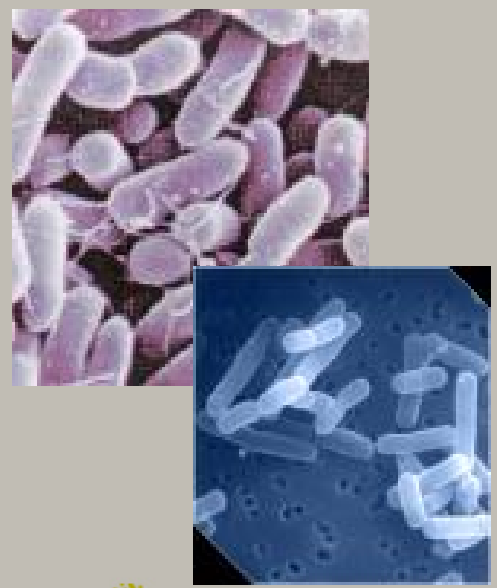
EU and Microfunction Project

Functional Assessment of Interactions Between the Human Gut Microbiota and the Host

Sofia Kolida



Brussels 2005

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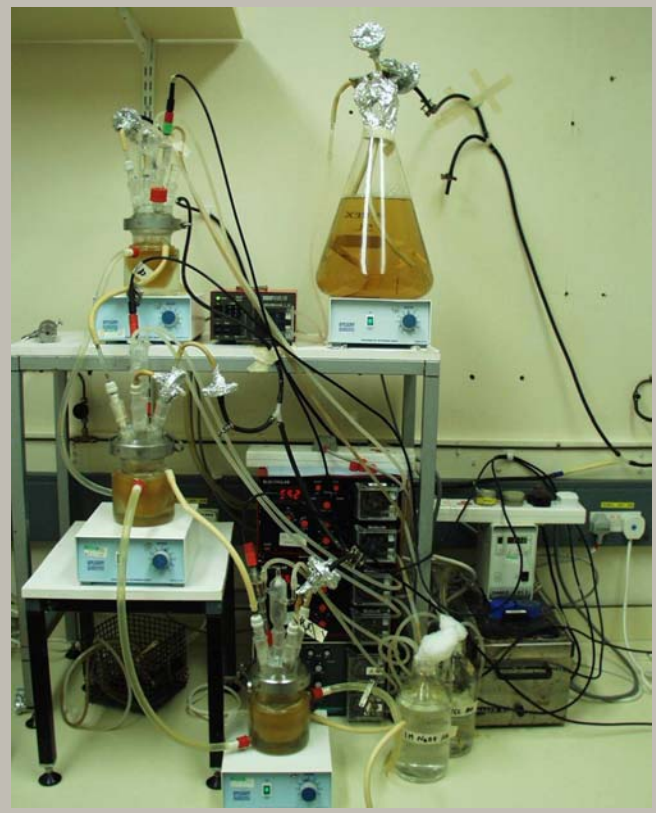
Project Objectives

- Compare the efficacy and safety of probiotics and prebiotics
- Determine effective dose/combination
- Identify mechanisms of action
- Investigate impact upon host function
- Determine effects in several model systems and human volunteers

Determine interactions between gut microbiota and the host



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Project Partners

- University of Reading, UK
- University of Turku, Finland
- Wageningen University, The Netherlands
- Lund University, Sweden
- University of Tartu, Estonia
- Orafti, Belgium
- Probi AB, Sweden
- University New South Wales, Australia

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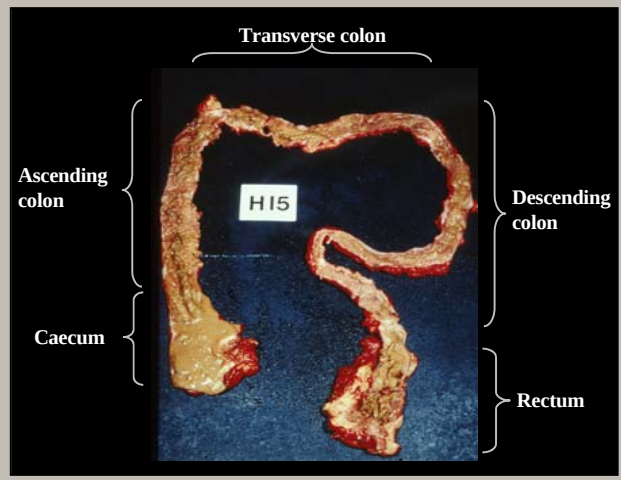
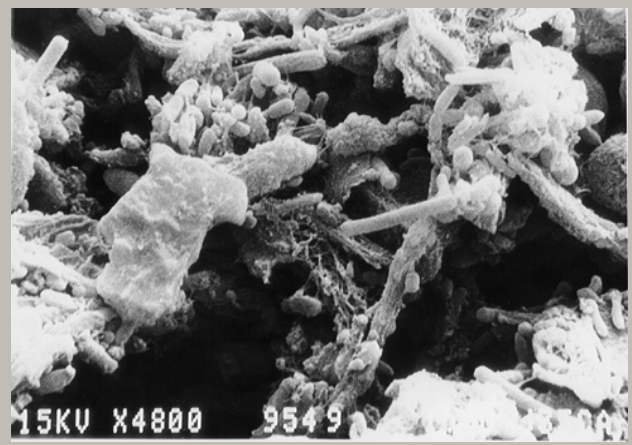
Prebiotic Efficacy WP1

