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Modulating the gut microbiota for better health

SP4
Klaudyna Borewicz
30/11/2017




Our own important “Micro-cosm”

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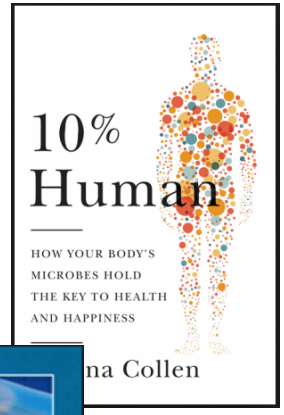
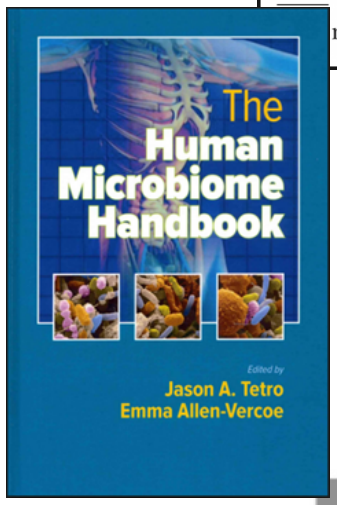
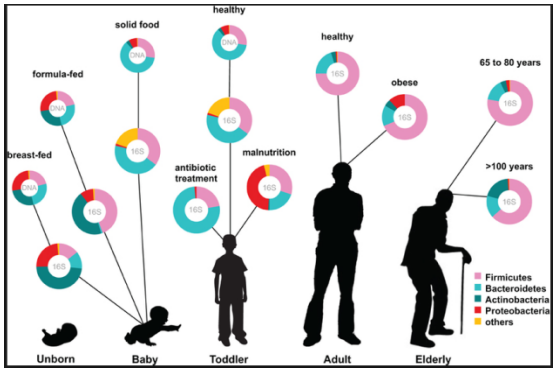
- We are covered with microbes – inside and out
- Microbiota research is “exciting, important and growing”
- The microbial ecosystems change through life
- Our bacteria keep us happy, healthy and alive







Microbiome papers published (1990-2016) (NCBI)

