Lactic Acid Bacteria and the development of microbiota

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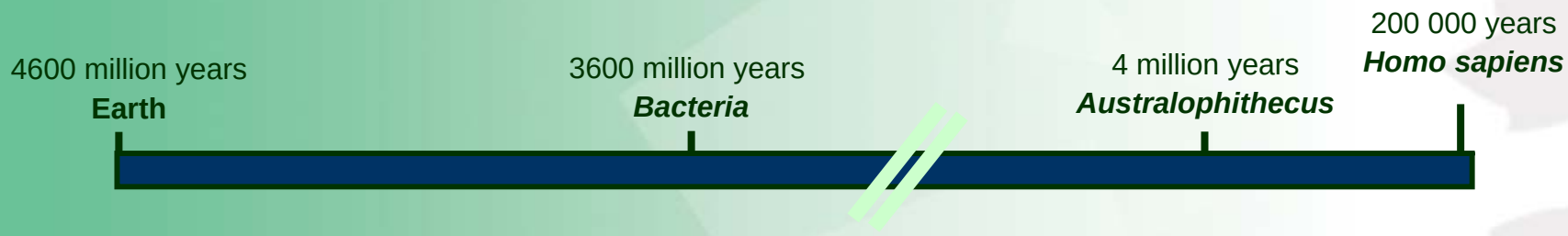
Miguel Gueimonde Fernández, PhD (IPLA-CSIC)

Instituto de Productos Lácteos de Asturias (IPLA)

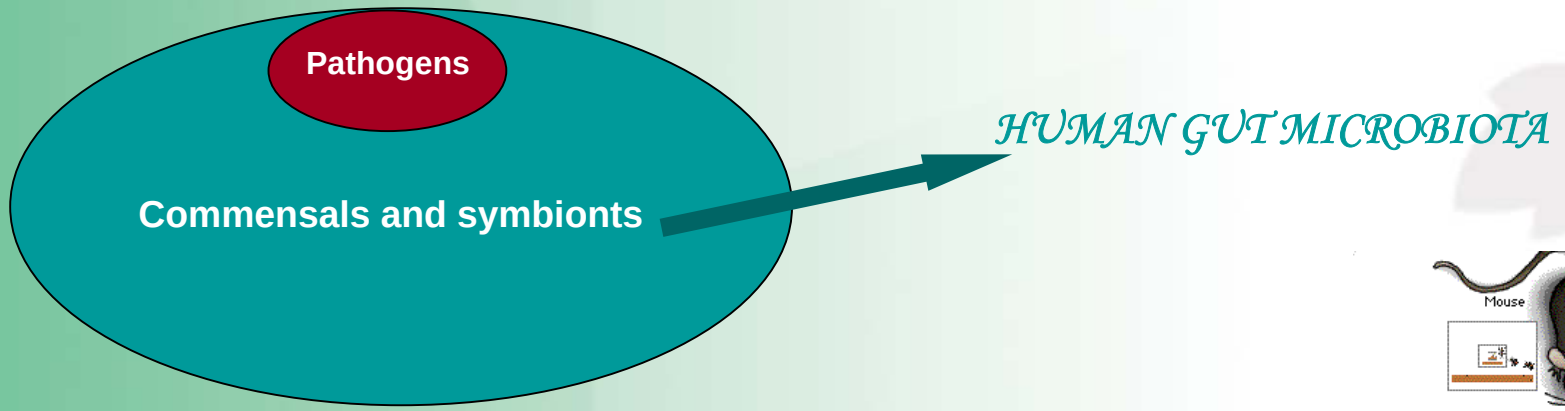
Consejo Superior de Investigaciones Científicas

CSIC

A microbial world



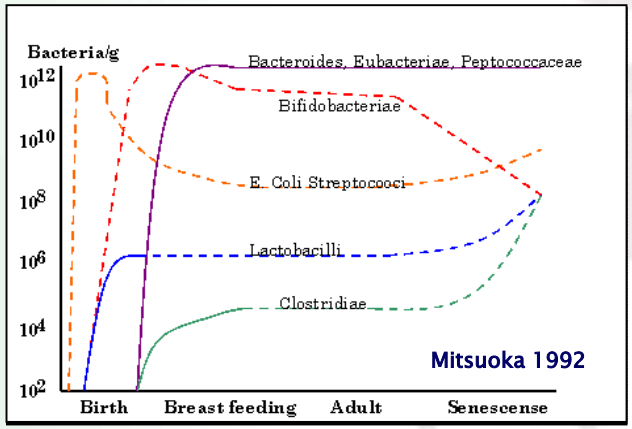
- *Evolution in a microbial world*
- *Microbes: a major evolutive force (innate immune system)*
- *Different relationships established with microbes*



