

# *Lactic Acid Bacteria and the development of microbiota*

*Regional Conference Fermented Milks- Technology and Nutrition*

*May 17, 2007. Moscow, Russia*

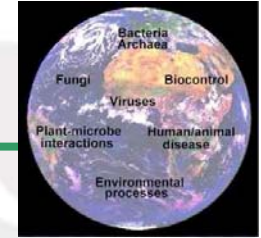
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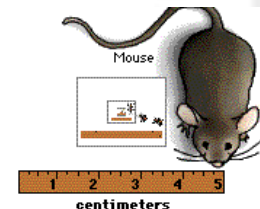
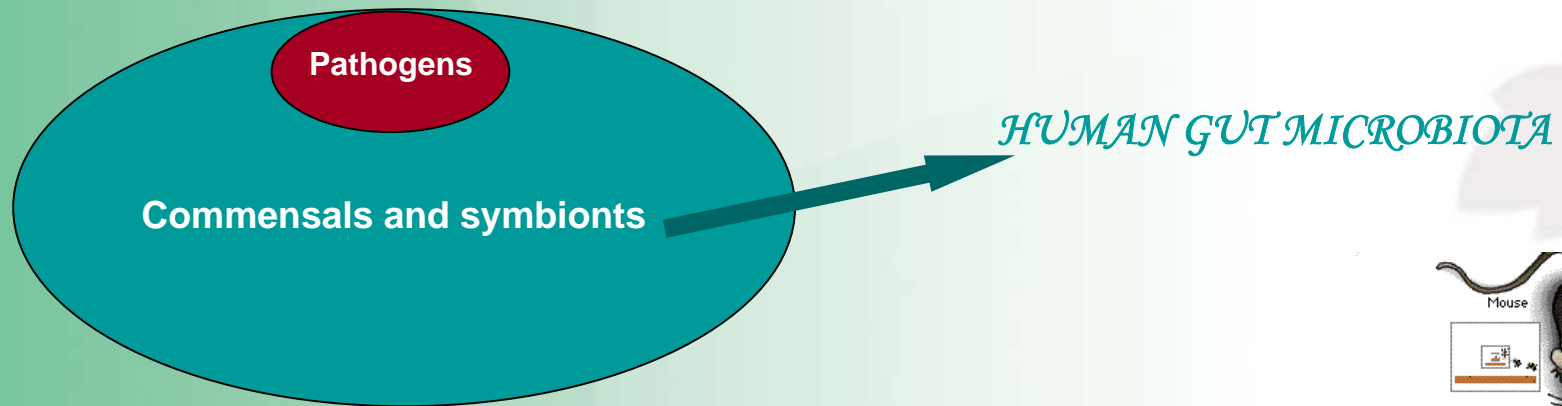
Consejo Superior de Investigaciones Científicas



# 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.)

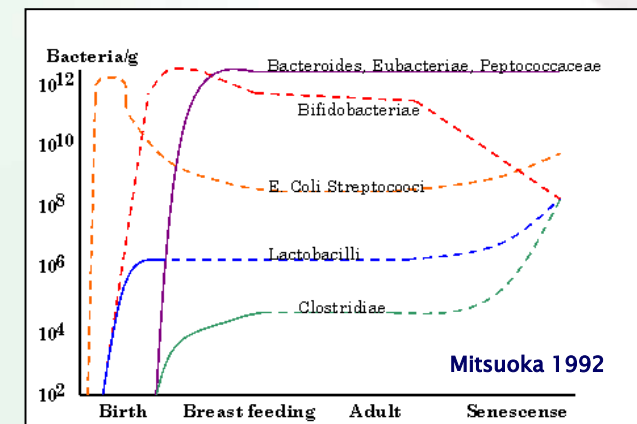
■  *$10^{12}$  microorganisms/g contents*