- Generation of a ‘pecking order’ for prebiotics
- Predictions in different colonic areas, for stimulation of a beneficial flora community
- Prebiotic Index = ΔBif + ΔLac - ΔBac - ΔClos
- 20 tests in the reporting period
- FOS type prebiotics performed well, site of fermentation depended upon molecular size
- Probiotic use had little effect





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Synbiotic Development WP2

Pure culture studies: ***L. plantarum WCFS1***
L. paracasei 8700:2
***L. fermentum* ME-3**
B. longum 46

} FOS (Raftilose®P95)

Batch cultures: **anaerobic, stirred pH/ temperature controlled (24h): Increase in bifidobacteria levels, probiotic levels remained stable throughout culture, decrease in eubacteria with the synbiotic**

Gut models: **simulate proximal (pH 5.5), transverse (pH 6.2) & distal (pH 6.8) colon.**

10^9 cfu of each probiotic strain + 6.6g FOS added daily to V1

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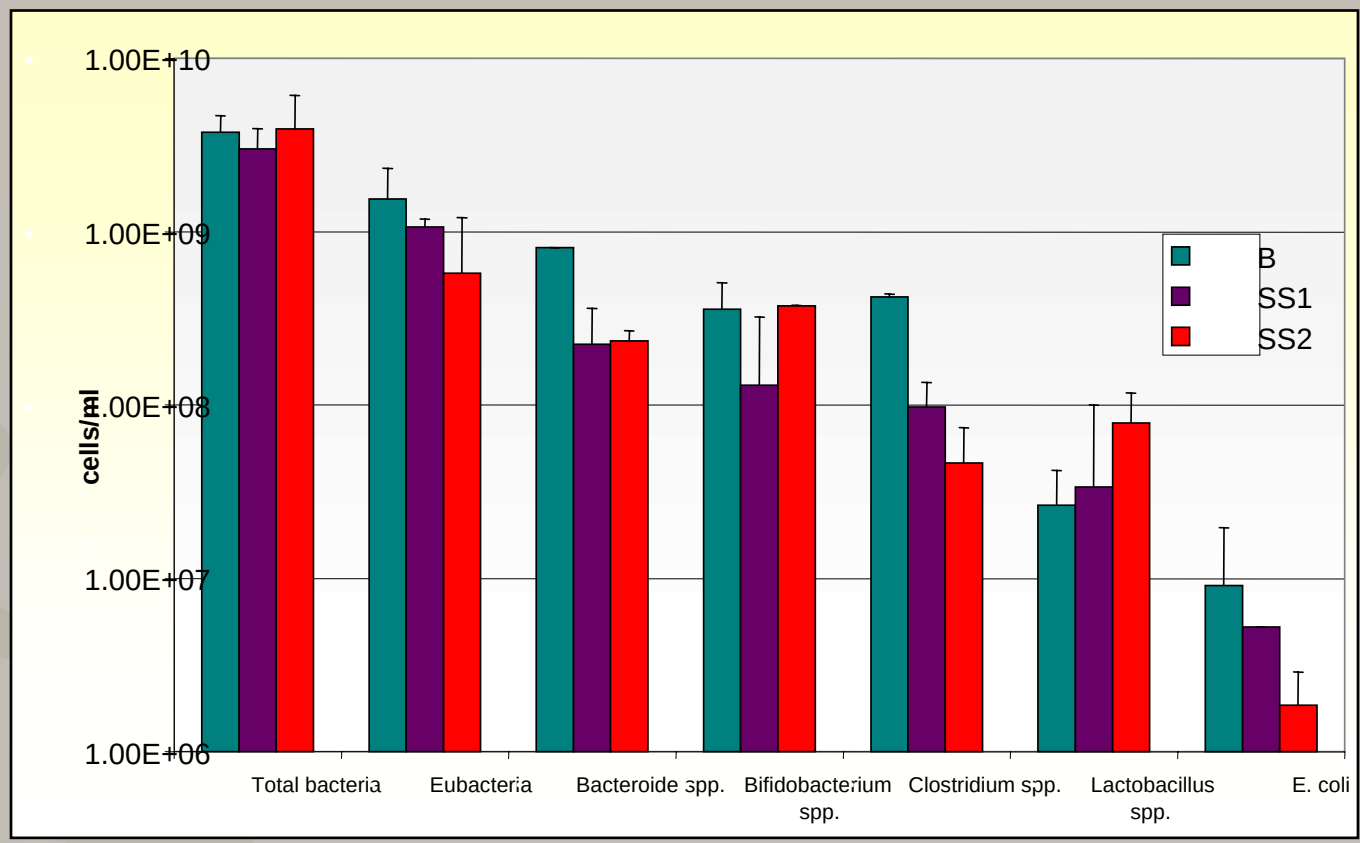
Gut Model Results

- **Bifidobacteria increased in all 3 vessels**
- **Probiotic survived throughout fermentation period**
- **Decrease in clostridia, eubacteria and *E. coli* in all vessels**

Bacterial populations in vessel 3 (pH6.8) at baseline, SS1 & SS2



GI Function and Translocation WP3

- Acute liver model (**D-galactosamine induces liver failure**)
- Colitis model (**DSS causes acute distal colitis**)
- Human study on bacterial translocation (**surgery, 60/66 recruited**)

Probiotics and/or prebiotics not only counteract adverse bacteria, they:

- **can improve the status of the mucosa**
- **can increase the barrier effect**

Bifidobacterium infantis strains with and without a combination of Oligofructose and Inulin (OFI) attenuate inflammation in DSS induced colitis in rats.

