved a feeding period of one year. The trial included patients in centres within Ireland, Spain and Finland with other aspects of care being standardised across the different centres. Both trials have recently closed and the data are currently being analysed by an independent statistical service, with the clinicians maintaining the blind while the final results are pending analysis. Whatever the outcome, it is noteworthy that the patients included in the trial were at the severe end of the clinical spectrum because they were selected on the basis of a requirement for corticosteroid therapy for induction of remission (despite ongoing mesalazine therapy) before being eligible for inclusion. The trial has already confirmed the safety of probiotics in intestinal disease with no reports of significant adverse effects attributable to the probiotics. In addition, it has provided important baseline and serial data which show informative microbiologic and immunologic features including the assessment of calprotectin as a monitor of disease activity in comparison with soluble serum cytokines. Finally, the trial provides unique insights into changes in the enteric flora over time and in relapse and remission.

POPULATION DYNAMICS AND DIVERSITY OF FECAL MICROBIOTA OF PATIENTS WITH ULCERATIVE COLITIS PARTICIPATING IN A PROBIOTIC TRIAL

Elaine E. Vaughan[1,2], Kaouther Ben-Amor[1], Suzanne Verhaegh[1], Ineke Heikamp-de Jong[1], Fergus Shanahan[3] & Willem M. de Vos[1]

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The human gastrointestinal tract is colonized with a myriad of microbial organisms termed the microbiota. Humans benefit from this mutualistic relationship whereby the microbiota contribute to host health by performing roles in nutrient uptake and processing, innate and adaptive immunity, and intestinal epithelial development and activity. However, the presence of these commensal microbes also appears to be involved in the pathogenesis of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Technological advancements that enable high throughput application of methods to monitor the diversity, activity and functionality of the intestinal microbiota can aid research into the mechanisms of probiotic bacteria. Rapid molecular techniques that bypass cultivation, especially those based on 16S rRNA genes such as PCR-denaturing gradient gel electrophoresis (DGGE) and fluorescent *in situ* hybridisation (FISH) allow rapid monitoring of the indigenous microbiota and the probiotic bacteria in human intestinal samples. The gastrointestinal microbiota of patients with ulcerative colitis participating in the PROGID double-blind clinical trial were monitored over time, while investigating the response to ingestion the probiotics, either *Lactobacillus salivarius* subsp. *salivarius* UCC118 or *Bifidobacterium infantis* 35624, or a placebo. The microbiota of fecal samples prior to and during the probiotic trial were analyzed by fluorescent *in situ* hybridization in combination with flow cytometry (FCM), and PCR-DGGE technique. The FISH probes revealed that the numbers of bifidobacteria and lactobacilli increased over time in all patients samples. The FISH analysis also revealed substantial temporal variation in the microbiota groups of each patient. The latter was supported by cluster analysis of the PCR-DGGE profiles and calculation of the similarity indices which confirmed the high temporal shifts, but indicated that the microbiota were stabilizing later in the trial. Further application of molecular techniques to study the activity and functionality of lactic acid bacteria in response to the intestinal environment will be presented.

TECHNOLOGY OF PRO- AND PREBIOTICS

Dietrich Knorr, Berlin University of Technology, Germany

The EU-funded project PROTECH (QLK1-CT-2000-30042) dealt with the selection and improvement of unique processing technologies related to the production and improvements of probiotics and prebiotics as well as the interaction of both in the production, distribution and utilization pipeline, in order to better maintain or even improve the health-related performance of these products.

The focus of the **fermentation study** was on the development of a general, milk-free fermentation medium as well as optimization of harvesting time with respect to yield improvement and post-harvesting stability (freeze-drying, storage, stability against acid or bile, etc.). The aforementioned fermentation medium contained food-grade components only and was found to support the growth of the probiotic lactobacilli equally to standard broth. Furthermore, it was found that additional modification on the composition of fermentation media could beneficially influence the viability/stability of probiotic strains

Both **freeze-drying** and **spray-drying** processes were evaluated, in terms of identification of suitable processing regimes, performance of different protectants in offering high survival during drying and storage, as well factors governing stability during storage. The possibility to produce dried synbiotic preparation was documented by trials on incorporation of prebiotics in the drying matrices of probiotics, which could be performed without substantially affecting the stability. Furthermore, efforts were made to elucidate the superiority of milk constituents in conferring protection during storage in dehydrated state.