■ *10 times more than human cells*

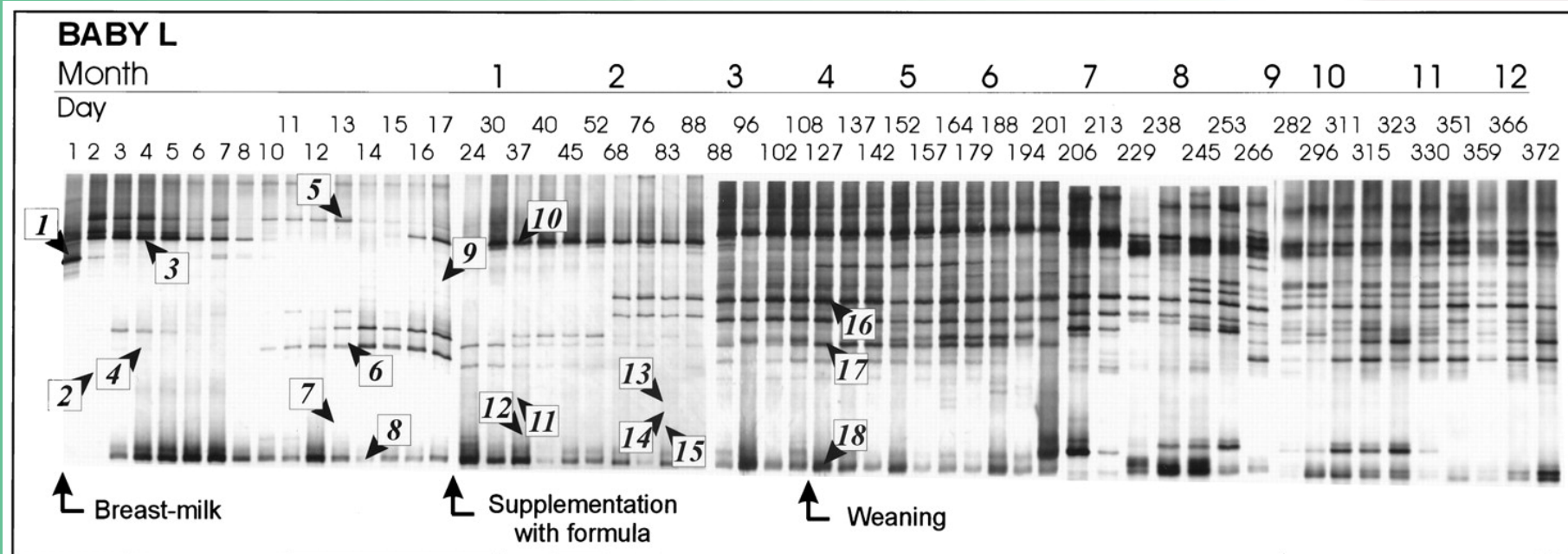
■ *10-100 times more genes*

■ *Up to 1.5 kg body weight*

■ *60% faecal weight*



# Establishment of gut microbiota



N	species	%	clones	GenBank	N	species	%	clones	GenBank
1	<i>Clostridium disporicum</i>	95	L1c	AF253386	10	<i>Ruminococcus torques</i>	94	L37A	AF253389
2	Uncultured bacterium adhufec 23 ( <i>Bacteroides thetaiotaomicron</i> )	93	L2e	AF253387	11	Uncultured bacterium adhufec 55 ( <i>Bacteroides</i> )	94	L37H	AF253393
3	<i>Enterobacter asburiae</i>	98	L4dl	AF253377	12	Uncultured bacterium adhufec 367 ( <i>Bacteroides</i> )	93	L37C	AF253390
4	<i>Streptococcus salivarius</i>	95	L4b	AF253388	13	<i>Enterococcus raffinosus</i>	98	L83M	AF253382
5	<i>Clostridium paraputrificum</i>	91	L13j	AF253381	14	<i>Bacteroides caccae</i>	98	L83D	AF253383
6	<i>Streptococcus salivarius</i>	97	L13dE	AF253378	15	<i>Bacteroides</i> sp. AR20	95	L83J	AF253385
7	<i>Bacteroides vulgatus</i>	96	L13dG	AF253379	16	Uncultured bacterium A57 ( <i>Ruminococcus</i> )	94	L127dB	AF253375
8	<i>Bifidobacterium pseudocatenulatum</i>	98	L14B	AF253372	17	Uncultured bacterium adhufec 171 ( <i>Ruminococcus</i> )	96	L127dA	AF253374
9	<i>Staphylococcus caprae/epidermidis</i>	96	L17j	AF253384	18	<i>Bifidobacterium pseudocatenulatum</i>	97	L127dC	AF253376

# *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  $2 \times 10^4$  to  $1 \times 10^5$*

Martin et al. 2003

# Breast-milk

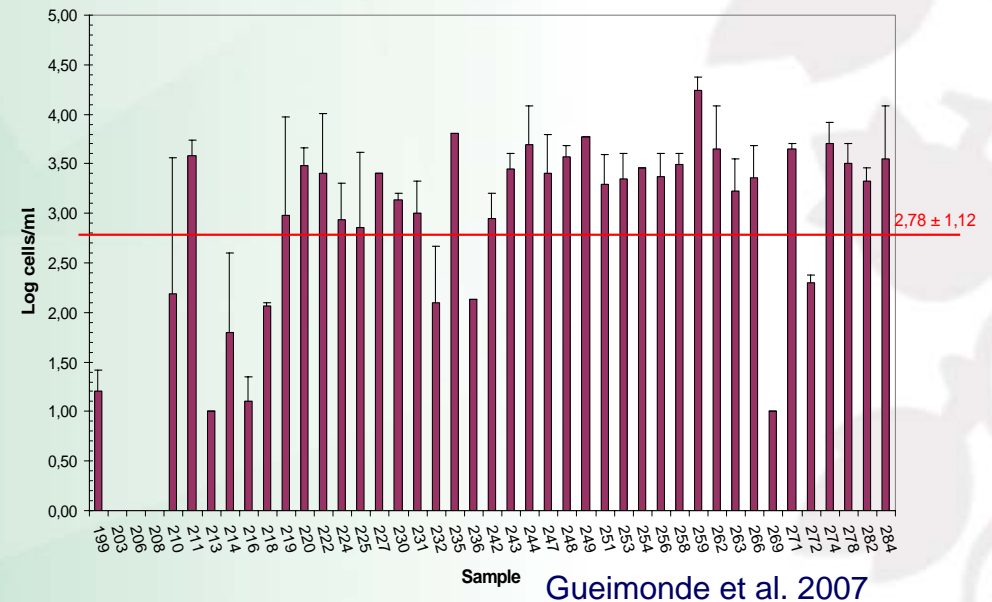
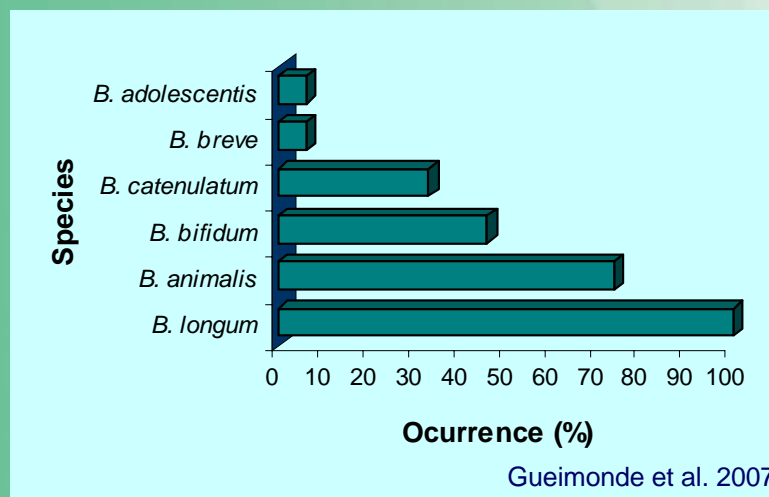
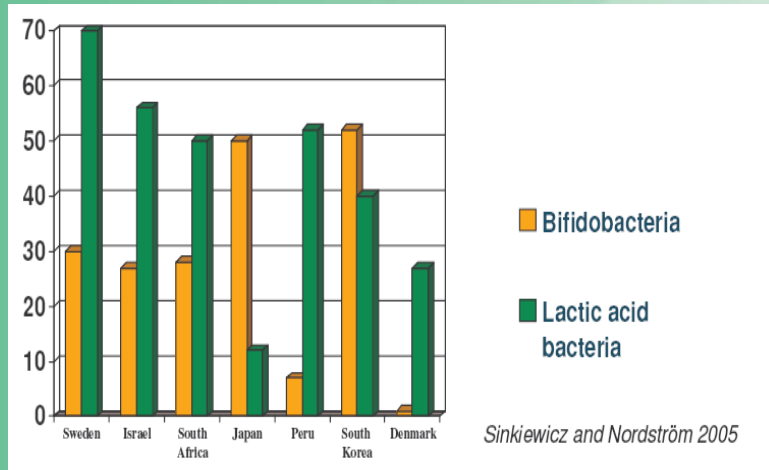
## ■ LABs commonly isolated from human milk