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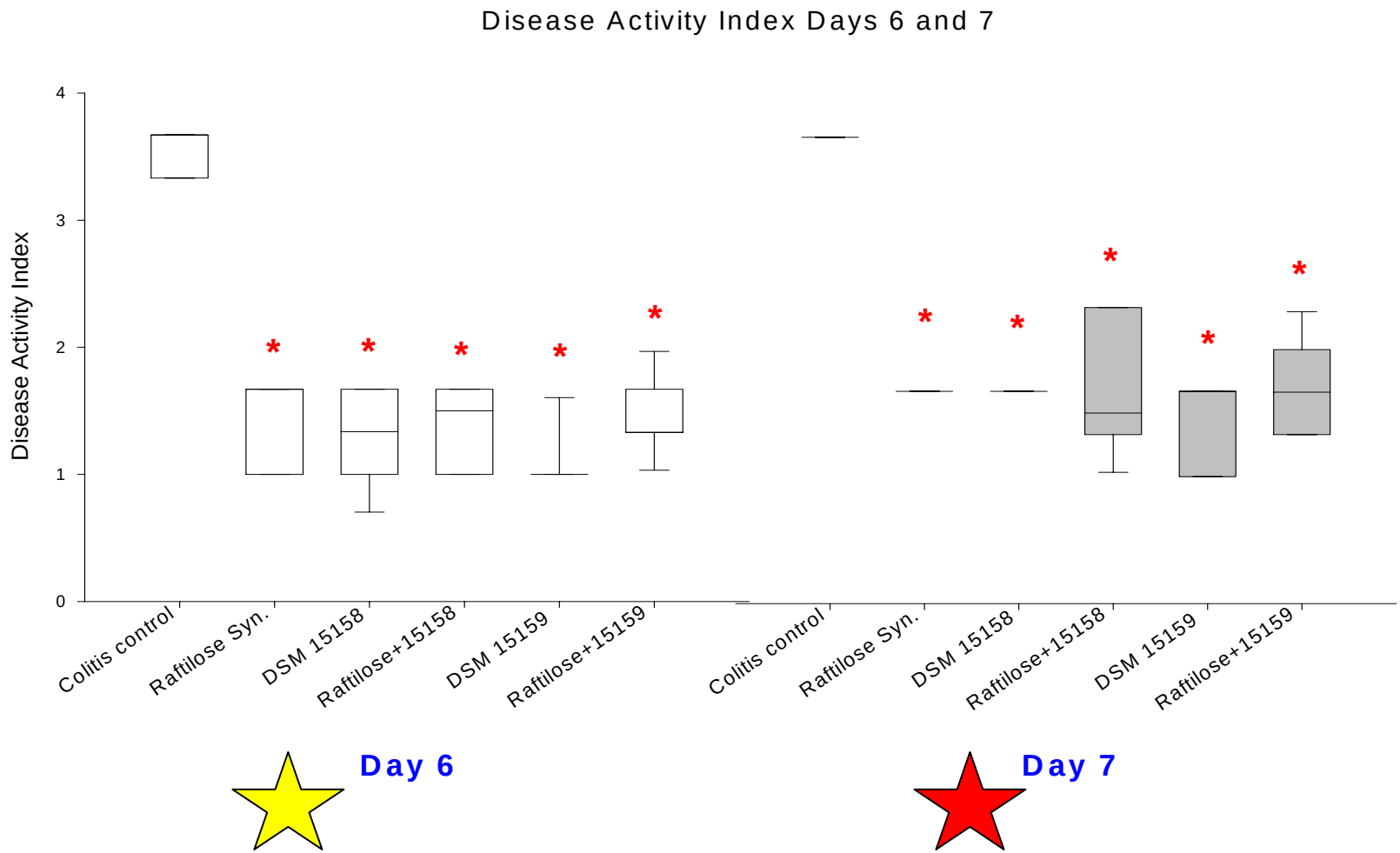
Disease Activity Index (Body weight, Stool consistency, Bleeding (Hemoccult test)