The use of **sub-lethal stress** – either by homologous or by heterologous stress factors – to improve technological behaviours as well as to enhance resistance against adverse conditions in the gastro intestinal tract was demonstrated. Different techniques including proteomic and genomic approaches were applied to understand the specific response of the cells following exposure to sub-lethal doses of heat, oxygen or bile.

Works had also been performed on **enzymatic modification of prebiotics** to allow further development of improved prebiotics. In particular, better understanding has been gained on the mechanism of galactan-modifying enzymes of bifidobacteria in the degradation galactan and galacto-oligosaccharide. Besides, works on **physical modification** of starch demonstrated the beneficial effect of high hydrostatic pressure treatment in enhancing the content of resistant starch, which is regarded to exert prebiotic effect.

Feeding trials of commercial or technologically modified prebiotics in combination with probiotics were performed to demonstrate the possibility of modulating beneficial changes on the degree of fermentation and short chain fatty acid patterns in different parts of the rat colon.

INHIBITION OF PATHOGENS BY PRO- AND PREBIOTICS

Luc De Vuyst

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Since a long time lactic acid bacteria (LAB) are used in fermented foods because they greatly influence their nutritional, organoleptic and shelf-life characteristics. Concerning the latter they inhibit both spoilage bacteria and food-borne pathogens. In addition, some strains of LAB have been shown to display interesting probiotic properties. One of these properties is the potential to inhibit pathogenic bacteria.

LAB are known to produce bioactive molecules such as organic acids, fatty acids, hydrogen peroxide, diacetyl, and ethanol. Many strains also produce bacteriocins, bacteriocin-like compounds, and other low-molecular-mass inhibitory substances (reuterin, reutericyclin, …). They all show antimicrobial activity against spoilage and pathogenic bacteria.

Several LAB strains have been shown to *in vitro* inhibit *Helicobacter pylori*, a Gram-negative pathogen causing gastritis, peptic and duodenal ulcers, and relating to the development of gastric cancer. Cell-free culture supernatant (CFCS) of such strains decreased *H. pylori* viability *in vitro*. Moreover, administration of certain *Lactobacillus* strains to *H. pylori*-infected mice resulted in a significant decrease of chronic and acute gastritis. The exact underlying mechanism of the anti-*H. pylori* activity exhibited by lactobacilli is not clear, although a number of reports point out that the organic acids produced and the resulting low pH play an important role in the antimicrobial activity. However, even after pH neutralization, CFCS from selected LAB have shown anti-*H. pylori* activity *in vitro*. Moreover, this activity was abolished after proteinase treatment but not after heat treatment. These data indicate that antibacterial substances other than lactic acid and possibly of a peptide nature are responsible for such inhibitory activity.

Similarly, several *Lactobacillus* strains have been shown to *in vitro* inhibit *Salmonella enterica* serovar Typhimurium, another Gram-negative pathogenic bacterium, causing gastroenteritis. CFCS from selected lactobacilli showed clear inhibition of *S.* Typhimurium, beyond the influence of lactic acid. Additionally, the CFCS of such strains significantly inhibited the invasion of *S.* Typhimurium into cultured human intestinal Caco-2 cells. Further, reduced *S.* Typhimurium levels were observed in the gastrointestinal tract and tissues of *Salmonella*-infected mice after administration of certain lactobacilli. The exact mechanism of this inhibitory activity is not known yet.