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

*What about bifidobacteria?*

# Breast-milk

## Breast-milk as a source of Bifidobacteria?



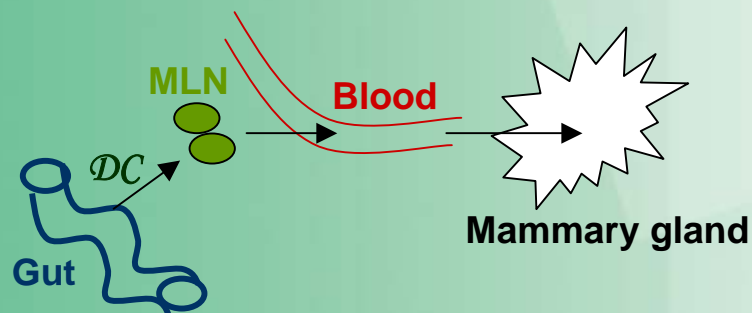
# Breast-milk

## ■ *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*

- *Mode of delivery*

- *Mother's microbiota*

- *Microbiota of hospital/nurse/staff*



- *Composition of breast milk?*

  - ✓ *Oligosaccharides*

  - ✓ *Fat*

  - ✓ *Bacteria*

- *Nutrition and environment*

- *Food microbiota (hygiene)*

- *Pro- and prebiotics*

- *Medication*




# *Intestinal Microbiota : functions*

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## *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

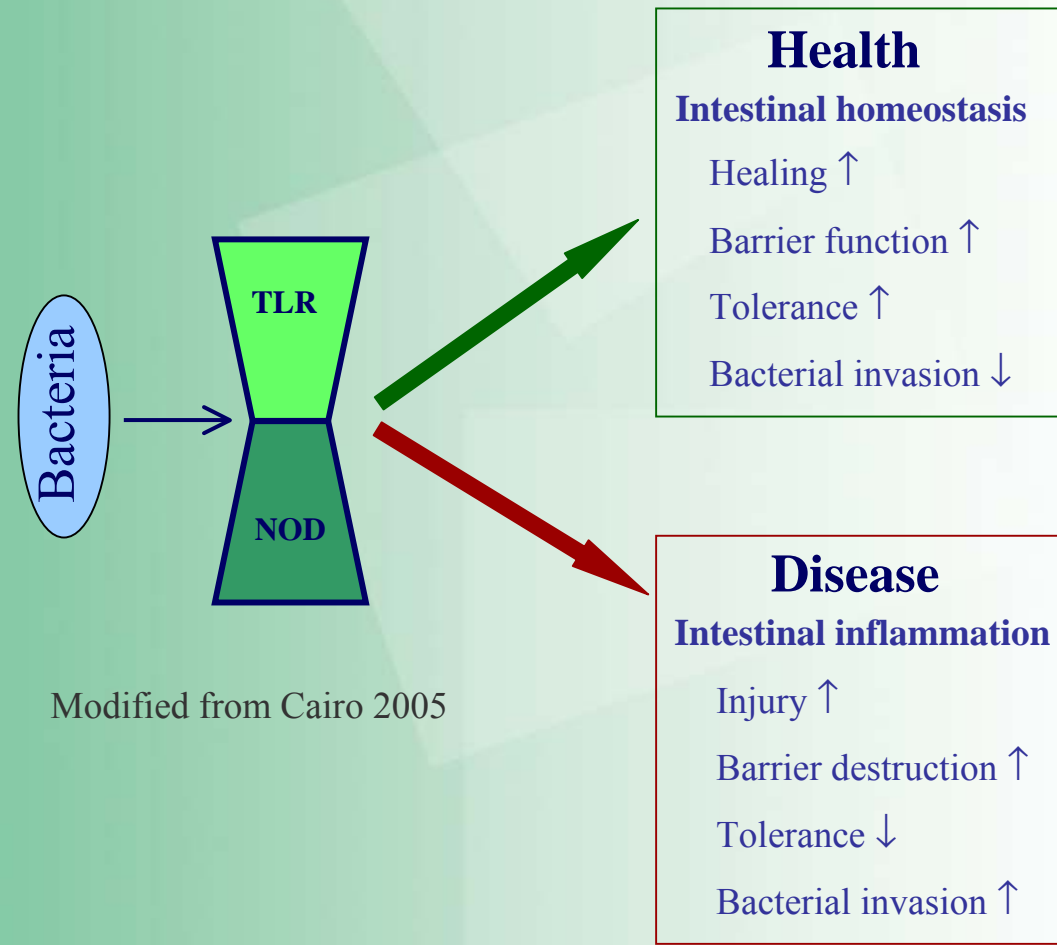
- *Establishment of oral tolerance* (Sudo et al. 1997)
- *Maintenance of intestinal homeostasis and healing of intestinal injuries* (Rakoff-Nahoum et al. 2004)
- *Regulates acquisition and storage of lipids* (Bäckhed et al. 2004)
- *Induces mucosal expresion of bactericidal proteins, establishment of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