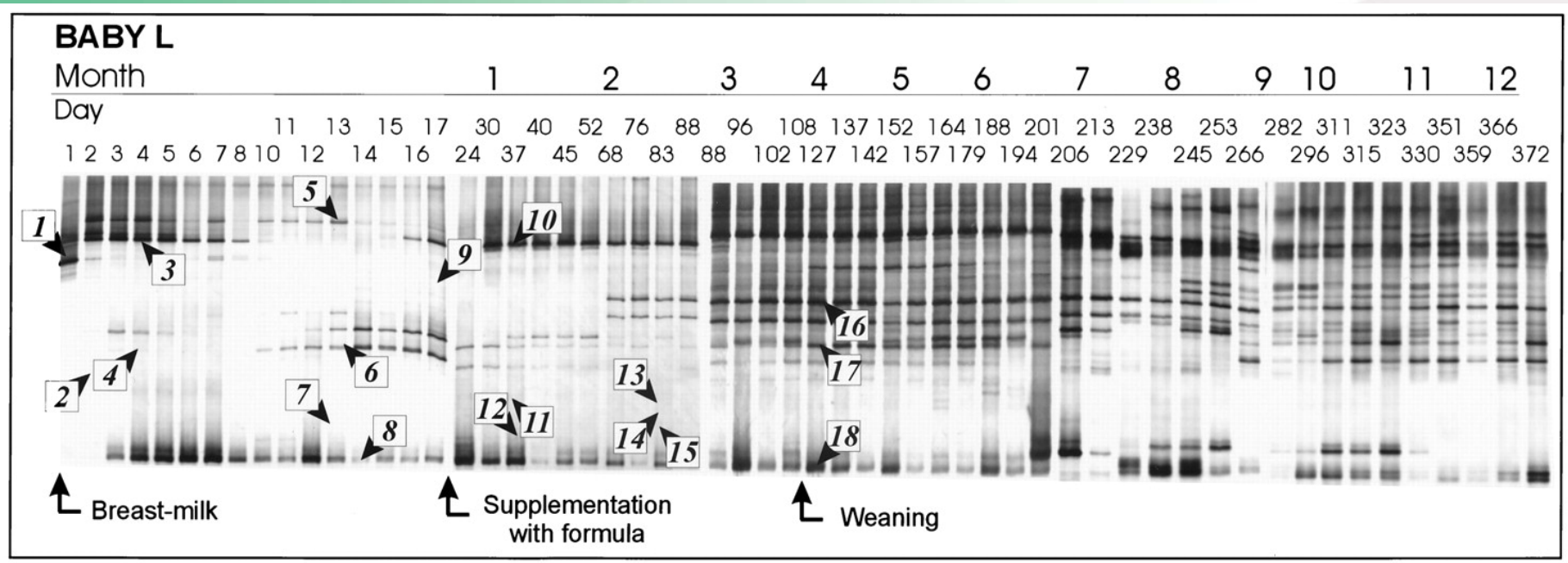
Intestinal Microbiota

Complex and dynamic community

- *Specific for each person (environmental and genetic factors)*
- *500-1000 species*
  - ✓ Predominantly anaerobic bacteria (*Bacteroides, Eubacterium, Bifidobacterium, Clostridium, Ruminococcus, Peptococcus* and *Peptostreptococcus*)
  - ✓ Aerobes (*Escherichia, Enterobacter, Lactobacillus*, etc.)
- *10^{12} microorganisms/g contents*
- *10 times more than human cells*
- *10-100 times more genes*
- *Up to 1.5 kg body weight*
- *60% faecal weight*



Establishment of gut microbiota



N	species	%	clones	GenBank	N	species	%	clones	GenBank
1	*Clostridium disporicum*	95	L1c	AF253386	10	*Ruminococcus torques*	94	L37A	AF253389
2	Uncultured bacterium adhufec 23 *(Bacteroides thetaiotaomicron)*	93	L2e	AF253387	11	Uncultured bacterium adhufec 55 *(Bacteroides)*	94	L37H	AF253393
3	*Enterobacter asburiae*	98	L4dI	AF253377	12	Uncultured bacterium adhufec 367 *(Bacteroides)*	93	L37C	AF253390
4	*Streptococcus salivarius*	95	L4b	AF253388	13	*Enterococcus raffinosus*	98	L83M	AF253382
5	*Clostridium paraputrificum*	91	L13j	AF253381	14	*Bacteroides caccae*	98	L83D	AF253383
6	*Streptococcus salivarius*	97	L13dE	AF253378	15	*Bacteroides* sp. AR20	95	L83J	AF253385
7	*Bacteroides vulgatus*	96	L13dG	AF253379	16	Uncultured bacterium A57 *(Ruminococcus)*	94	L127dB	AF253375
8	*Bifidobacterium pseudocatenulatum*	98	L14B	AF253372	17	Uncultured bacterium adhufec 171*(Ruminococcus)*	96	L127dA	AF253374
9	*Staphylococcus caprae/epidermidis*	96	L17j	AF253384	18	*Bifidobacterium pseudocatenulatum*	97	L127dC	AF253376

Favier et al. 2002

Microbiota development in infants

- Genetic background
- Mode of delivery
- Mother's microbiota
- Microbiota of hospital/nurse/staff

- Composition of breast milk?
  - ✓ Oligosaccharides
  - ✓ Fat
  - ✓ Microorganisms

Breast-milk

- *Breast-milk as a source of LAB?*
  - *LAB displaying identical RAPD profiles isolated from breast-milk and infant faeces*
  - *L. gasseri and E. faecium*
  - *Counts in breast-milk ranged from 2×10^4 to 1×10^5*

Martin et al. 2003

Breast-milk

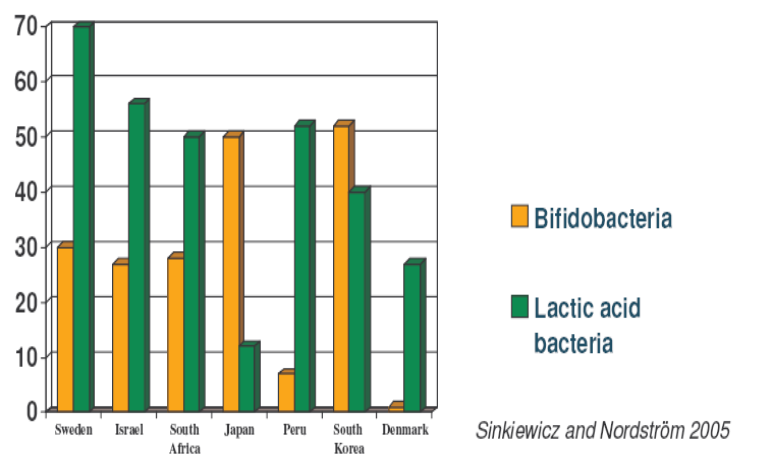
LABs commonly isolated from human milk

Bacterial group	Species
Staphylococcus	*S. epidermidis* *S. hominis* *S. capitis* *S. aureus*
Streptococcus	*S. salivarius* *S. mitis* *S. parasanguis* *S. peroris*
Lactobacillus	*L. gasseri* *L. rhamnosus* *L. acidophilus* *L. plantarum* *L. fermentum*
Enterococcus	*E. faecium* *E. faecalis*

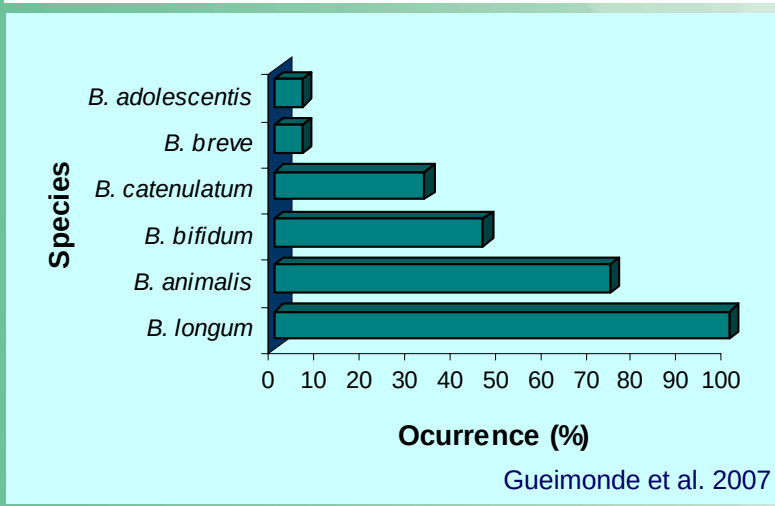
What about bifidobacteria?