Gut microbiota importance in physical and mental health

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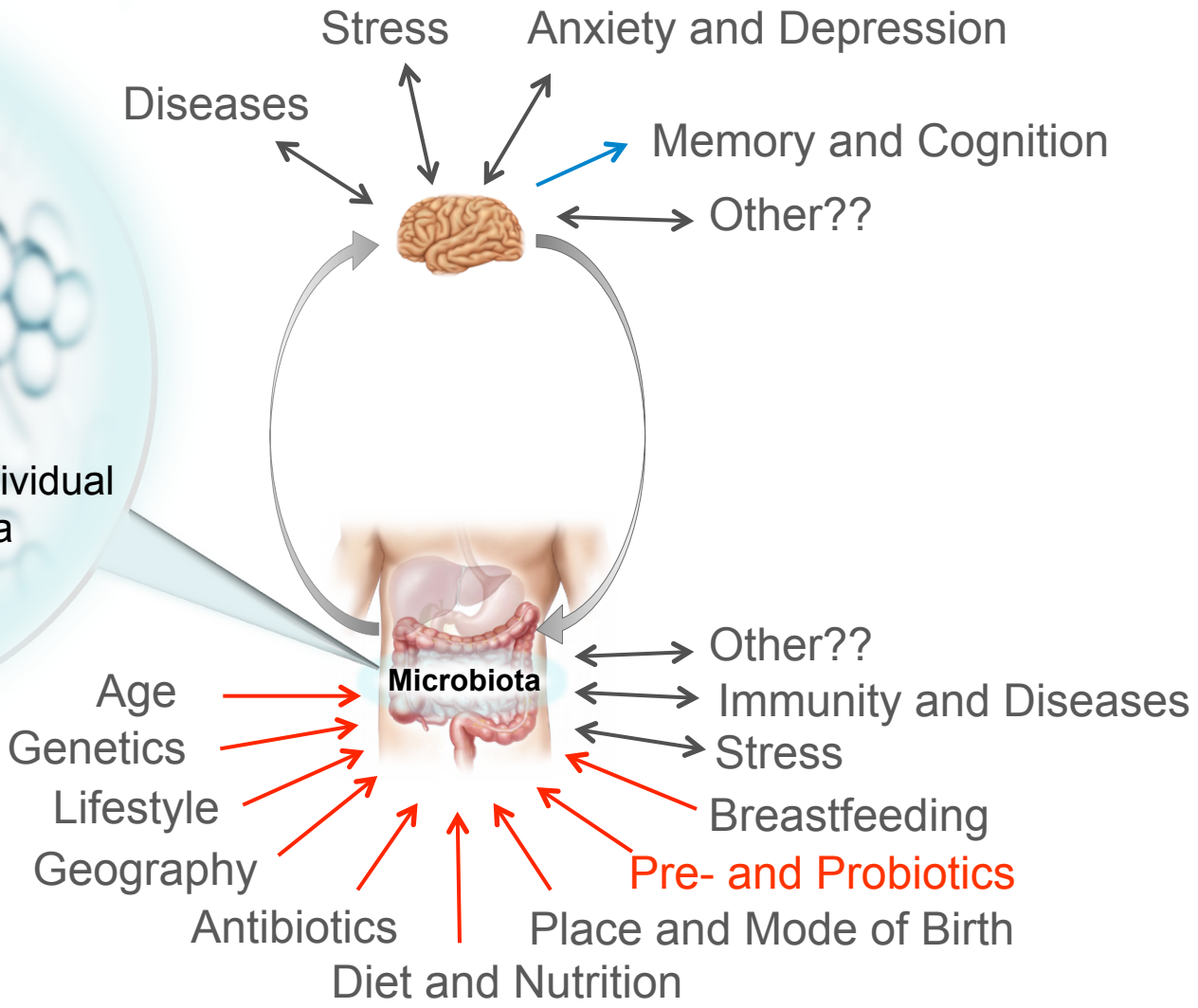
<u>Gut Microbiota:</u>

- Bacteria
- Archaea
- Fungi
- Protozoa
- Viruses
- Bacteriophages

- Unique to each individual
- 95% of our bacteria
- 1-2 kg
- 500 -1000 species



Prebiotics, Probiotics and Synbiotics

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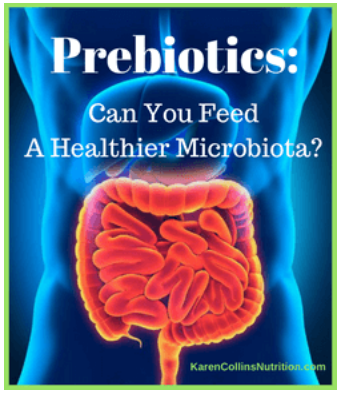
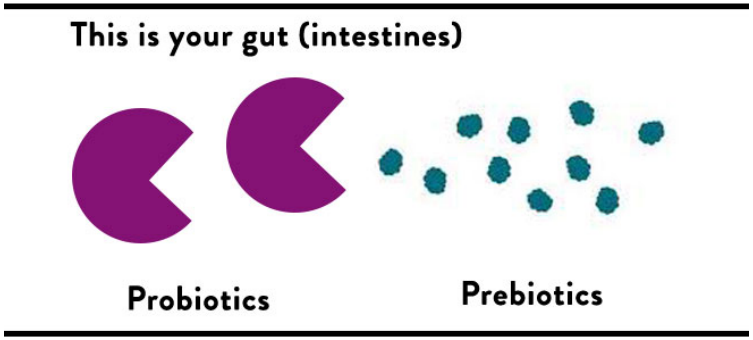


Prebiotics = the "food for good bacteria":
•eg. GOS, FOS, IMMP, Inulin, HMOs

Probiotics = the "good bacteria"
•eg. *Bifidobacterium (B. longum, B. lactis, B. animalis, B. breve); Lactobacillus (L. reuteri, L. acidophilus)*

Synbiotics = Prebiotics + Probiotics
•eg. Breastmilk

Health benefits:
- improve nutrient absorption
- improved bowel function
- enhanced innate immunity
- reduced inflammation
- help prevent cancer
- ...