* denotes p<0.05 compared to Colitis control

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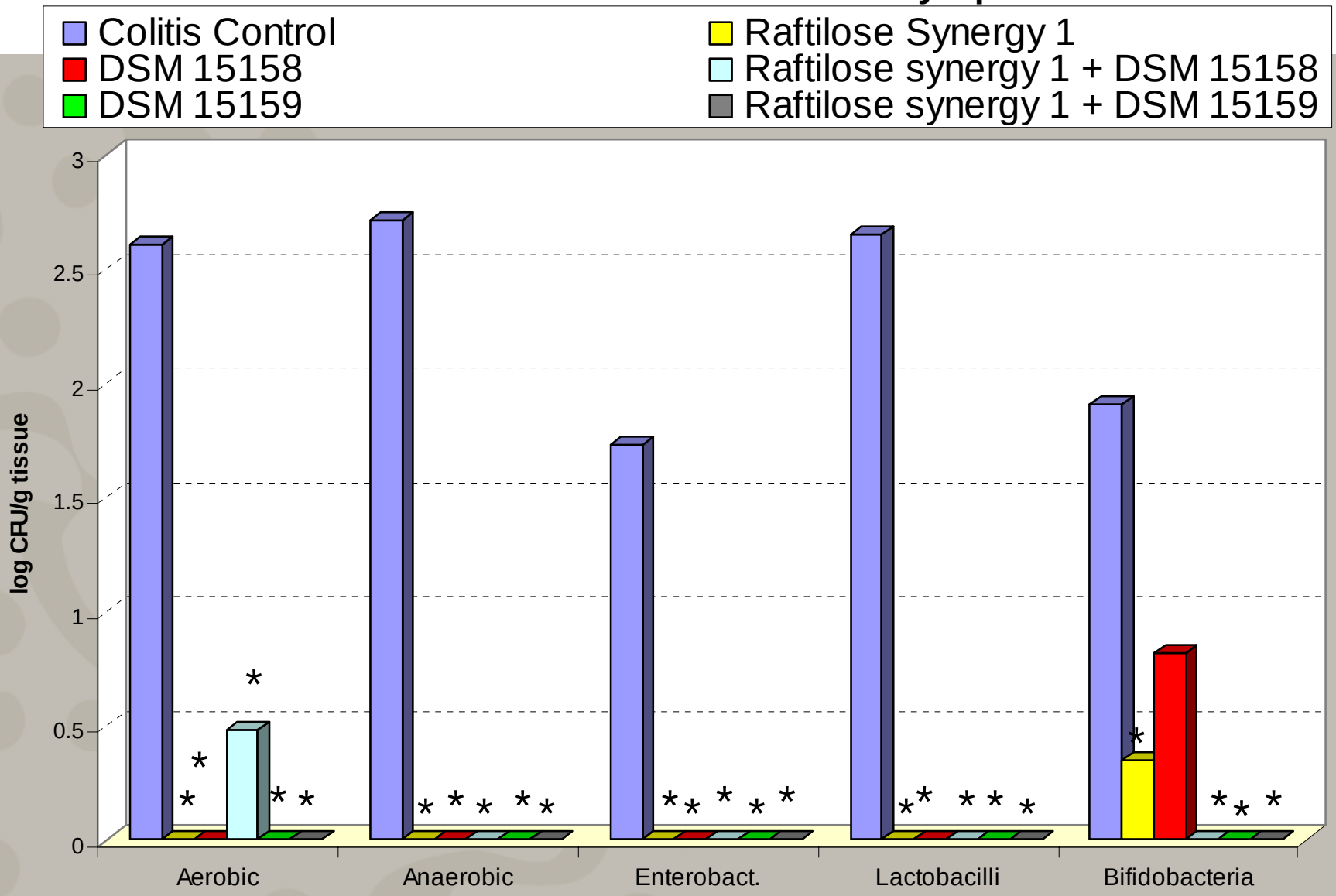
Bacterial translocation to the mesenteric lymph nodes



* denotes p<0.05 compared to Colitis control

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Conclusions WP3

- **Administration of certain strains of bifidobacteria with and without Raftilose Synergy 1 improved significantly the DAI in the DSS-induced colitis model and decreased colonic Myeloperoxidase activity**

- **Mesenteric lymph nodes bacterial translocation decreased significantly in all the groups compared to colitis control. Bifidobacteria translocation significantly decreased in all the groups except *B. infantis* DSM 15158 group**

- **Both probiotics and prebiotics can counteract intestinal inflammation, alone and in combination.**

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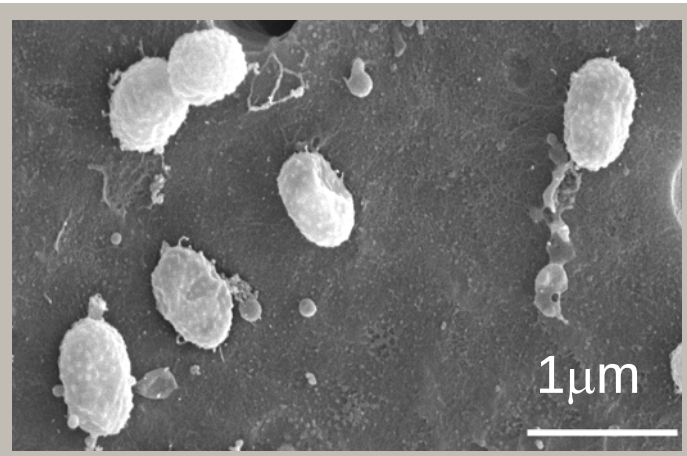
Host – Gene Interactions WP4