Breast-milk

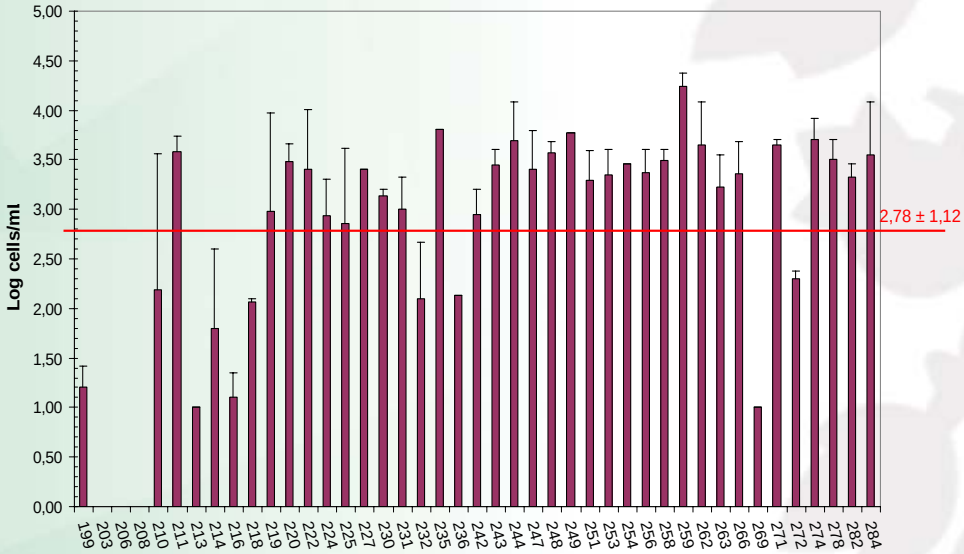
Breast-milk as a source of Bifidobacteria?



Sinkiewicz and Nordström 2005



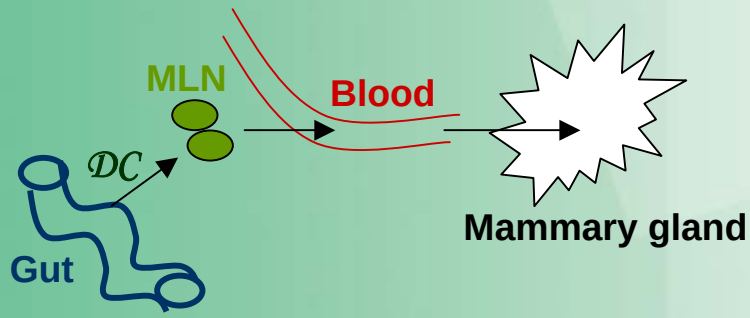
Gueimonde et al. 2007



Gueimonde et al. 2007

Breast-milk

- Breast-milk as a source of bacteria?
  - *B. longum* DNA detected (TGGE) in PBMC, Milk cells, infant and maternal faeces
  - *S. thermophilus* and *St. epidermidis* DNA detected in milk cells and infant faeces
  - $<10^3$ CFU/mL breast-milk



- Increased translocation in pregnant mice

Perez et al. 2007

Microbiota development in infants

- Genetic background
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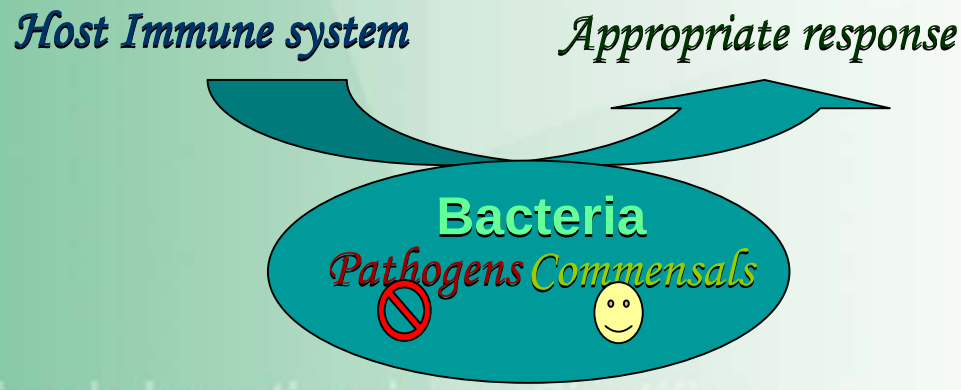
- Composition of breast milk?
  - ✓ Oligosaccharides
  - ✓ Fat
  - ✓ Bacteria
- Nutrition and environment
- Food microbiota (hygiene)
- Pro- and prebiotics
- Medication

Intestinal Microbiota : functions

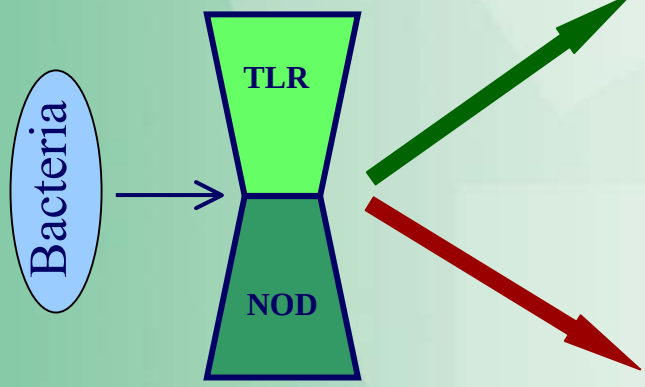
- **Metabolic**
  - Fermentation (food components, mucus, etc.)
  - Production of substances (SCFAs, vitamins, etc.)
- **Morphologic**
  - Control of epithelial cell proliferation and differentiation
  - Development of the mucosal irrigation network (angiogenesis)
  - Development of the immune system
- **Protective**
  - Competition (attachment sites, nutrients, etc.)
  - Production of antimicrobial substances (bacteriocins, organic acids, etc.)