Key aims of CCC SP4

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- Characterize microbial networks responsible for fibre fermentation, with main focus on prebiotic fibers
- Track place & products of fermentation
- Identify carbohydrates that stimulate specific microbes & products (e.g. butyrate producers)
- Personalized ingredients - Differences between individuals/enterotypes?

Infant Projects within SP4

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KOALA - STUDY

- **KOALA and BINGO cohort studies**

  - To gain an insight on faecal microbial community of infants and investigate the link between milk oligosaccharides and gut microbiota composition changes (in collaboration with SP3)

  - Joint publication(s) hinging on correlations between microbiota & HMO fermentation profiles

  - KOALA -1 month old infants, BINGO - cohort followed in time at 2, 6, 12 weeks

KOALA (2003) Samples group by feeding mode (PCA)

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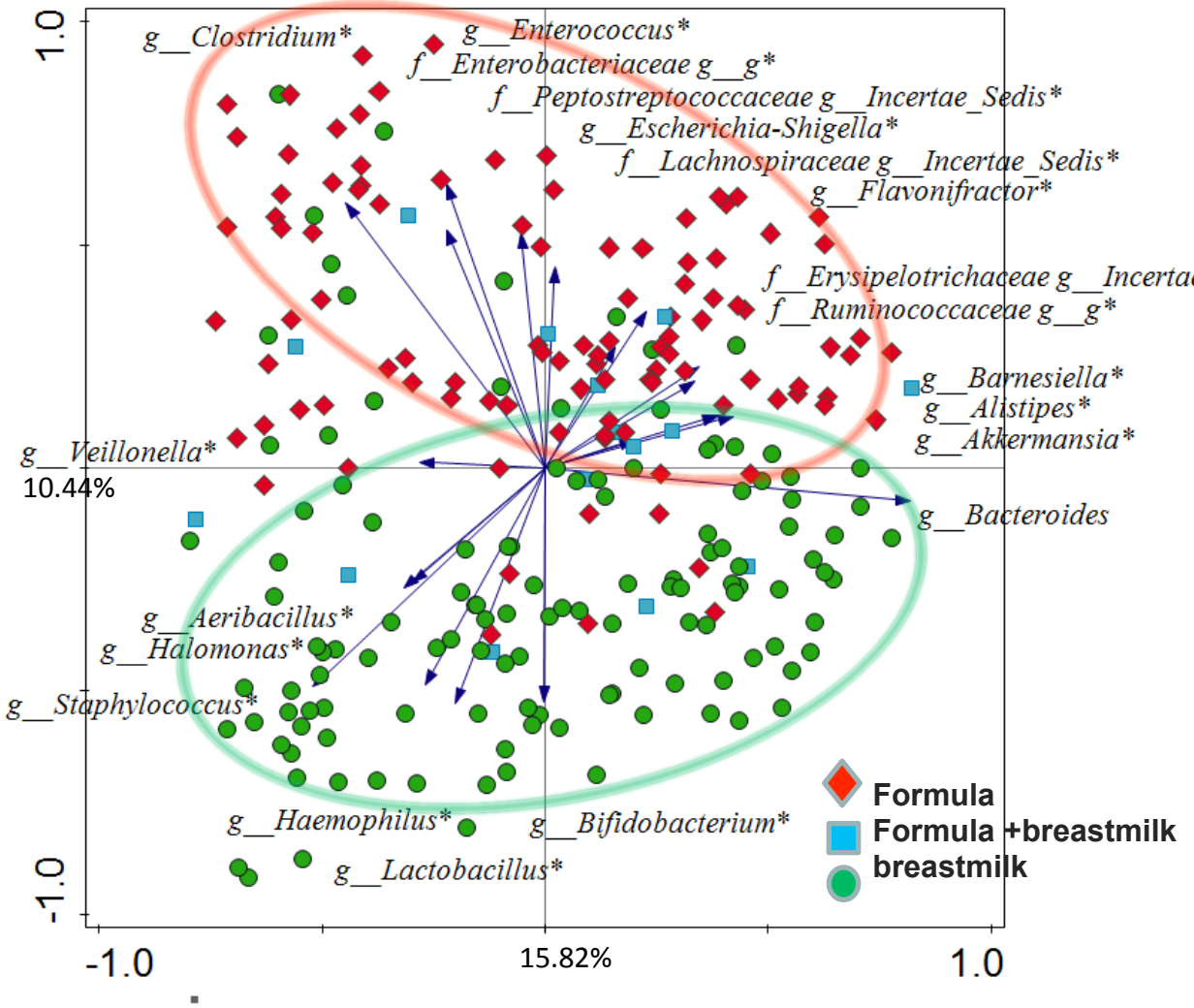
KOALA - STUDY



