



# Newsletter No. 4

May 2004

## Food, GI-tract Functionality and Human Health Cluster



### 3<sup>rd</sup> PROEUHEALTH Workshop Sitges, Spain (15–18 March, 2004)

The first PROEUHEALTH workshop was held as north as possible, in Saariselkä, Finnish Lapland. The second was held as south as possible, at Taormina, Sicily. So this year it had to be somewhere in between. Sitges, a little holiday resort next to Barcelona was chosen due to its perfect location on the coast of the Mediterranean Sea. The Workshop was held in the Hotel Meliá Gran Sitges, which is located near to the centre of Sitges.



### The cluster over halfway approaching the end

In her opening words Prof. Tiina Mattila-Sandholm reminded that five out of the eight projects are already nearly finished and lots of data and knowledge have already been acquired. The cluster is therefore approaching the most active phase for result dissemination.

During the first day of the Workshop, the PROEUHEALTH co-ordinators presented the results obtained for each of the 8 projects so far. The projects are described in the workshop proceedings, which can be ordered from the Cluster co-ordinator at VTT Biotechnology.

## HIGHLIGHT RESULTS — PROEUHEALTH CLUSTER

### MICROBE DIAGNOSTICS (3<sup>rd</sup> year)

Project Co-ordinator: Prof. Michael Blaut  
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1) improved coverage of biodiversity of human gut microbiota:

- Isolation of 800 fecal strains, 70 isolates did not correspond to described species, 22 novel species
- Over 1000 fecal 16S rRNA clones have been analyzed
- Web site of GI-tract diversity:  
[www.food.rdg.ac.uk/people/afs99pal/index.htm](http://www.food.rdg.ac.uk/people/afs99pal/index.htm)

2) Culture-independent automated enumeration of fecal bacteria:

- Improved protocols for microscopical sample preparation, image capture and image analysis
- Further development of high throughput DNA-microarrays, 4169 probes (~1000 bacteria) will soon be printed on a microarray



- Panel of 18 oligonucleotide probes used with FISH – flow cytometry cover over 75% of fecal microbiota
- *Desulfovibrio* spp. and *Desulfobacter* spp. were found to be the most dominant sulfate reducing bacteria in the human GI-tract

### EU & MICROFUNCTION (2<sup>nd</sup> year)

Project Co-ordinator: Prof. Glenn R. Gibson  
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- New mucus-degrading bacterium, *Akkermansia muciniphila*, isolated and characterized
- An oligonucleotide probe for *A. muciniphila* developed and validated and *A. muciniphila* quantified from fecal samples with FISH - flow cytometry
- Synbiotic development; 5 bifidobacteria and 12 prebiotics → *Bifidobacterium adolescentis* in combination with IMO (isomaltooligosaccharides) increased the proportion of bifidobacteria in an *in-vitro* model



- Dairy originating *Bifidobacterium* strains and *Lactobacillus* strains adhere stronger to intestinal mucus than clinical and fecal isolates

### DEPROHEALTH (3<sup>rd</sup> year)

Project Co-ordinator: Dr. Annick Mercenier  
[annick.mercenier@rdls.nestle.com](mailto:annick.mercenier@rdls.nestle.com)

- Construction of cell wall mutants; target strain *Lactobacillus plantarum*
- Oral administration of *Lactococcus lactis* secreting hIL10 prevents and heals chronic colitis in three different mouse models
- Biocontained *L. lactis* secreting hIL 10 do not survive in the environment
- Biocontained *L. lactis* strain was approved by the Dutch authority for physically contained clinical trial in 12 Crohn's disease patients





## PROTECH (3<sup>rd</sup> year)

Project Co-ordinator: Prof. Dietrich Knorr  
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- Viability and stability of probiotic bacteria is strain specific and propagating and downstream processing steps should be tailor-made for each strain
- Processing conditions, packaging material and storage conditions all have an impact whether the probiotic bacteria will survive in the product
- Treatments such as stress-treatments can be utilized to improve the viability of the strains (so far tested in pilot-scale – it is possible to perform this in an industrial scale too)



- Spray-drying appears to be a promising downstream processing method for robust probiotic strains

## PROPATH (2<sup>nd</sup> year)

Project Co-ordinator: Prof. Luc De Vuyst  
[ldvuyst@vub.ac.be](mailto:ldvuyst@vub.ac.be)

### 1) *Helicobacter pylori* SS1 infection mouse model

- The gastritis scores in both chronic gastritis or chronic active gastritis were reduced after consumption of probiotics although no difference was observed in *H. pylori* gastric sample colonization
- The probiotic effect was strain specific (*L. johnsonii* La1 had the most prominent effect)

### 2) *L. johnsonii* La1 produced protease-sensitive compounds inhibitory to both lactobacilli and *H. pylori*



## PROGID (3<sup>rd</sup> year)

Project Co-ordinator: Prof. Fergus Shanahan  
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- The effect of *Lactobacillus salivarius* and *Bifidobacterium infantis* to help maintain remission in ulcerative colitis and Crohn's disease has been studied
  - Clinical studies have been completed and analysis of the data is in progress
- The diversity of *Desulfovibrio* spp. was found to be higher in ulcerative colitis patients than in healthy subjects
- *Desulfovibrio* profiles of UC patients are not stable in time, but tend to get simpler in some patients who stayed in remission (probiotic effect?)