Microbiota-Host interactions

- *Establishment of oral tolerance* (Sudo et al. 1997)
- *Mainteinance of intestinal homeostasis and healing of intestinal injuries* (Rakoff-Nahoum et al. 2004)
- *Regulates acquisition and storage of lipids* (Bäckhed et al. 2004)
- *Induces mucosal expresion of bactericidal proteins, establishment of mucosal barrier* (Hooper et al. 2003)
- *Drives gut development, eg. angiogenesis* (Stappenbeck et al. 2002)
- *Development of mucosal immune system* (MacDonald & Monteleone 2002)



TLR/NOD: Functional vs. aberrant



Health

Intestinal homeostasis

- Healing ↑
- Barrier function ↑
- Tolerance ↑
- Bacterial invasion ↓

Disease

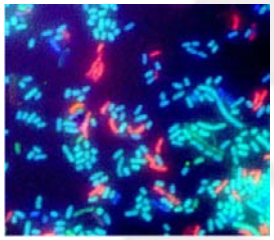
Intestinal inflammation

- Injury ↑
- Barrier destruction ↑
- Tolerance ↓
- Bacterial invasion ↑

Modified from Cairo 2005

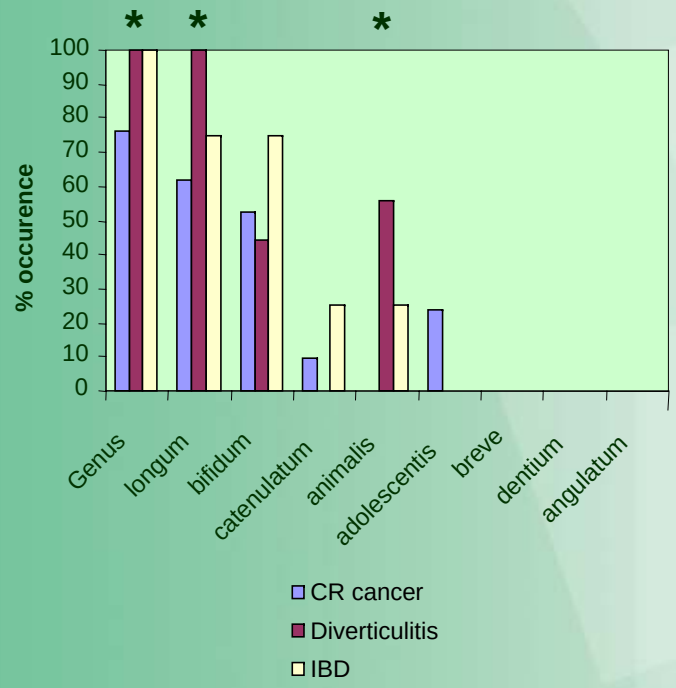
Intestinal Microbiota: role in health and disease

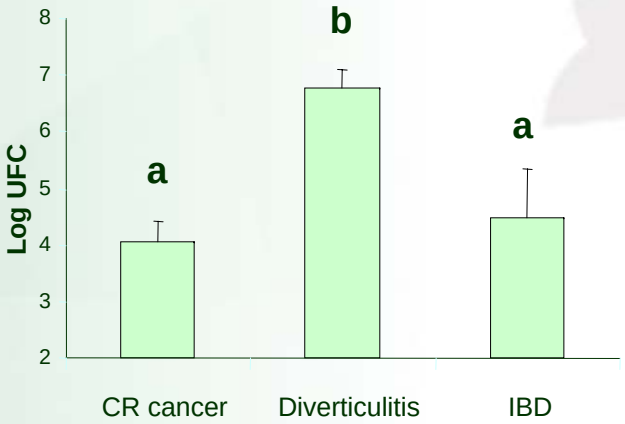
- **The intestinal microbiota is related to many diseases at both intestinal and extra-intestinal levels**
  - Atopic diseases
  - Other autoimmune diseases (rheumatoid arthritis)
  - Inflammatory bowel diseases (Crohn´s, ulcerative colitis, etc.)
  - Overgrowth of Clostridium difficile (pseudomembranous colitis, antibiotic associated diarrhoea)
  - Colon cancer
  - Autism
  - Obesity?