*Host Immune system*

*Appropriate response*



# TLR/NOD: Functional vs. aberrant

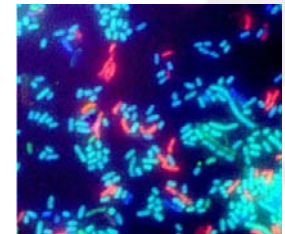


Modified from Cairo 2005

# *Intestinal Microbiota: role in health and disease*

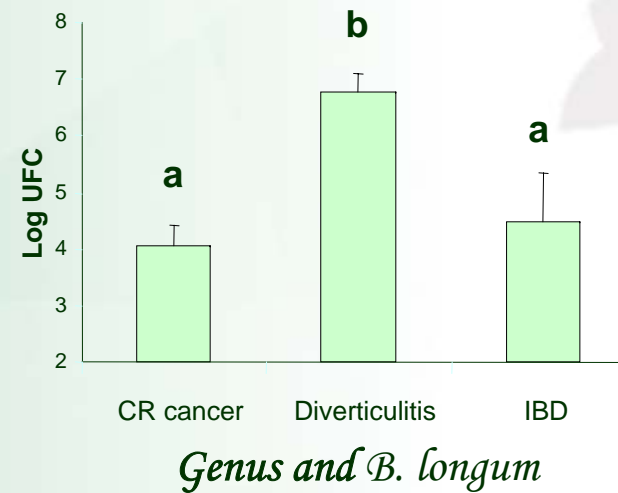
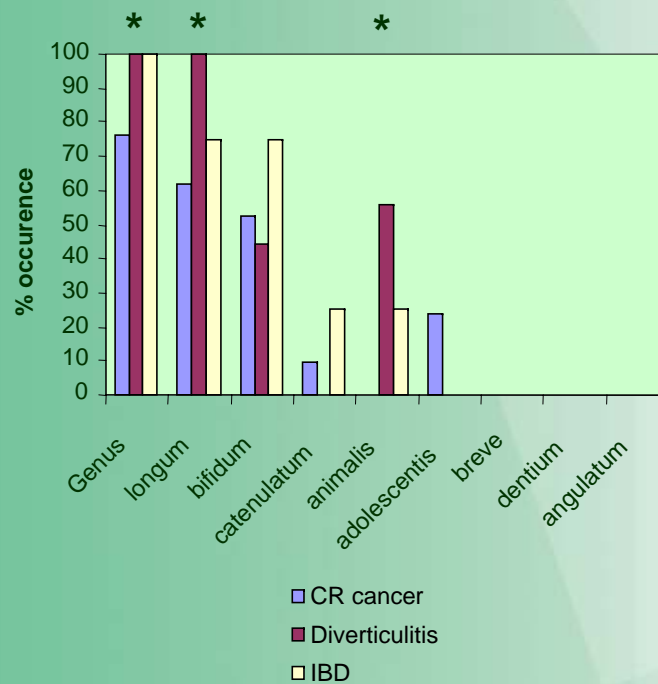
■ *The intestinal microbiota is related to many diseases at both intestinal and extra-intestinal levels*