Finally, several *Bifidobacterium* strains, including commercial probiotic bacteria, were found to exert strong inhibitory activity towards *S.* Typhimurium and *Escherichia coli*. The inhibitory mechanism was shown to be dependent on the lowering of pH and the production of organic acids, in particular acetic acid and lactic acid. Some strains also produced formic acid and succinic acid. When particular strains were grown on inulin-type fructans instead of glucose, the amounts of acetic acid and formic acid increased at the cost of lactic acid.

BIOSAFETY OF PROBIOTIC LACTIC ACID BACTERIA (LAB)

Herman Goossens[1,8], Vanessa Vankerckhoven[1], Carl Vael[1], Tim Van Autgaerden[1], Geert Huys[2], Marc Vancanneyt[3], Jean Swings[2,3], Ingo Klare[4], Wolfgang Witte[4], Marie-Bénédicte Romond[5], Philippe Moreillon[6], Jan Knol[7], Richèle Wind[7], Emmanuel Wiertz[8].
University of Antwerp[1], Ghent University[2], BCCM/LMG Bacteria Collection[3], Belgium; Robert Koch Institute[4], Germany; University of Lille[5], France; University of Lausanne[6], Switzerland; Research NUMICO[7] and the University of Leiden[8], The Netherlands.

The final PROSAFE strain collection comprises 834 isolates: 279 nutritional isolates, including 186 probiotic LAB (as defined by the depositor or isolated from product), 57 probiotic strains still under research and 36 food isolates; 540 human isolates, including strains isolated from normal flora (of faecal origin), and sterile isolates (from the blood); 7 animal strains, and 8 'non-categorised' strains.

All 834 strains have been successfully identified at the species level. Different levels of confirmation of identification have been defined. Either the depositor correctly identified a strain at the genus level leading to a possible confirmation of a correct identification at the species level, or the genus identification from the depositor could not be confirmed. Overall, the confirmation rates were high, with 95% of confirmation at the genus level and 90% at the species level. In particular, for the probiotic strains, a total 162 out of 186 (87%) strains were confirmed at the genus level, of which again 87% was confirmed at the species level.

Typing of *Lactobacillus rhamnosus, L. paracasei, L. plantarum, L. gasseri,* and *L. acidophilus,* using AFLP and PFGE was completed. AFLP was shown to generate a reliable grouping at the species and intra-species level. Both for *L. rhamnosus* and *L. plantarum*, and *Enterococcus faecium* PFGE confirmed a very high genomic relationship among strains from probiotic and human (blood) origin within delineated AFLP groups. Moreover, AFLP profiles of *E. faecium* isolates revealed 2 intragenomic groups, which correlated with previously described clusters. For *Bifidobacterium* culture conditions and PFGE parameters are being optimised.

Using the broth microdilution test for enterococci (NCCLS) and the newly developed broth microdilution method for non-enterococcal LAB, 746 PROSAFE strains were tested for their antibiotic susceptibilities to 16 antibiotics representing nearly all antibiotic classes. Microbiological cut-offs were determined at the species level for *L. rhamnosus, L. paracasei, L. plantarum, L. fermentum, L. gasseri, L. acidophilus, L. delbrueckii, L. reuteri, L. johnsonii, L. crispatus, Pediococcus acidilactici, P. pentosaceus, Bifidobacterium breve, B. animalis B. longum,* and *B. bifidum*. For *Lactococcus* and the other species of *Lactobacillus* and *Bifidobacterium* less than 10 strains were tested which is insufficient to set cut-off values. The wild type population was determined as WT $\leq$z mg/L and non-wild type as NWT >z mg/L. Among the nutritional strains only very few probiotic and research probiotic isolates contained genes for antibiotic resistance: *B. animalis* subsp. *lactis* (5), *L. reuteri* (1), *L. plantarum* (1) and *L. curvatus* (1 food isolate) to tetracycline [*tet*(M)group-positive] and each one strain of *L. crispatus* and of *L. acidophilus* group to erythromycin/clindamycin [*erm*(B)-positive] plus tetracycline [*tet*(M)group-positive].
We did not detect any of the described virulence properties in probiotic *E. faecium* strains using the newly developed multiplex PCR. One probiotic *E. faecalis* strain was found positive for 3 (*asa1, gelE, esp*) of the 5 virulence genes.