Akkermansia muciniphila Muc^T

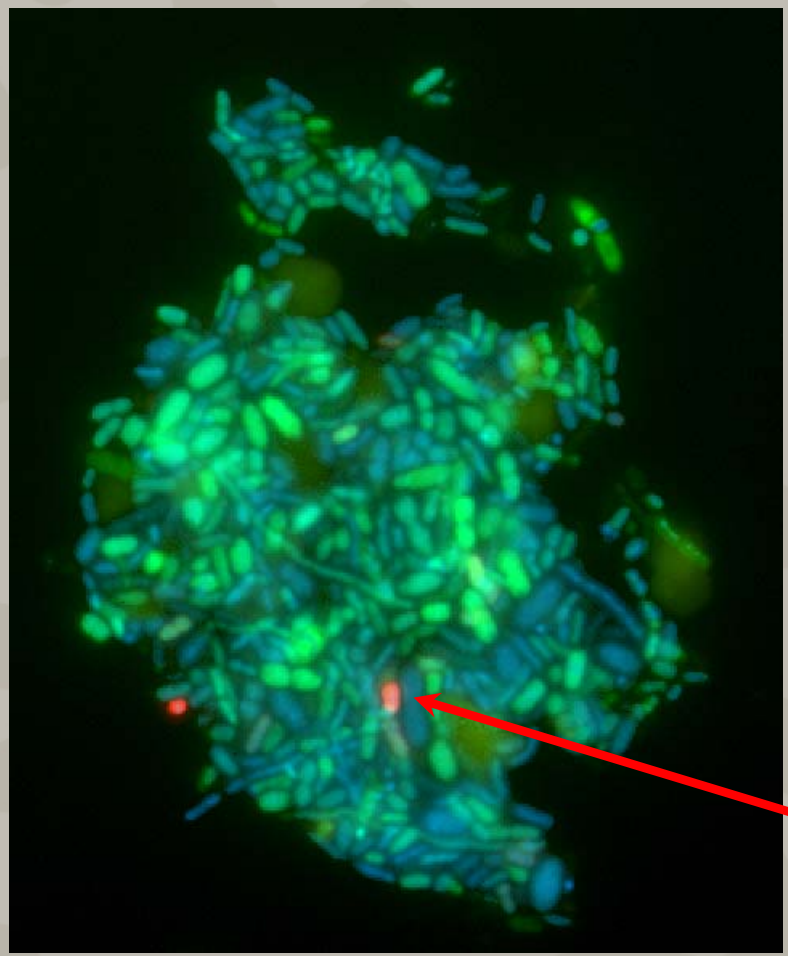


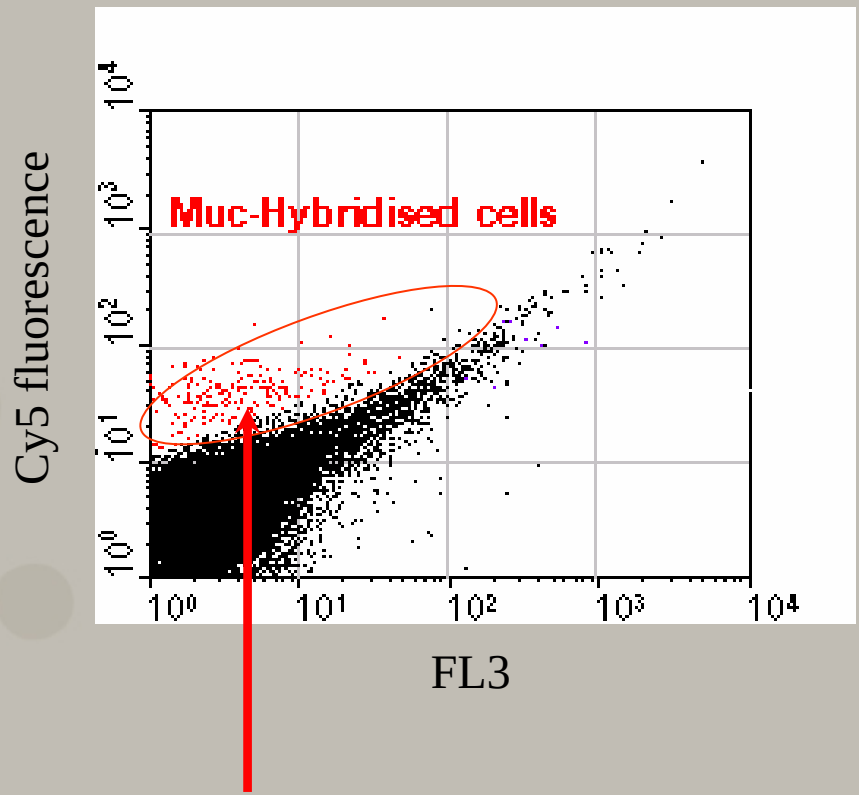
- Isolated from human fecal sample on mucin
- Gram negative, strict anaerobic
- Non motile, non spore-forming
- Highly specific on mucin (C and N source)
- Production of acetate, propionate

- Member of the *Verrucomicrobium* phylum

(Derrien *et al*, IJSEM, 54: 1469-1476)

Quantify *A. muciniphila* in 20 fecal samples from healthy adults





- *A. muciniphila*

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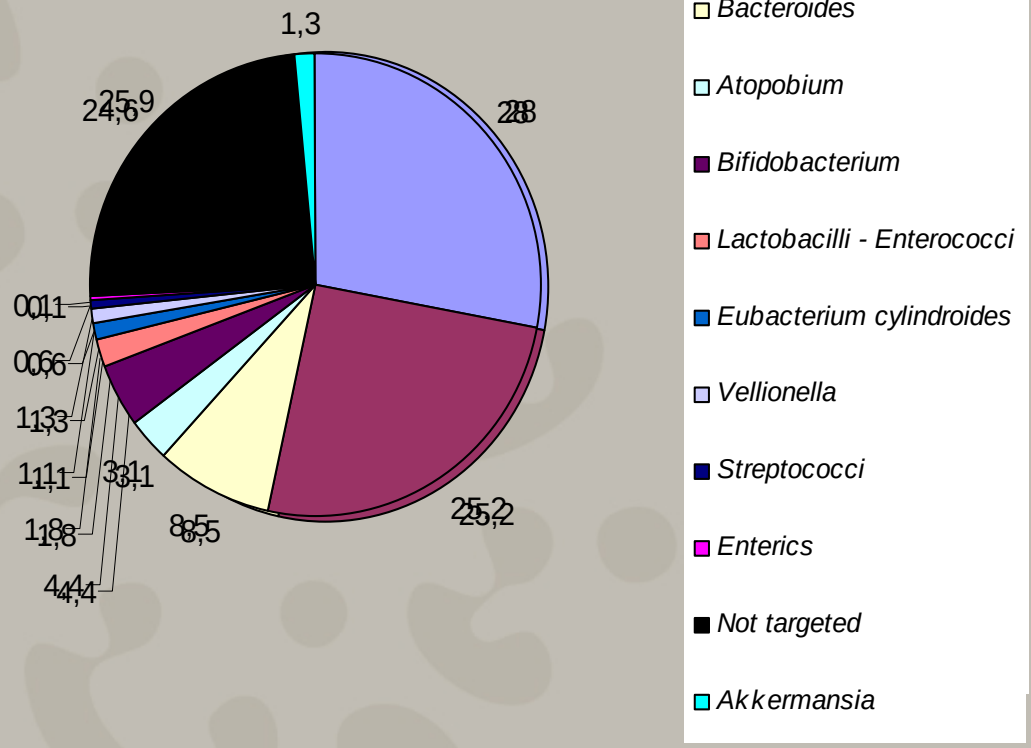
Quantify *A. muciniphila* in 20 fecal samples from healthy adults