- *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

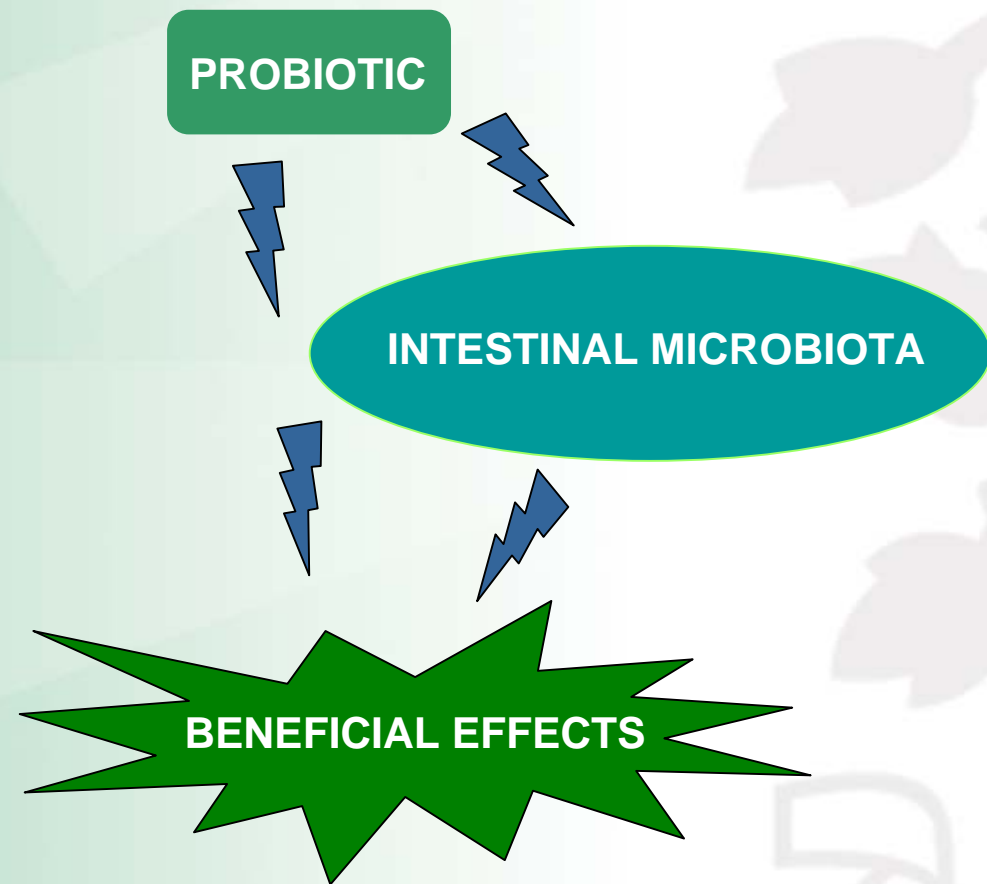
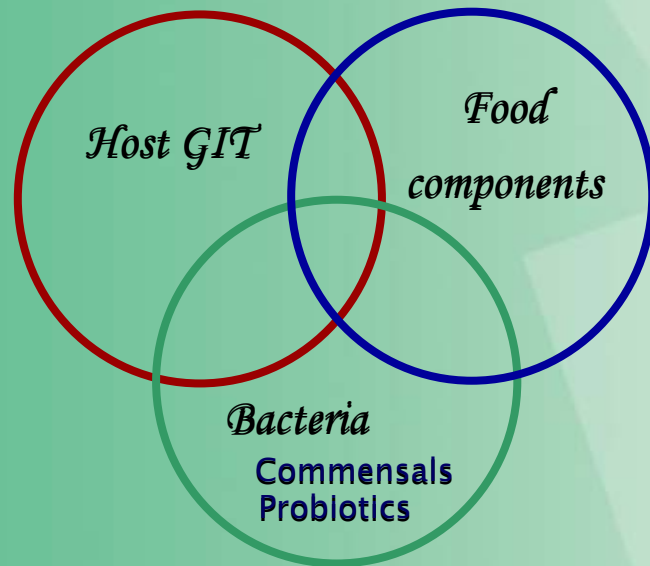
## ■ Qualitative and quantitative alterations of bifidobacterial microbiota



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)