Adhesion of probiotic, clinical and faecal lactic acid bacteria was assessed using three different cell lines (Caco-2, HT-29MTX, Girardi). A high variability was seen between triplicate results, probably caused by one or more variables, such as the number of CFU of the inoculum, the researcher performing the experiments, the incubation time of the plate, the incubation time of the strains tested, the species assayed. The results question the relevance of adhesion studies widely reported in the literature.

The EAE mouse model has been established and validated. Probiotic strains will now be assayed in the model. Infectivity of *L. rhamnosus* and *L. paracasei* was assessed in a rat model of endocarditis. With one exception, probiotic strains were shown to infect and multiply less in the rat endocarditis model. A trend was seen for all *Lactobacillus* strains assayed: endocarditis was not induced, but the spleens were infected. On the contrary, this trend is not seen for the non-pathogenic *Lactococcus lactis* control strain. In an in-vitro colon model a probiotic and a clinical strain have been tested. The model showed that the probiotic strain created a more acidic environment and produced more acetate compared to the control and the clinical strain. Plating showed no differences between the probiotic and the clinical isolate, while qPCR showed that the clinical isolate retains longer in the colon model and the number of bifidobacteria increased during the run for both strains.

We continued to enter the data retrieved and encountered by the various efforts of the PROSAFE partners in the master database, ensuring that the objectives are complied with, and achieving the expected integration. During the final year, a human colonization study will be conducted and guidelines concerning the biosafety evaluation of probiotic lactic acid bacteria used for human consumption will be written.

PROEUHEALTH: INDUSTRY BENEFITS – THE VIEW OF THE LACTIC ACID BACTERIA INDUSTRIAL PLATFORM

A.M. Ledeboer, Unilever R&D Vlaardingen, The Netherlands

Consumers are becoming more and more interested in foods that have a health benefit beyond basic nutrition and the food industry is trying to fulfil these wishes by developing products with measurable health benefits. Foods that contain pro- pre- or syn-biotics are part of these and are getting special interest of the consumer. Health claims of such products are related to improvement of the gastrointestinal health and strengthening of resistance.

In the PROEUHEALTH cluster 64 research partners from university, research institutes and industry have worked together over a period of 4 years in 8 large research projects to substantiate these health claims further. The different aspects that have been studied vary from the description of the human microbial flora, modulation of the flora, safety, effects of probiotics on elderly or in IBD patients, efficacy against pathogens and methods to optimize survival during processing.
Specific results that will benefit the food industry can be divided in 3 groups:

Aspects related to health
- Inhibitory effects against pathogens such as *H. pylori* or rotavirus (GMO and non-GMO)
- Effects of certain probiotics on maintaining remission in IBD patients
- Effects of pro- pre or syn-biotics on the microflora of elderly people
- Mechanisms of immunomodulation

Aspects related to microbial function
- Finding suitable synbiotic pairs
- Determination of shifts in the microbial flora and metabolism of the flora
- Safety aspects of probiotics

Aspects related to technological properties
- Taxonomical analysis of the microbial flora
- Optimization of processing conditions to maintain viability, stability and functionality

All these aspects are important to the food industry, if the goal is to bring a wide variety of consumer products on the market containing viable probiotics with proven health claims. If the industry wants the consumer to have trust in products with health functionality, then results that come out of these and future projects will certainly be needed.