Intestinal Microbiota: role in health and disease

Qualitative and quantitative alterations of bifidobacterial microbiota



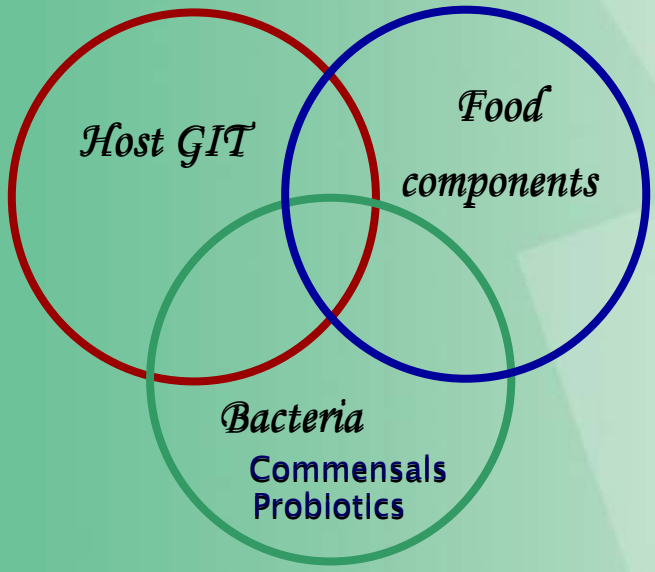


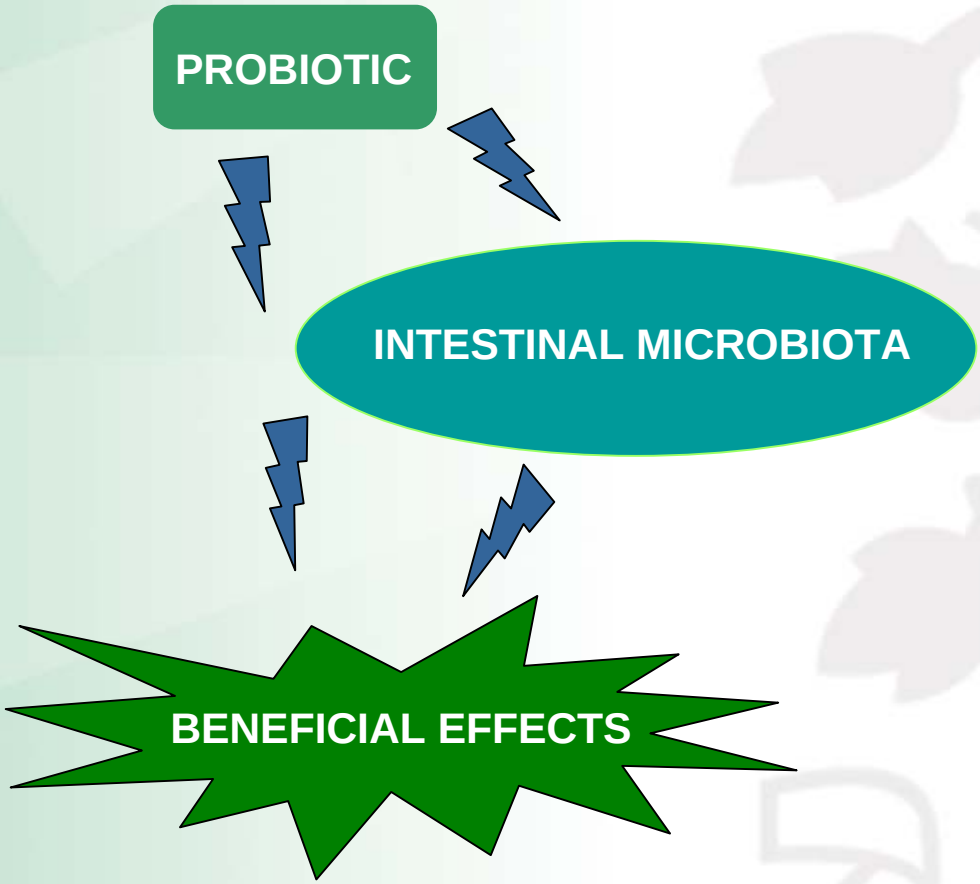
Genus and B. longum

Gueimonde et al. 2007 (In press)

Intestinal Microbiota: target of probiotic action

Intestinal interactions





Probiotic

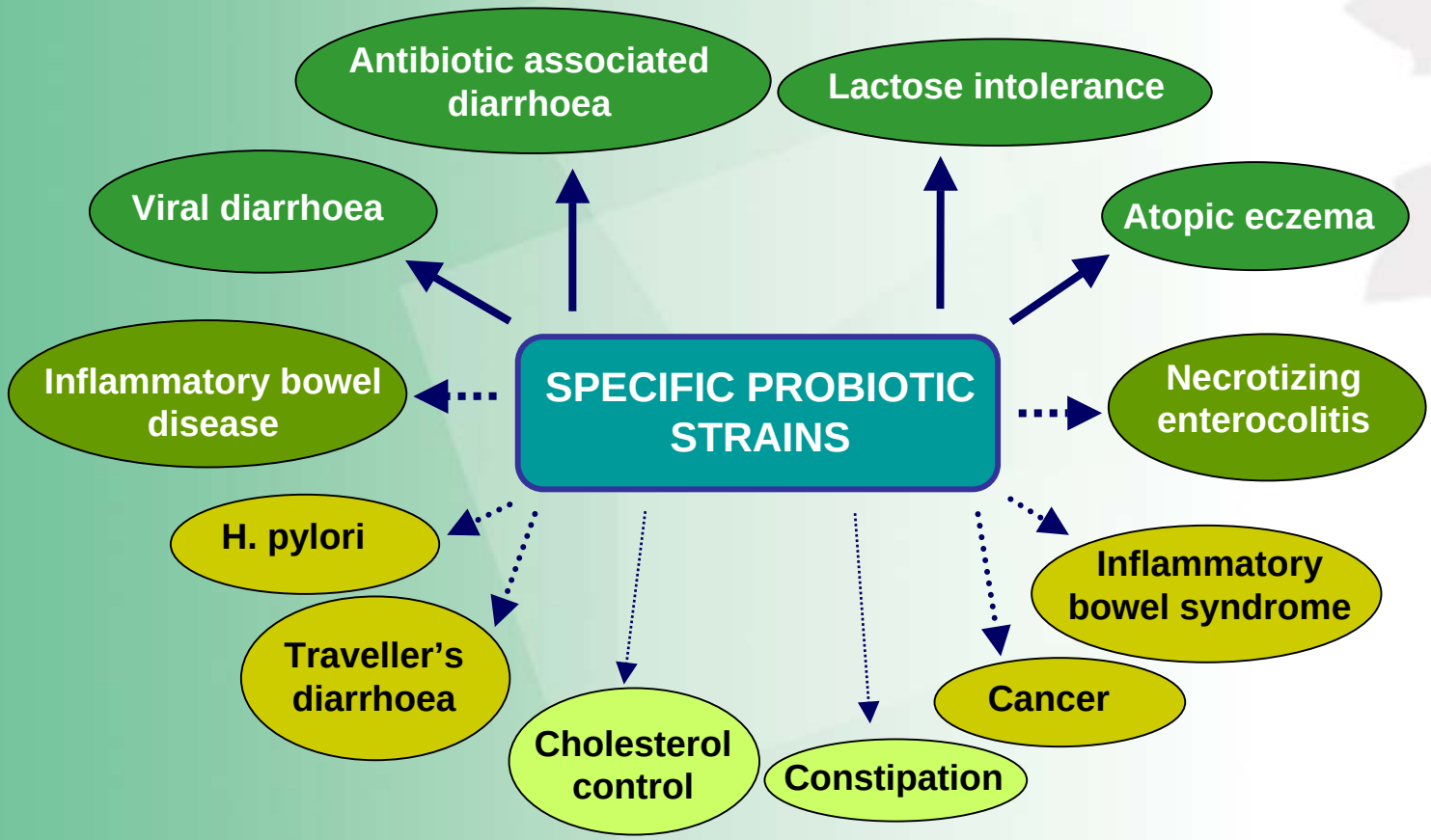
"Live microorganism which when administered in adequate amounts confer a benefit on the host"

(FAO/WHO 2002)

- *Lactobacillus*
- *Bifidobacterium*
- *Enterococcus*
- *Saccharomyces boulardii*
- *Escherichia coli*
- *Bacillus cereus*
- *Clostridium butyricum*

Efficacy and Safety have to be Scientifically demonstrated!