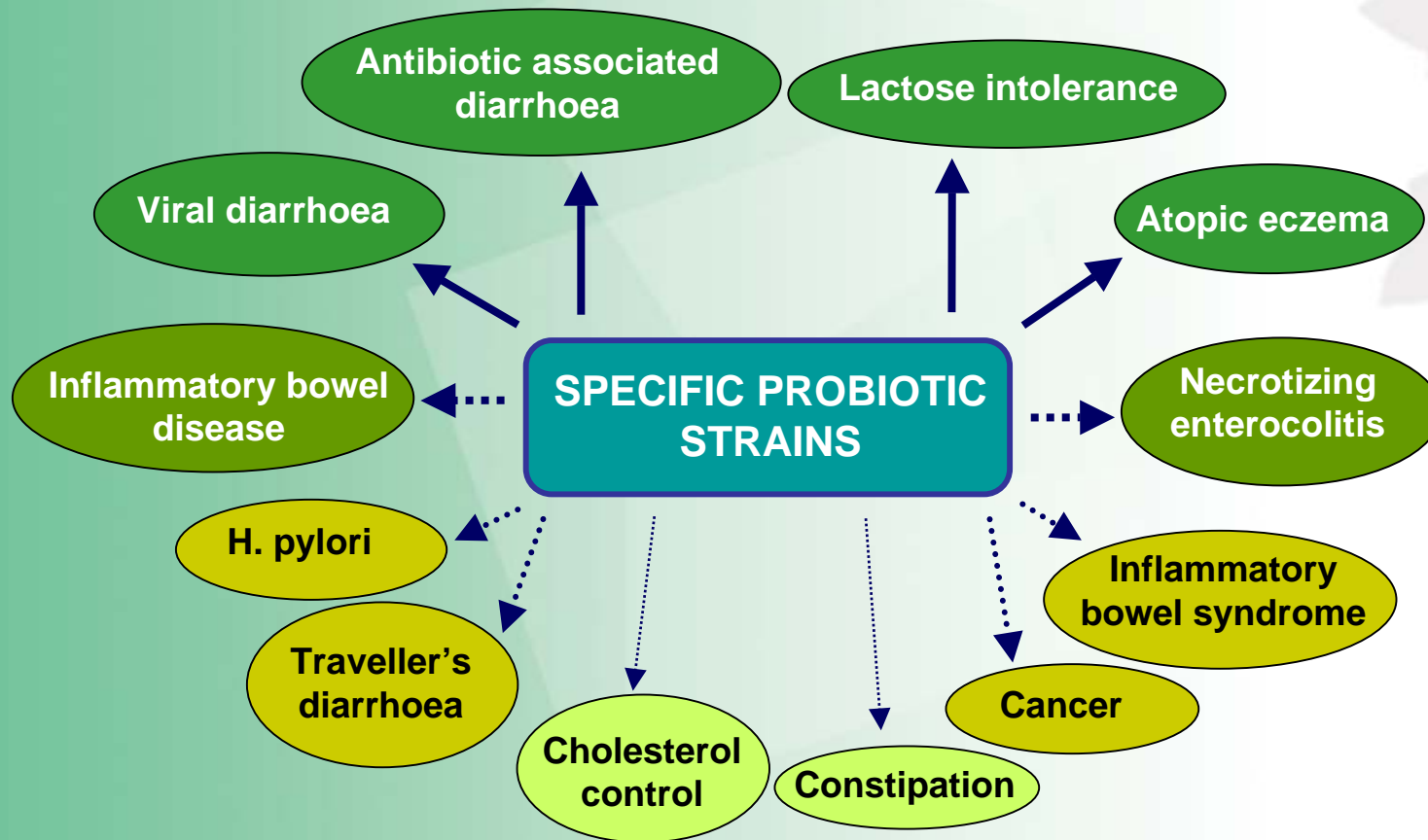


-  *Lactobacillus*
-  *Bifidobacterium*
-  *Enterococcus*
-  *Saccharomyces boulardii*
-  *Escherichia coli*
-  *Bacillus cereus*
-  *Clostridium butyricum*



*Efficacy and Safety have to be Scientifically demonstrated!*

# Health benefits of probiotic strains



# *Probiotics: effects on lactose intolerance*

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- *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  $\beta$ -galactosidase entering the small intestine*

## *Considerations*

- ✓ *Strain-specific  $\beta$ -galactosidase activities are highly variable*
- ✓ *The  $\beta$ -galactosidase is an inducible enzyme*

# *Probiotics: effects on diarrhoea*

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## ■ *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*

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*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*)*

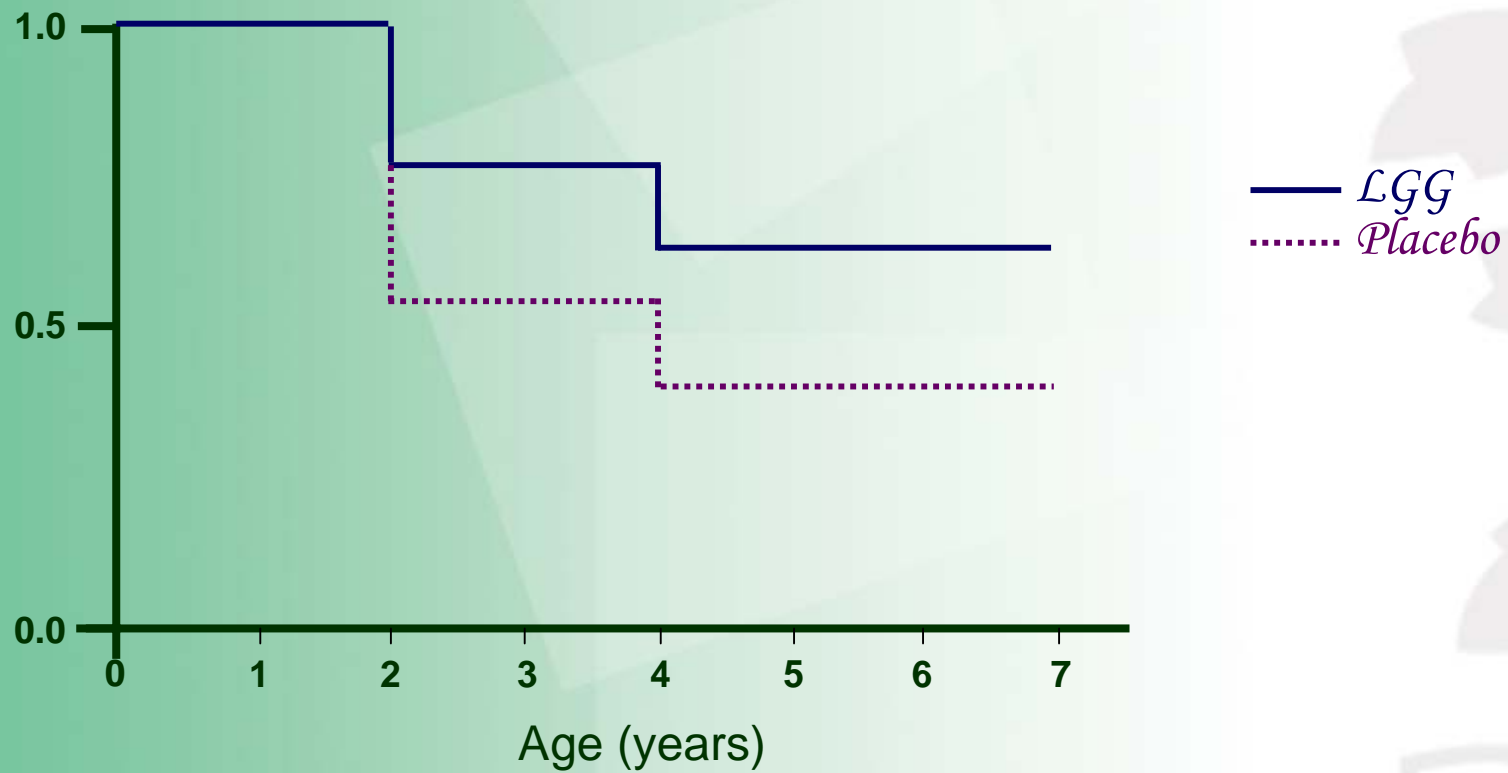
✓ *Healthy infants: *B. breve*, *B. infantis*, *B. bifidum**