## PROSAFE (2<sup>nd</sup> year)

Project Co-ordinator: Prof. Herman Goossens  
[herman.goossens@uza.be](mailto:herman.goossens@uza.be)

- Creation of a database on safety of lactic acid bacteria and bifidobacteria
- Establishment of the PROSAFE collection: 907 strains obtained from the industry and scientists
- Identification of 87% of isolates performed
- AFLP (three primer combination) and PFGE (two restriction enzymes) fingerprinting of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* performed
- A new method was developed for susceptibility testing of lactobacilli, pediococci, lactococci and bifidobacteria



- Microbiological breakpoints were determined for several genera of LAB
- Virulence factors in enterococci have been studied

## CROWNALIFE (3<sup>rd</sup> year)

Project Co-ordinator: Dr. Joël Doré  
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### 1) Characterization of the fecal microbiota of the elderly

- Increased bacterial diversity with age
- Baseline study of microflora composition and microflora gut function with 240 subjects is finished
- Sequencing of 16S rDNA of 10 clone libraries from elderly fecal samples; total of 1580 exploitable clones, 393 different phylotypes, distributing into 25 phylogenetic groups
- Assessment of composition of the elderly gut flora with FISH-flow cytometry using a panel of 19 probes

### 2) Functionality of microbiota

- enzymatic activity, SCFA, etc. –biomarkers for colonic health ?



- ### 3) Intervention study; a synbiotic trial with *Bifidobacterium animalis* and Raftilose Synergy1 will be finished in September



## Oral poster presentations – Day 2

On Tuesday morning some young – and also some senior – scientists had an opportunity to share the highlights of their work as oral presentations in a poster session chaired by Dr. Maria Saarela. The following posters were presented:

- **Pjotr Heczko:** *Involvement of bacteria in pathogenesis of inflammatory bowel disease (IBD)*
- **Hanna-Leena Alakomi:** *Application of microplate scale fluorochrome staining assay for assessment of viability and stability of probiotic Bifidobacterium sp.*
- **Cecile Cinquin:** *Comparative effects of exopolysaccharides and fructooligosaccharides in an infant in vitro colonic fermentation model with immobilized fecal microbiota*
- **Matteo Fallani:** *Design and validation of 16S rRNA probes to enumerate Clostridium difficile and Clostridium perfringens species in infant fecal microflora*
- **Domitille Fayo-Messaoudi:** *Lactobacilli strains examined by PROPATH project produce unknown antibacterial compound(s)*
- **Jana Hejnova:** *Genomic characterization of the Escherichia coli probiotic strain O83:K24:H31*
- **Roger Hutson:** *A new genus Anaerofustis stercorihominis from human feces*
- **Pawel Namsolleck:** *Percoll™ gradient DGGE – a novel two dimensional approach to study microbial community*
- **Mirjana Rajilić:** *Diversity of sulfate-reducing bacteria in the human gastro-intestinal tract in relation to ulcerative colitis assessed by 16S rDNA analysis*
- **Kieran Tuohy:** *Generation of a novel synbiotic specifically designed for the elderly population*
- **Katerina Vaculova:** *Czech research activities on bactoreal synbiotics*



## Industry statements and Consumer platform – Day 2

The afternoon was reserved for "Industry statements and Consumer platform" – session, which Prof. Charlie Daly and Dr. Liisa Lähteenmäki chaired. The session started with short viewpoint talks given by the industrial partners and Consumer statements presented by selected consumer organizations (The abstracts of the statements are in the workshop proceedings).

The participants in the panel discussion were (from left to right in the pictures):

- 1\*. Rod Mitchell, EFCCA, United Kingdom
2. Esben Laulund, Chr. Hansen, Denmark
3. Aat Ledeboer, Unilever, Netherlands
4. Colette Shortt, Yakult, United Kingdom
5. Bart Degeest, Yakult, United Kingdom (not in the picture)
6. Jean-Michel Antoine, Danone, France
7. Christoph Cavadini, Nestlé, Switzerland
- 8\*. Gemma Trigueros, Technical Department OCU, Spain
- 9\*. Beate Kettlitz, BEUC, Belgium
10. Jan van Loo, Orafti, Belgium (not in the picture)

\* representatives from consumer & patient organizations



Afterwards the floor was open for questions raised by the public and for panel discussion. In the latter part of the session there was a lively debate between consumer organizations, industrial participants and the audience. Some excerpts from the questions raised and answers given:

What kind of claims can be made and what kind of claims are needed?

- *Consumers want and need claims that can be proven*
- *Consumer need to understand the message*
- *Consumers need to be educated and the claims should be clear*
- *The information provided should be true and based on scientific data (consumers need simple sentences)*

Are probiotics safe?