Health benefits of probiotic strains



Probiotics: effects on lactose intolerance

- *Fewer symptoms if milk is replaced with fermented dairy products* (Kolars et al. 1984)

Mechanisms

- ✓ Lower lactose concentration in the product
- ✓ Lower transit time
- ✓ Higher β-galactosidase entering the small intestine

Considerations

- ✓ Strain-specific β-galactosidase activities are highly variable
- ✓ The β-galactosidase is an inducible enzyme

Probiotics: effects on diarrhoea

- **Effects have been reported in rotavirus and antibiotic associated diarrhoea (acute diarrhoea)**

There are several studies (Siitonen et al. 1990, Vanderhoof et al. 1999, De Roos and Katan 2000, Kotowska et al. 2005, Weizman et al. 2005), *including multicentre* (Guandalini et al. 2000) *and meta-analysis* (Huang et al. 2002, Van Niel et al. 2002, D´Souza et al. 2002 [S. boulardi and adults], Cremonini et al. 2002, Allen et al. 2004, Johnson et al. 2006[infants], McFarland 2006 Sazawal et al. 2006) *studies, confirming the results.*

- ✓ *Prevention and treatment*
- ✓ *Normalise microbiota/shorten duration by 50%*
- ✓ *Specific strains of L. rhamnosus, B. lactis, L. reuteri, S. boulardi*

Probiotics: effects on allergy

Probiotic administration (prenatally to mothers and during first months of life to infants) *reduced the incidence of atopic eczema in infants*

(Kalliomäki et al. 2001, 2003, 2007)

Supplementation of extensively hydrolysed formula with probiotics was effective on eczema alleviation in infants

(Isolauri et al. 2000, Rosenfeldt et al. 2003, Viljanen et al. 2005, Weston et al. 2005)

Brower et al. 2006: NO EFFECT of L. rhamnosus in children < 5 months (DBPC, 17 infants per group)

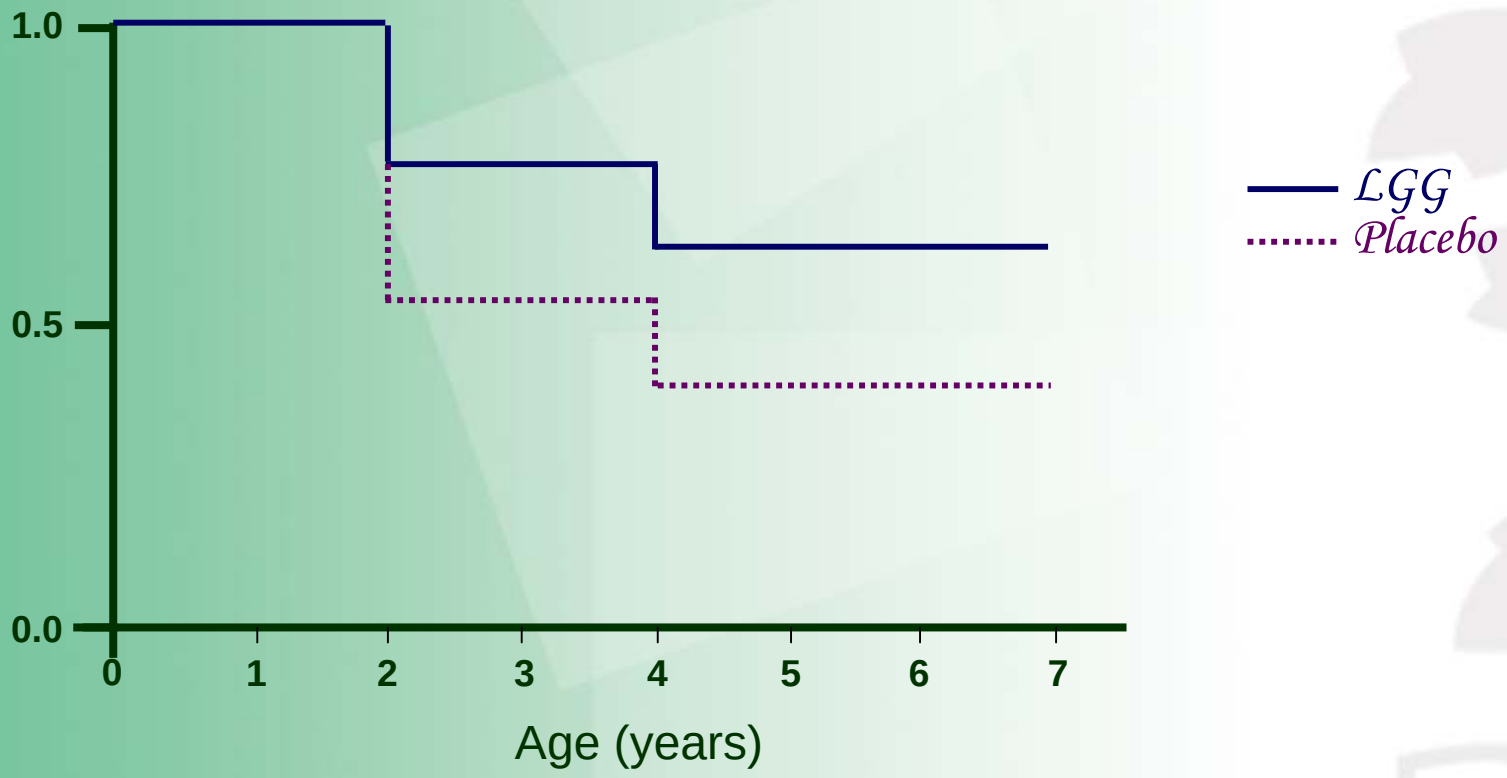
No beneficial effect of was found in adolescents suffering from pollen allergy (Helin et al. 2002) *but a subjective benefit in pollen allergy* (Ishida et al. 2005, Xiao et al. 2006) *and allergic rhinitis* (Wang et al. 2004, Ishida et al. 2005, Peng et al. 2005)

Probiotic targets: microbiota deviations

- *Microbiota differs between allergic and healthy infants*
  - *Low levels of LAB and bifidobacteria*
  - *More diverse/complex microbiota in healthy infants*
  - *Distinct bifidobacterial microbiota*
    - ✓ *Allergic infants: adult like bifidobacterial microbiota (B. adolescentis)*
    - ✓ *Healthy infants: B. breve, B. infantis, B. bifidum*
  - *Differences precede the development of atopic disease* (Kalliomaki et al. 2001)
  - *Relation to the hygiene hypothesis* (Rautava et al. 2004)

Microbiota establishment in early infancy provides crucial signals for the development of the immune system in infancy, influencing the immunological homeostasis later in life ⇒ it is the first key step!