■ *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*

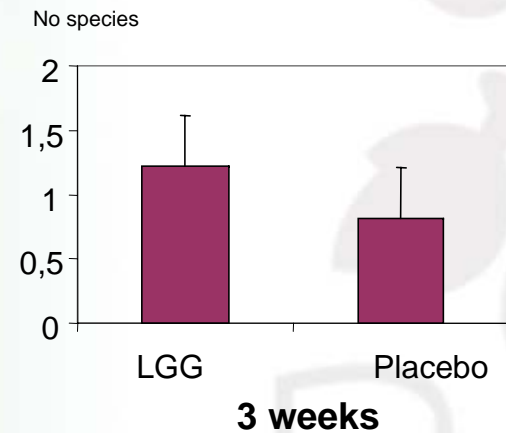
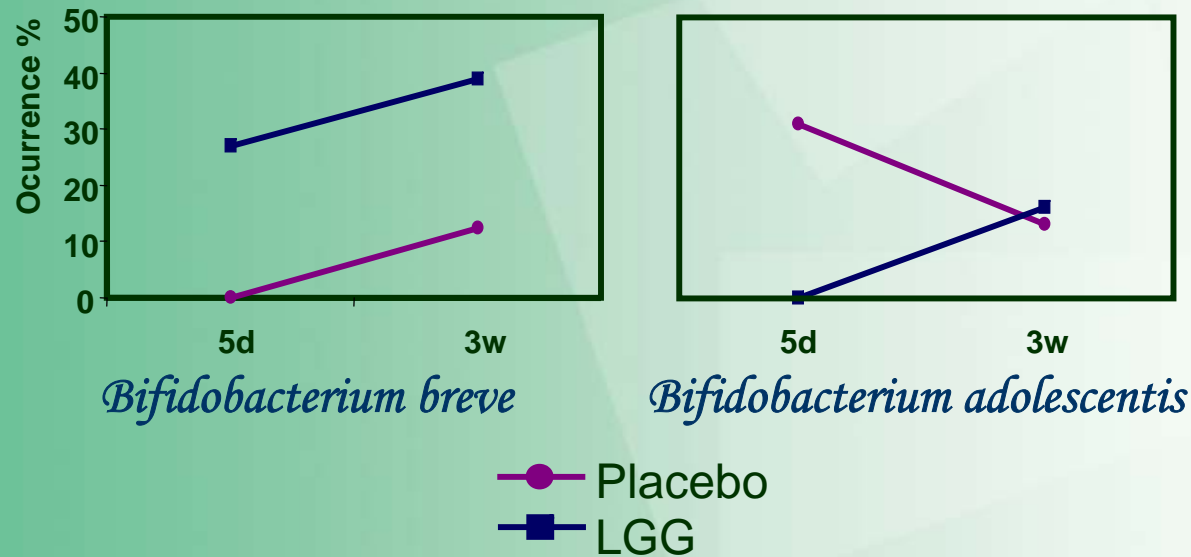
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 *With vaginal delivery the mother is the main source of bacteria for establishment and further development of gut microbiota*

 *Can we affect these processes by feeding probiotics perinatally 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

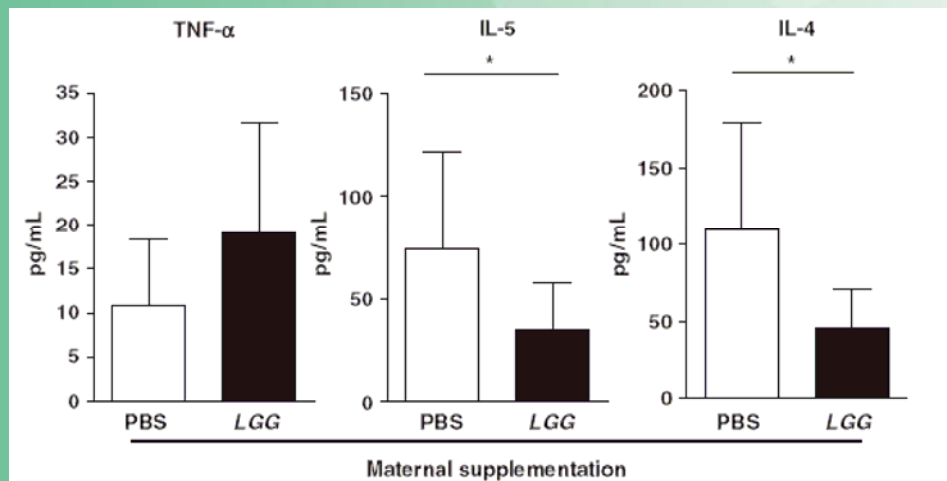


Gueimonde et al. 2006

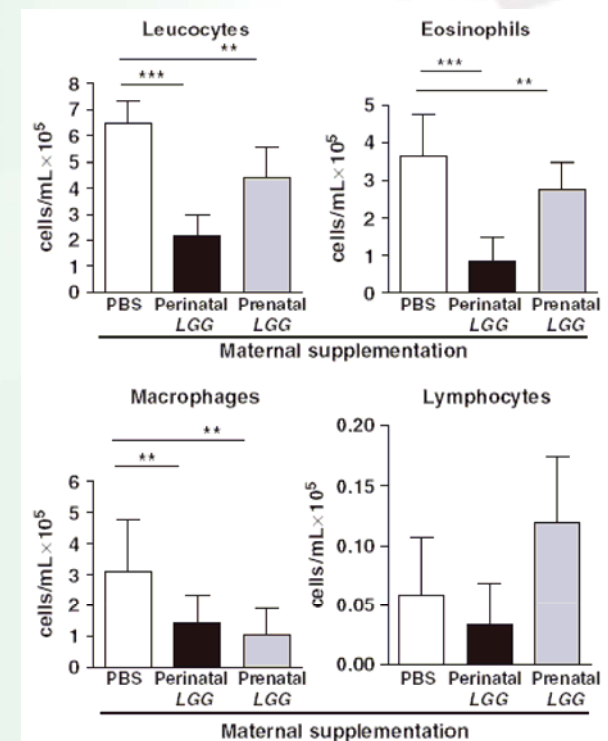
# Establishment of gut microbiota: intervention