- *Probiotics are regarded as safe,*
- *Long-term effects are inadequately known, especially with young children*
- *Information about the portion (daily intake) should be provided*

Importance of viability and stability in probiotic products?

- *Bacteria in the products can be alive/stressed/dead*
- *Tailored technologies for each strain are needed*
- *Live bacteria in the product are important*
- *Activity of dead bacteria?*

It was concluded that the panel discussions need to continue with the co-operation of EFSA and SANCO hopefully in the near future. Furthermore, it was agreed that statements, questions and answers are a good basis for future research and product development actions.



**A gala-dinner was organized on Tuesday evening at Finca Mas Solers**, which was built at the end of the 18<sup>th</sup> century as a “country house”. After dangerous dinner, project co-ordinators – or delegated project partners – were obliged to present their TIPs (technical implementation plan). Every project was given a 10 € note on Sunday morning, which they could use to buy something that characterizes their project – what comes “out of the tube” after the project is finished - and what could be used as part of the TIP.



The scientific board that graded each TIP, consisted of Prof. Willem de Vos, Prof. Charlie Daly, Dr. Aat Ledebouer and Dr. Liisa Lähteenmäki (from left to right).



The scientific board chose PROSAFE as “Dr. PROEUHEALTH”, CROWNALIFE as the first runner up and PROPATH as the second runner up. MICROBE DIAGNOSTICS got the least points – since it had prepared the annual report as first - and was awarded for the last position [However, the Press liked Microbe Diagnostics’ molecular tool TIP]. The winning team – PROSAFE - had bought a pink music box (safe) with “Tiina the ballerina” inside of it and they had also made a song for Tiina. The original hand-written lyrics were enclosed in the music box, which will be chained until the end of the PROSAFE project...



In addition to scientific board, the audience and the press (Newsletter staff) had also an opportunity to vote for their favorite. CROWNALIFE was chosen as “Dr. Choice of the Audience” and as “Dr. Choice of the Press”. The magnificent song (Ne me quittez pas !) performed by Dr. Joël Doré also gave some extra points.



Prof. Tiina Mattila-Sandholm received all the TIPs that were still in one piece after the demonstrations. The TIPs of each project (with 10 €) were:

**Microbe Diagnostics:**

- toy fish (FISH), tube + magnifying glass (flow cytometry), potato chips (chips)

**Crownalife:**

- toilet paper (milk and longevity), song to celebrate crown of life

**Protech:**

- hammer to stress and knock out bugs

**Progid:**

- stool basin (launching of new hat), toy fish

**Deprohealth:**

- the Simpson family (first comes Homer, then the second generation; Lisa)

**Propath:**

- shooting balloons with bow and arrows (mechanisms how to kill bugs)

**Prosafe**

- see the text alongside

**EU & microfunction**

- Glenn-toy with yogurt-package in his head (model)

## 6<sup>th</sup> framework programme & new member states – day 3

On Wednesday morning two on-going EU-projects were presented. Dr. Jerry Wells started with Highlights of the LABEL project, in which LAB have been shown to be promising vehicles for the prevention and treatment of type I allergy using a murine model of birch pollen allergy.

Dr. Jerome Panes continued with CRAFT project on microencapsulation of probiotic products. Pre-adaptation, testing of different encapsulation methods, stability testing in different food and feed products and evaluation of the release of micro-encapsulated probiotics in the GI-tract are studied in the project.

After EU-project presentations new actions from candidate countries were presented. Dr. Tsona Stefanova, from LB Bulgaricum, presented information about the only state owned dairy in Bulgaria, which produces dairy products from their own local lactic acid bacteria strains. LB Bulgaricum studies the technological performance and health benefits of yoghurt bacteria; thermophilic lactobacilli and bifidobacteria. They have a large strain collection of LAB and starter cultures, consisting of over 200 lactobacilli and 160 bifidobacteria.

The next speaker, Prof. Maria Bielecka from Poland, told about their studies, in which the effectiveness of probiotics, prebiotics and synbiotics were examined in vivo on healthy and *Salmonella* -challenged rats and broiler chickens. The developed synergistic sets of probiotic strains have been applied for development of new probiotic products. The last speaker from candidate countries was Dr. Włodzimierz Grajek from Poland, who presented the new projects concerning pre- and probiotics at their University.

In addition, all the speakers from candidate countries presented methods that are used in their laboratories for probiotic, probiotic and/or synbiotic research.

Last and still the fastest speaker – as session chair Dr. Annick Mercenier pointed out – of the workshop was Prof. Willem de Vos, who talked about future prospects for the gut health. As Prof. de Vos pointed out, industry, consumers and scientists share the same interests concerning functional foods; does it sell? Does it work? How does it work? With genomic approaches it is possible to gain more understanding of host-microbe interactions and microbial functions in the gut and how to exploit those interactions. Microbial responses are studied in model systems and real life to help the understanding of host responses.

As the workshop ended, Dr. Mercenier showed a modification of the TIP of the DEPROHEALTH (Lisa Simpson; second generation), which fits Prof. de Vos, herself, and Prof. Mattila-Sandholm (from left to right).



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