Reduction of atopic eczema risk



Kalliomaki et al. 2007

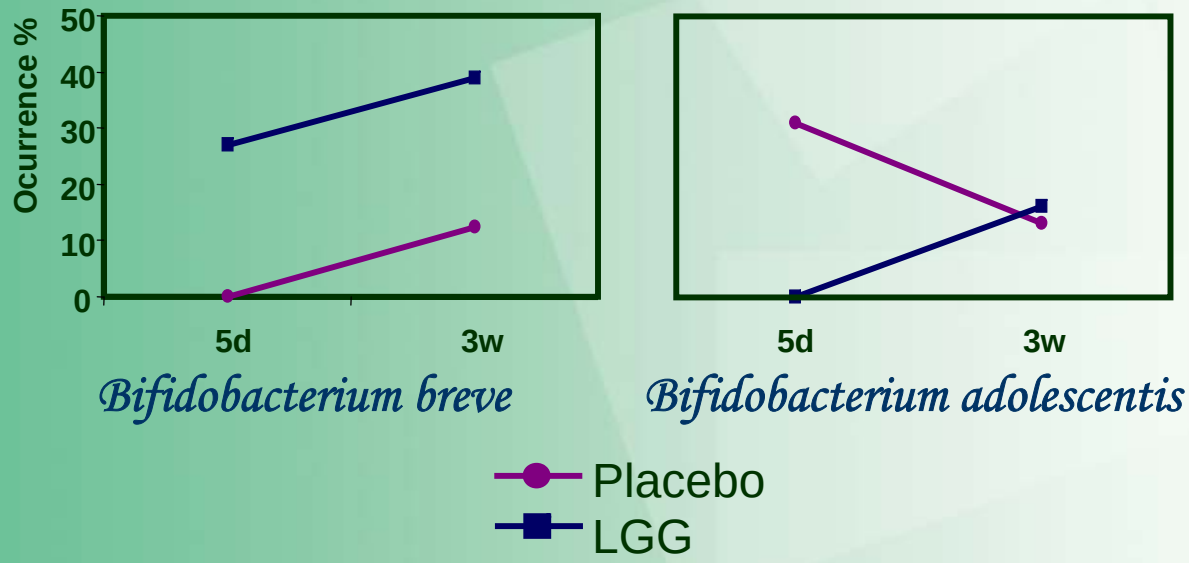
Establishment of gut microbiota: intervention

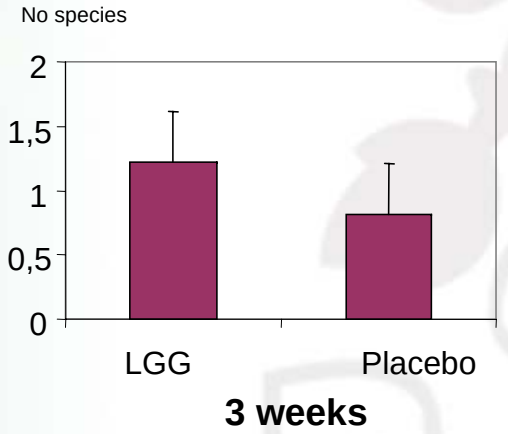
- With vaginal delivery the mother is the main source of bacteria for establishment and further development of gut microbiota

- Can we affect these processes by feeding probiotics perinataly to the mother? This would open new opportunities for infant microbiota modulation

Establishment of gut microbiota: intervention

- *Effect of maternal perinatal LGG consumption on bifidobacterial microbiota transfer and establishment*



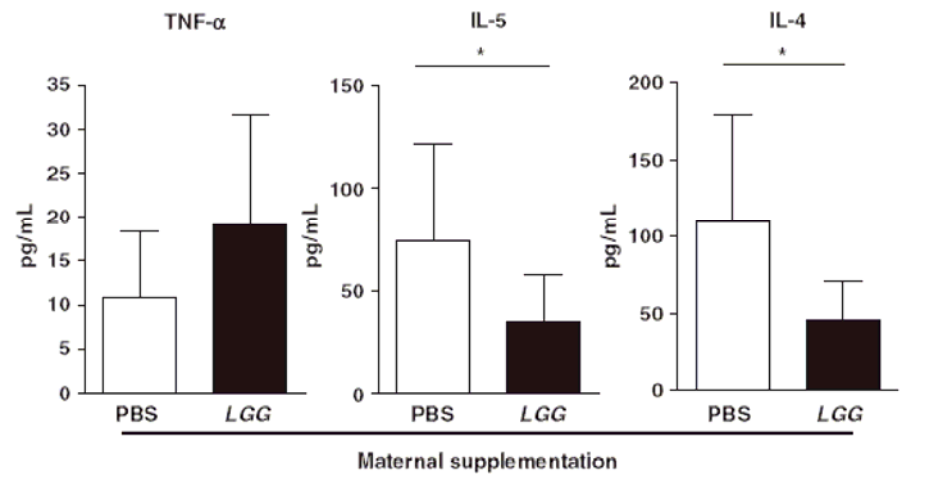


Gueimonde et al. 2006

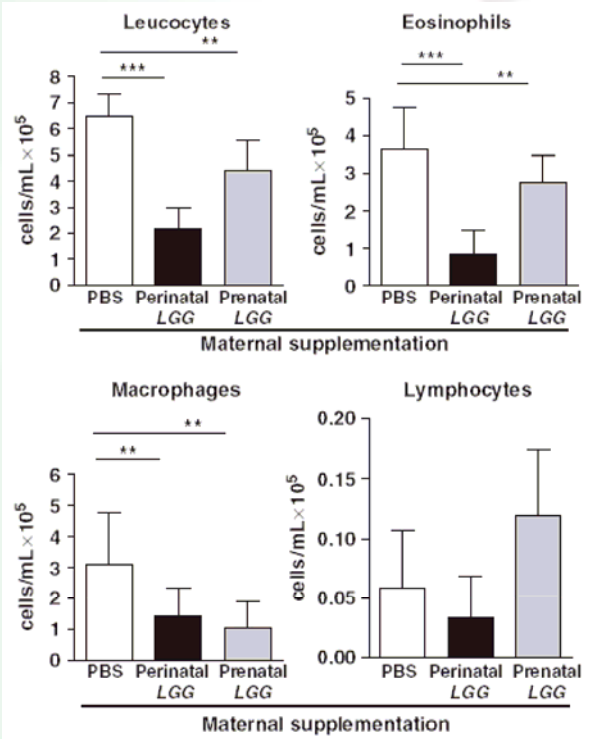
Establishment of gut microbiota: intervention