- 1.33 % (±0.82) of total fecal cells
- $1.07x10^9$/ g feces (wet weight)



Lay *et al* (Unpublished)

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Safety Assessment of Probiotics WP5

- Various safety determinants
- Clinical and intestinal lactobacilli, bifidobacteria
- Adhesion, enzymes, serum killing, respiratory burst, haemolysis
- In vivo studies, rats

A safety assessment that compares new strains, existing probiotics and different origins

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Translocation of (clinical) *L. rhamnosus* in rats

Five *L. rhamnosus* strains

1. *L. rhamnosus* GG
2. *L. rhamnosus* T-21162
3. *L. rhamnosus* T-4846
4. *L. rhamnosus* T-31846
5. *L. rhamnosus* L42

\+ Control

Aims

- Translocation
- Intestinal permeability
- Helicobacter infection

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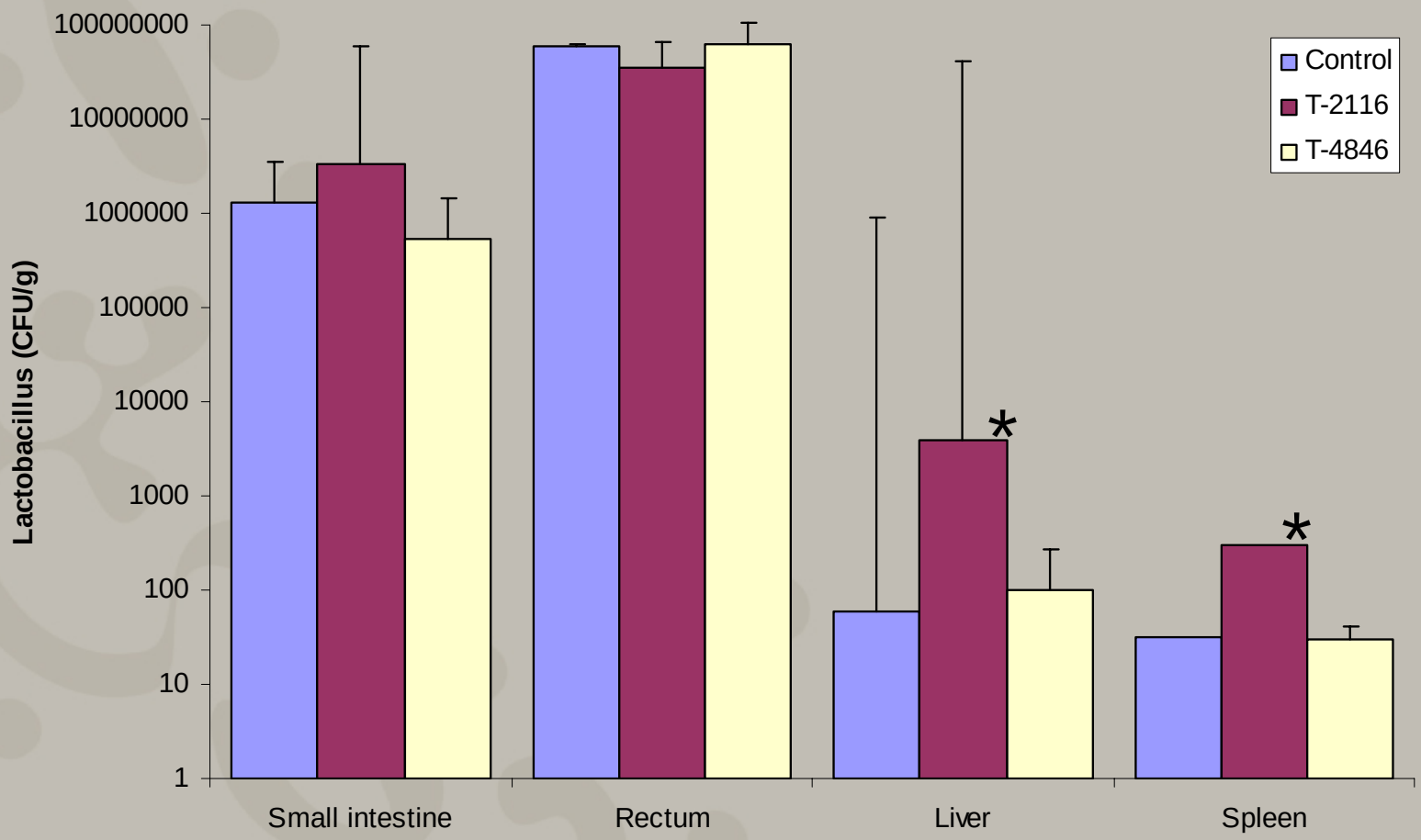
Results: *Lactobacillus* translocation



- Chronic inflammation in rats colonised by *L. rhamnosus* T-2116

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In vivo synbiotic evaluation in humans WP 6

The best combination out of 7 probiotics was selected:

***Bifidobacterium longum* B46**
***Lactobacillus paracasei* 8700:2**
***Lactobacillus fermentum* ME-3**

- anti-infectious **- antioxidative**

- **Test synbiotic:** Probiotic combination with FOS (Raftilose P95)
- randomised, double-blind, placebo controlled, cross-over trial

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Clinical study design

Inclusion criteria:

50 healthy volunteers

- wish to participate in the study
- age 20-60 years (1/3 males and females)
- healthy (i.e. no known health problems and no medical conditions that require drug therapy)
- informed consent obtained(APP1)

Exclusion criteria:

- history of any gastrointestinal disease
- use of any antimicrobial drug within last month
- use of any regular concomitant medication, incl. medical preparations
- pregnancy / breastfeeding
- food allergy

Reasons for withdrawal

- acute disease and antimicrobial treatment
- any allergy
- nausea, vomiting, diarrhoea
- non-compliance

Randomly allocated by computer to receive either:

- 2 capsules of probiotic mixture

dose 6 x10^9 CFU

1 sack of prebiotic or

- Placebo capsule + placebo maltodextrin

for 3 + (2) + 3 weeks

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Clinical study sample analysis

Selected general health indices:

- **General welfare**
- **Intestinal function**
- **Blood pressure**
- **Bone density**
- **Body height/ weight**

Additional marker: **Food intake** generally before trial

Measurable markers of synbiotic functionality

- **Improving IMF composition**
- **Lowering the excessive oxidative stress markers**
- **Reducing colonisation by selected pathogens :**

Helicobacter pylori

Blood sera:

- **the serum level of oxidised LDL (by ELISA assay)**
- **resistance of LDL to oxidation (lag phase of LDL by time-course assay of diene conjugates in LDL particles)**
- **antioxidative activity blood sera by TAS-test (Randox kit)**
- **glutathione content and glutathione red-ox ratio (spectrophotometrically)**

Urine: cadaverine, putrescine (GC, HPLC)

Faeces: microbial/ molecular/ SCFA analysis/ *H. pylori* (ImmunoCard STAT! HpSA test)

40 volunteers recruited, 20 already started

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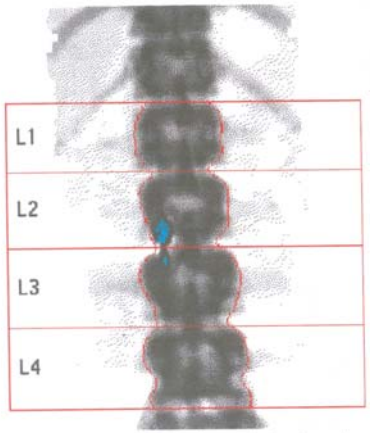
Bone density

ROOTS, IRJA

AP SPINE BONE DENSITY

Facility: TÜ MBL
45 years 13.11.1959
162.0cm 71.0kg White Female
Physician: MIKELSAAR

Acquired: 17.02.2005 (4.5c)
Analyzed: 17.02.2005 (4.5c)
Printed: 17.02.2005 (4.5c)
rootsi00.s90



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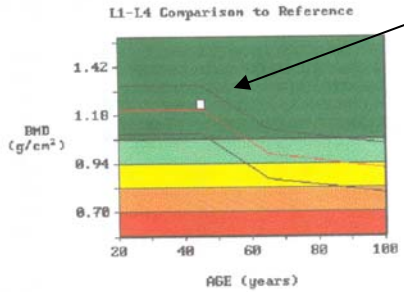


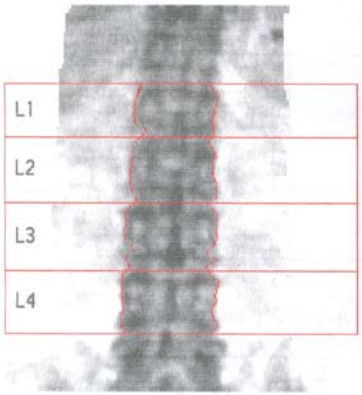
Region	BMD g/cm²	Young-Adult %	Young-Adult T	Age-Matched %	Age-Matched Z
L1-L4	1.236	105	0.5	103	0.3

ROOTS, MALLE

AP SPINE BONE DENSITY

Facility: KARDIOLOOGIA
68 years 16.08.1933
162.0cm 74.0kg White Female
Physician: SIIM

Acquired: 23.05.2002 (4.5c)
Analyzed: 23.05.2002 (4.5c)
Printed: 28.05.2002 (4.5c)
rootsm00.s90



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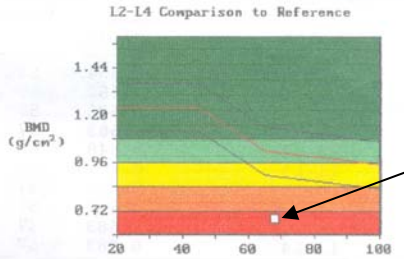

Region	BMD g/cm²	Young-Adult %	Young-Adult T	Age-Matched %	Age-Matched Z
L2-L4	0.683	57	-4.3	68	-2.7