THE BENEFIT OF PROEUHEALTH TO ORAFTI

Jan Van Loo – ORAFTI

ORAFTI is a company that (this year 10 years ago) grew out of an R&D lab, and is a producer of natural functional food ingredients that are sold on world wide scale. Actual products are derived from chicory roots and from rice. These 'active food ingredients' have several particular and interesting nutritional properties. Extensive nutritional research on chicory fructans initiated by Orafti resulted in the creation of the 'prebiotic' concept, demonstration of effects of these food ingredients on mineral absorption in young and old, as well as on bone mineralisation. Anticancer effects were demonstrated, in which the impact of the immune function is not negligible. Inflammation is suppressed in IBD. Lipid metabolism has the tendency to be modulated and there are indications of reduced risk for factors related to syndrome X. Suppression of oxidative stress, improved feeling of well-being and increased satiety effect are topics that are under further evaluation.

These nutritional interesting properties are the "added value" of this kind of products. Research to support these functional food claims is central in the existence and further development of this company, and of many other European functional food companies are in the same case. Orafti spends an important part of its turnover in R&D, in which exploration and consolidation of physiological effects of its existing ingredients and products in development have highest priority.

The research some functional food companies as Orafti initiate however has consequences beyond common business goals: it has become clear that the research has tangible impact on public health, which especially in the ageing population will become an issue the following decennia. It is not the mission of a company to invest in public health issues. Therefore it is justified that governmental institutions contribute by making possible long-term large-scale confirmation studies with established functional food ingredients in human volunteers. It is in this context that specifically pre- and probiotic related initiatives as the PROEUHEALTH 'cluster' (in FP V; 'IP's' in FPVI and FPVII?), are and will be of big importance. They are think tanks, bring together European critical scientific mass, and are breeding grounds for further innovation. As such they contribute significantly to the progress of the EU by strengthening this developing industry and by clarifying nutritional topics related to public health.

MOLECULAR TECHNIQUES

P.S.T. Tan, Microscreen BV

Microscreen is an ISO-9001-2000 certified organisation, focussed on the commercialization of rapid detection and identification of micro-organisms using molecular biological techniques.
Within the project Microscreen has collaborated with its partners to develop and improve molecular biological techniques in order to monitor the dynamics of complex populations.
A short outline of the techniques will be presented and a short overview of the products currently available will be given as well.

PROEUHEALTH BENEFITS: SAFETY OF PROBIOTICS

Jan Knol, Numico Research B.V., the Netherlands

Probiotic species, like different lactic acid bacteria and bifidobacteria, are in general considered harmless micro-organisms devoid of any pathogenicity. This is supported by their commensal status. Also, the traditional and abundant use in food preservation or food processing is without any apparent public health problem. Thus, these species are considered 'GRAS' (= Generally Regarded As Safe). Several studies on related species, however, have indicated possible adverse effects. Bacterial species have been isolated in a number of disease situations, suggestive of the opportunistic pathogenicity of these bacteria.

From the current scientific literature it can be concluded that the overall health risk remains very low. Nevertheless, safety aspects have always to be considered, especially with novel probiotic strains. Possible adverse effects (specifically in high risk groups, including diseased) should be addressed when probiotic strains are selected (risk benefit analysis). Thus, extensive testing of probiotic strains is necessary to build a firm basis for both safety and health claims.

Within the current EU program the collaboration of several independent institutes in Europe, addresses the safety of currently commercially available probiotic strains as well as novel putative probiotics using different *in vitro* and *in vivo* tests. This project will yield insides in the safety evaluation of probiotics with a specific focus on identification, taxonomy, antibiotic resistance, virulence and will possibly identify new safety markers. This is considered important for the selection of probiotic strains in the future.

PROEUHEALTH BENEFITS: PROBIOTIC TECHNOLOGY

Tiina Mattila-Sandholm, Valio, Finland

Valio was a partner in the PROTECH project which aimed at better applicability of pro- and prebiotics. The following deliverables of PROTECH will facilitate further probiotic product development in industry:

Cell production:

- Development of edible medium for lactobacilli and bifidobacteria
- Optimization of industrial scale fermentation and freeze drying process
- Spray drying of probiotic cultures
- Probiotic survival in food matrices
- Influence of processing parameters on the properties of probiotics related to the survival in the upper GI-tract
- Application of flow cytometry and fluorometry to viability studies
- Preadaptation treatment for stress induction to improve tolerance against harsh conditions

Molecular level studies:

- Genome-wide gene expression analysis for analysis of stress response
- Insertional mutagenesis to improve probiotic stress tolerance

Since all technological applications are strain-specific the results of PROTECH – obtained with mainly 4 probiotic strains – will function as a basis for the further probiotic technology development work.