- *Effect of maternal perinatal LGG consumption on immune parameters*

  - *Bronchoalveolar lavage from BALB/C mice*

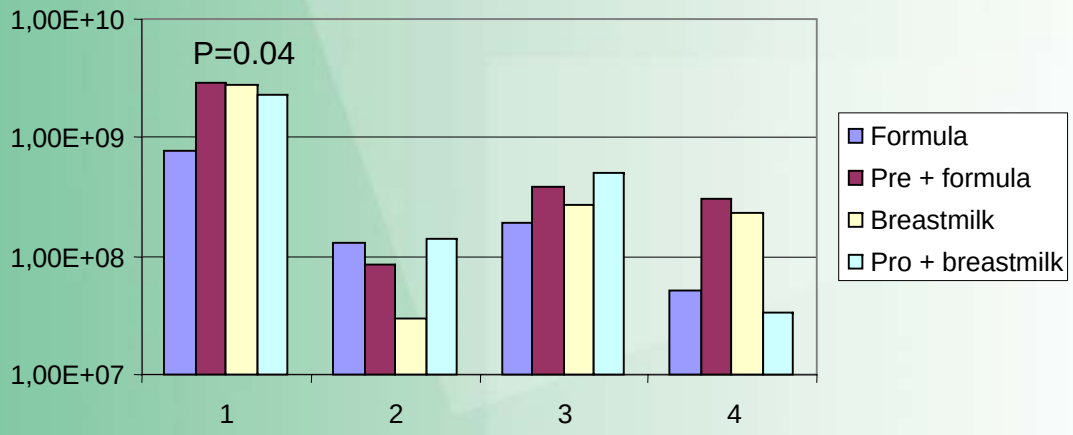


Blümer et al. 2007



Establishment of gut microbiota: intervention

- *Effect of formula supplemented with prebiotics*
  - *Quantitative microbiota composition resembles that of breast-fed babies*
  - *Qualitative bifidobacterial composition similar to that of breast-fed babies*



Bacteria: 1: Bifidobacteria, 2: Clostridia, 3: Lactobacilli/enterococci, 4: Bacteroides

Rinne et al. 2005

Mechanisms: Microbe-Host interactions

Probiotic-host cross-talk

Effect of LGG on gene expression in small bowel (Di Caro et al. 2005)

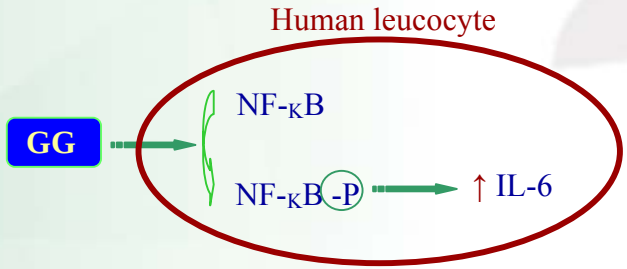
↑ 334 ↓92

Immune response/inflammation

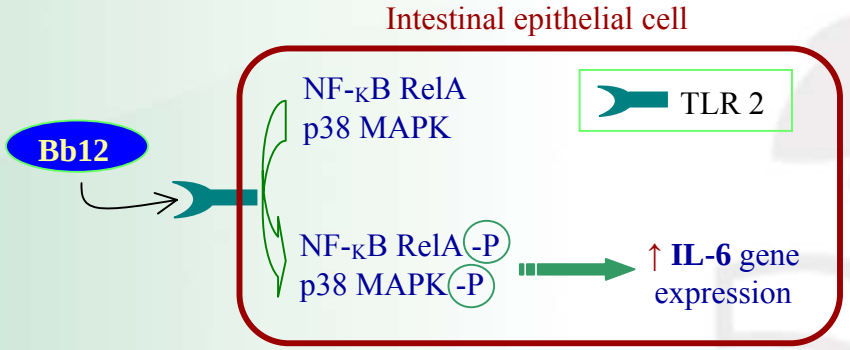
Cell adhesion

Cell signaling

Cell growth and differentation, apoptosis, ...



Miettinen et al. 2000



Ruiz et al. 2005

What is needed?

- **Knowledge on intestinal microbiota composition and activities**

  Intestinal microbiota

  - *Complex microbial comunity specific for each individual*
  - *More than 500 different species, 10^{12} cells/g contents*
  - *10 times more that eucaryotic cells in the body*
  - *≈1.5 kg, up to a 65% of faecal weight*
  - *Related to many diseases at intestinal and extra-intestinal levels*

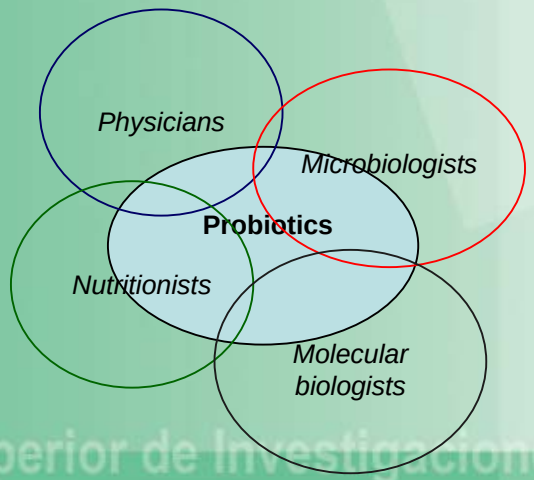
- **Understand mechanisms of action**

  Eg. Microbiota-host, role of TLR, Nod on immune stimulation

  - *DNA (GC) from probiotic strains increase secretion of anti-imflammatory cytokines* (Lammers et al. 2003).
  - *Interaction of DNA with TLR9 appears to mediate this effect* (Rachmilewitz et al. 2004).

Future opportunities

- *New targets and Probiotics with better characteristics for prevention or treatment of disease*
  - *Site-selected microorganisms*
  - *High adhesion at the target site/disease*
  - *Disease specific action*
  - *Highly specific in the interaction with the host (even at molecular level)*
  - *Search for more effective combinations for disease-risk reduction*



Multidisciplinary approach!!!!!

Thank you very much for your attention!!

Prof. Seppo Salminen

Asturian Dairy Products Institute (IPLA)