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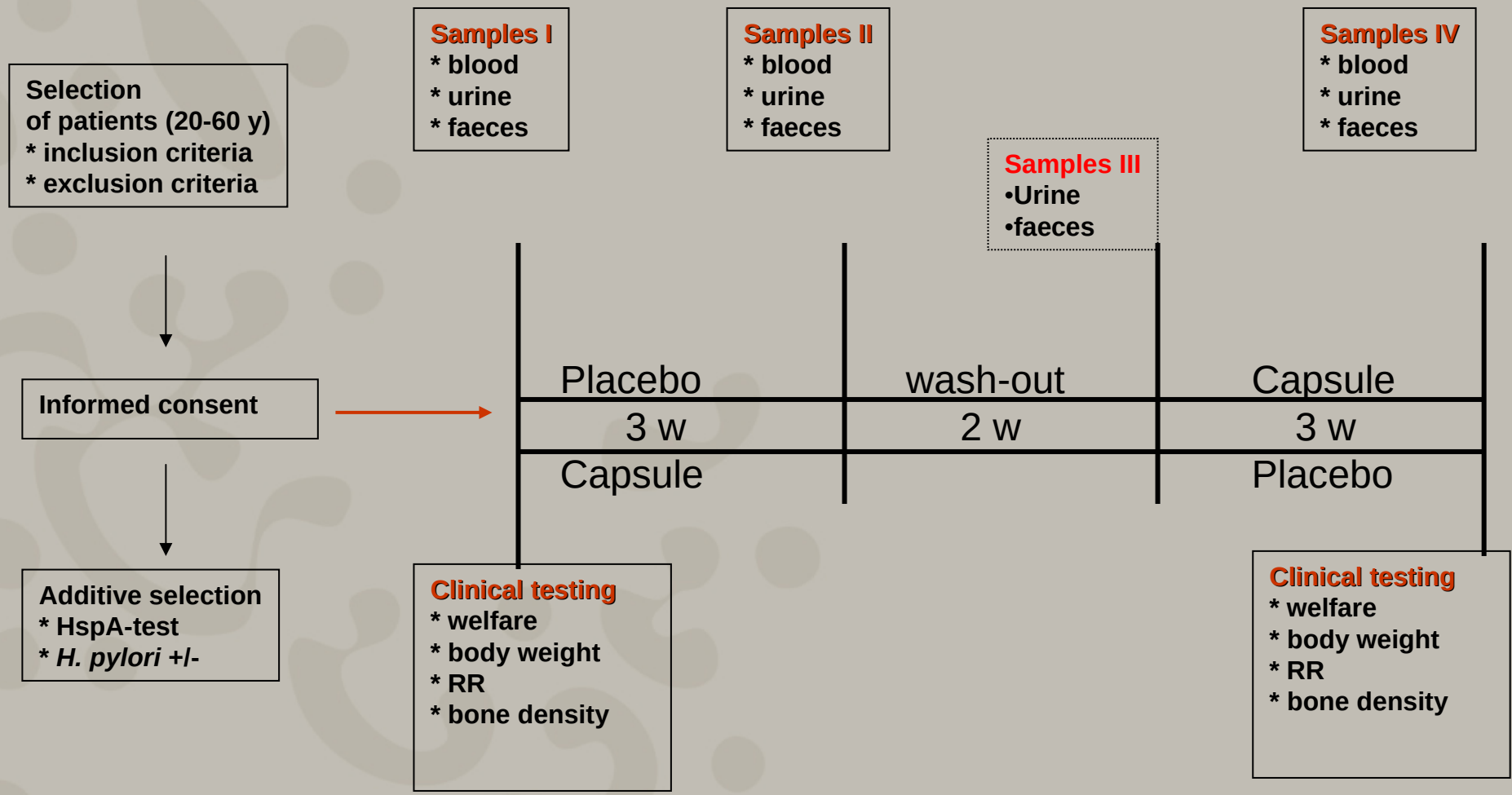
Clinical Study Timeline



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Publications

WP1

•**Kolida** S, *et al. In vitro* evaluation of potential prebiotics in a 3-stage model of the human colon using the prebiotic index. Manuscript ready for submission

WP3

•**Osman** N, *et al*. Modulation of the effect of Dextran Sulfate Sodium-induced Acute Colitis by the Administration of Different Probiotic Strains of *Lactobacillus* and *Bifidobacteria*. *Digestive Diseases and Science* 2004; 49(2): 320-327.

•**Osman** N, *et al*. Probiotic strains of *Lactobacillus* and *Bifidobacterium* affect differently the translocation and intestinal load of *Enterobacteriaceae* after D-galactosamine-induced liver injury in rats. *Microbial Ecology in Health and Disease* 2005; In press

•**Osman** N, *et al*. *Bifidobacterium infantis* strains with and without A combination of Oligofructose and Inulin (OFI) attenuate inflammation in DSS-induced colitis in rats. Manuscript ready for submission.

WP4

▪Derrien *et al*, IJSEM, 54: 1469-1476

WP5

▪**Ouwehand**, A.C *et al*. (2004) Assessment of suggested virulence factors and related properties of clinical, faecal and dairy *Bifidobacterium* isolates. Bioscience and Microflora 23:37-42

▪**Gueimonde**, M., *et al*. (2004) Safety of Probiotics. Scandinavian Journal of Nutrition 48:42-48

▪**Derrien**, M., *et al*. (2004) The intestinal mucosa as a habitat of the intestinal microbiota and a target for probiotic function and safety. Microbial Ecology in Health and Disease 16:137-14

▪**Ouwehand**, A.C., *et al*. (2004) Phenotypic differences between commercial *Lactobacillus rhamnosus* GG and *L. rhamnosus* strains recovered from blood. Clinical Infectious Diseases 39:1858-1860

WP6

▪**Truusalu**, K., *et al.*(2004) The influence of antibacterial and antioxidative probiotic lactobacilli on gut mucosa in a mouse model of *Salmonella* infection. Microbial Ecology in Health and Disease