CONSUMER BENEFITS

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The objective of the consumer platform in the PROEUHEALTH-cluster has been to translate the scientific work carried out in the eight research projects into lay language and thereby enhance European consumers' understanding of factors that influence our gut health. This task has included producing a leaflet with a short description of the cluster, progress reports on the on-going research, and by organising workshops within cluster meetings to promote the open discussion between scientists, industry and the consumers. The crucial tool for carrying out these activities has been the PROEUHEALTH website where the consumer platform materials have been available to the public and documents can be downloaded. The one-page leaflets and progress reports have been translated into 11 European languages.

The public interest in gut health and the possible role of probiotics and prebiotics in promoting our well-being has been wide. The projects study a field that both improves our understanding of what is the normal variety in our gut microbiota and finds possible new therapeutic tools for treating intestinal disorders and diseases. In the workshop held in the previous cluster meeting at Sitges in 2004, the representatives from consumer organisations in their viewpoints emphasised the need to have sound scientific background for all claims that are attached to food products, and this cluster has promoted that goal. However, they have also pointed out that in aim to build and maintain consumer trust, the products should deliver what they promise. The claims should clearly state what are the possible benefits and information on the required amount of product should be given in an easy and comprehensible way without underestimating consumers' ability to understand them. These all are challenges that food manufacturers have to respond to.

The consumer platform has provided additional information about the research directly to consumers. The aim has been to support the scientists working in the projects by translating hard scientific facts into consumer language without loosing the main message. Due to the limited resources the website has been the central tool in distributing information, but assessing its real impact is a complicated task. The most active consumers in contacting the platform have been those who suffer from intestinal disorders. The response and activity in visiting the website has shown that this kind of service has been useful and allowed consumers to get an idea of what is studied

Further information: http://proeuhealth.vtt.fi/

SCIENCE BENEFITS IN THE GUT HEALTH AREA

Willem M. de Vos, Wageningen University and Wageningen Centre for Food Sciences, The Netherlands willem.devos@wur.nl

Most scientific activities within the PROEUHEALTH cluster have focused on the human intestinal tract. This metabolically most active organ of the body is colonized by a vast array of microbial communities that show specific temporal, spatial and functional development. Major attention has been given on the diversity of these microbes, how their presence can be affected by dietary interventions with probiotic and other lactic acid bacteria, and what the effects are on intestinal health. In addition, major advances have been realized in the production, preservation and safety of these lactic acid bacteria. Finally, a critical mass of a competent and collaborating scientific community has been established. This provides a platform for asking questions related to where and how this science domain of gut health can be further developed while addressing the needs of the society, including those of the large and small food industries, EU consumers and patient groups. This presentation aims to summarize the most important and critical issues.

COMMISSION BENEFITS AND THE FUTURE OF GUT HEALTH RESEARCH

C. Patermann and J. Lucas

European Commission, Research Directorate-General, Biotechnology, Agriculture and Food Research

The European Commission has been funding projects on gut health and probiotics since the earlier framework programmes, thereby also stimulating other stakeholders' initiatives and laying the ground for regulatory measures such as the proposal for the health and nutrition claims directive. Within the funded projects on gut health, work on basic research has been reduced gradually in favour of the development of specific probiotic foods as well as demonstration and exploitation over the years, culminating in the PROEUHEALTH cluster of FP5 by now. In FP6, a Specific Support Activity (SSA) on networking in the New Member States is running, and another SSA for a virtual technology platform is under negotiation. The future of gut health research is represented in the call 2005 through a topic for an Integrated Project on milk and dairy products with optimised bioactivity.