## Effect of maternal perinatal LGG consumption on immune parameters

### Bronchoalveolar lavage from BALB/C mice



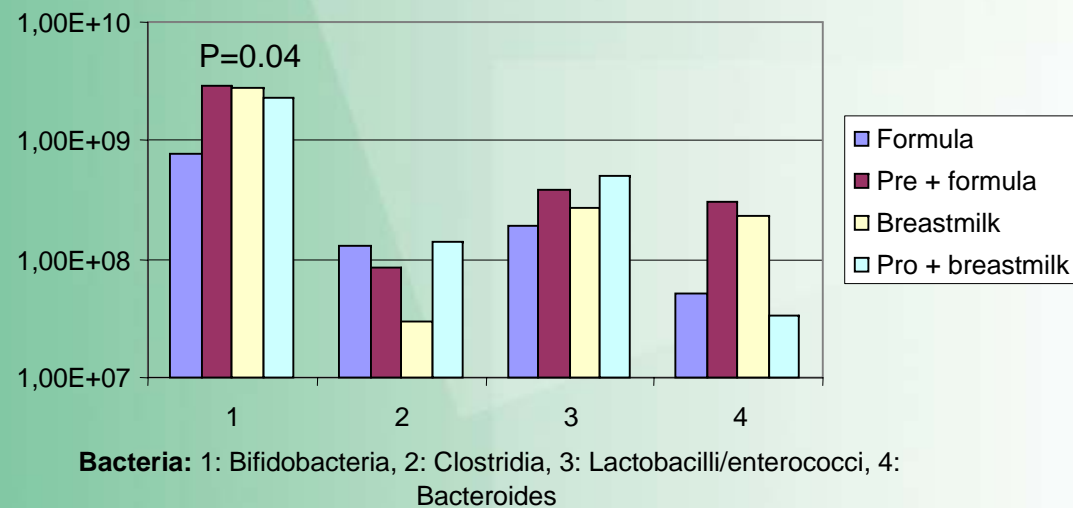
Blümer et al. 2007



# 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*



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)

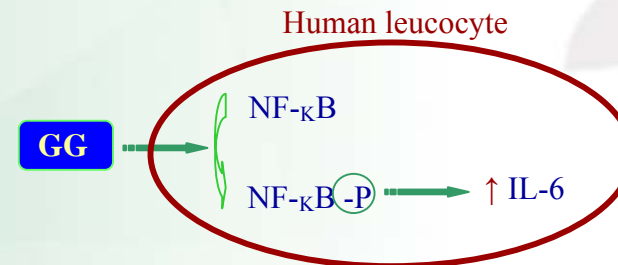
↑ 334 ↓ 92

*Immune response/inflammation*

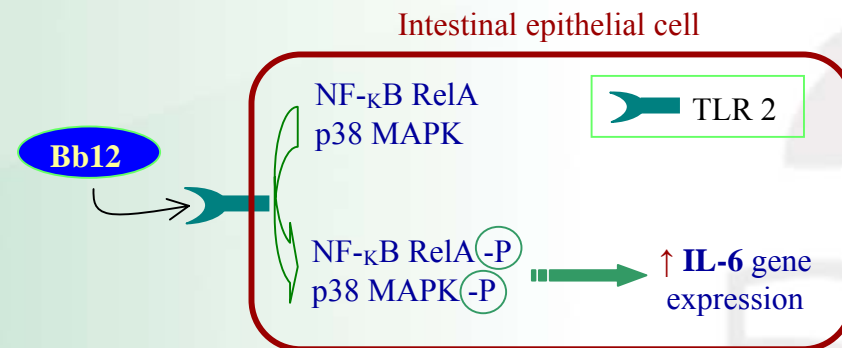
*Cell adhesion*

*Cell signaling*

*Cell growth and differentiation, apoptosis, ...*



*Miettinen et al. 2000*








*Ruiz et al. 2005*

# *What is needed?*



## *Knowledge on intestinal microbiota composition and activities*

### *Intestinal microbiota*

-  *Complex microbial community specific for each individual*
-  *More than 500 different species,  $10^{12}$  cells/g contents*
-  *10 times more than 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-inflammatory 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*

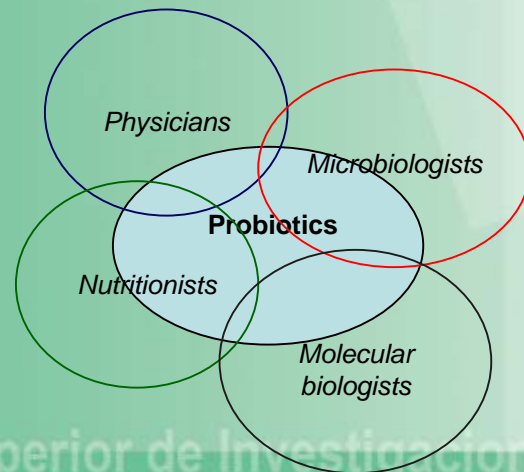
■ *Site-selected microorganisms*

■ *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)**