Fecal microbial community was different in infants fed formula (FF)

FF infants had more bacterial genera and their distribution was more even

19 genus level taxa differed between BF and FF infants

FF infants had lower levels of *Bifidobacterium*

Formula feeding has smaller effect in BINGO (2015) samples

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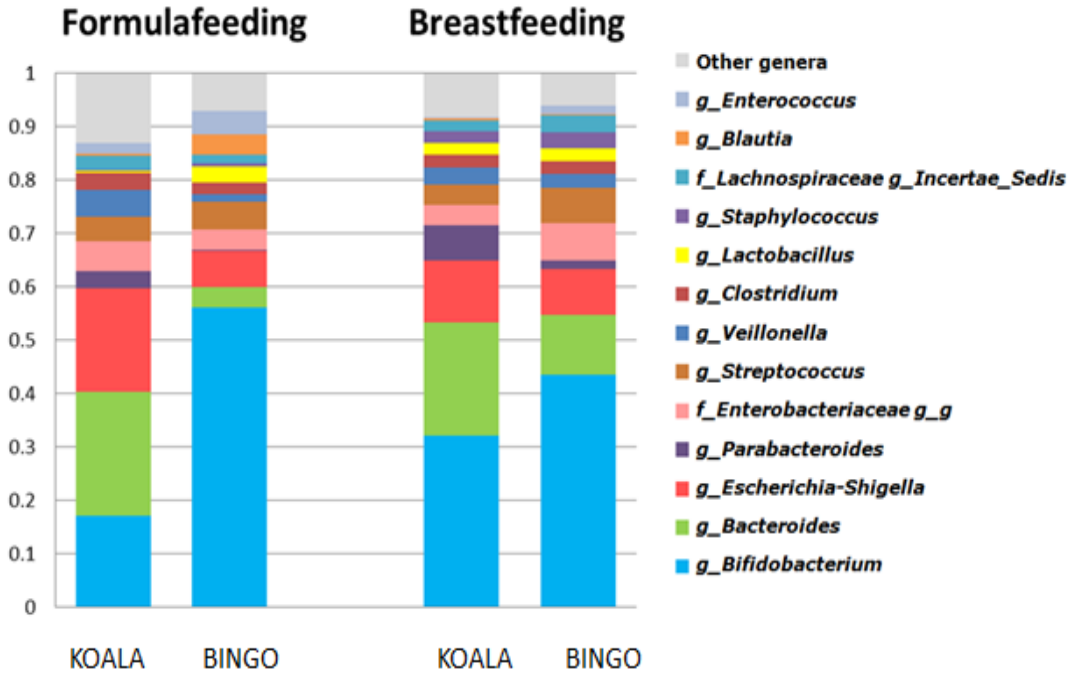
Modern formulas are fortified with prebiotics (eg. GOS and FOS)

Microbial community still different between FF and BF infants

FF results in more genera but the RA of main groups is similar to BF

FF results in overall high level of *Bifidobacterium*

BF infants have higher levels of *Staphylococcus* and *Streptococcus*



Temporal dynamics of microbiota in BF infants (BINGO Study)

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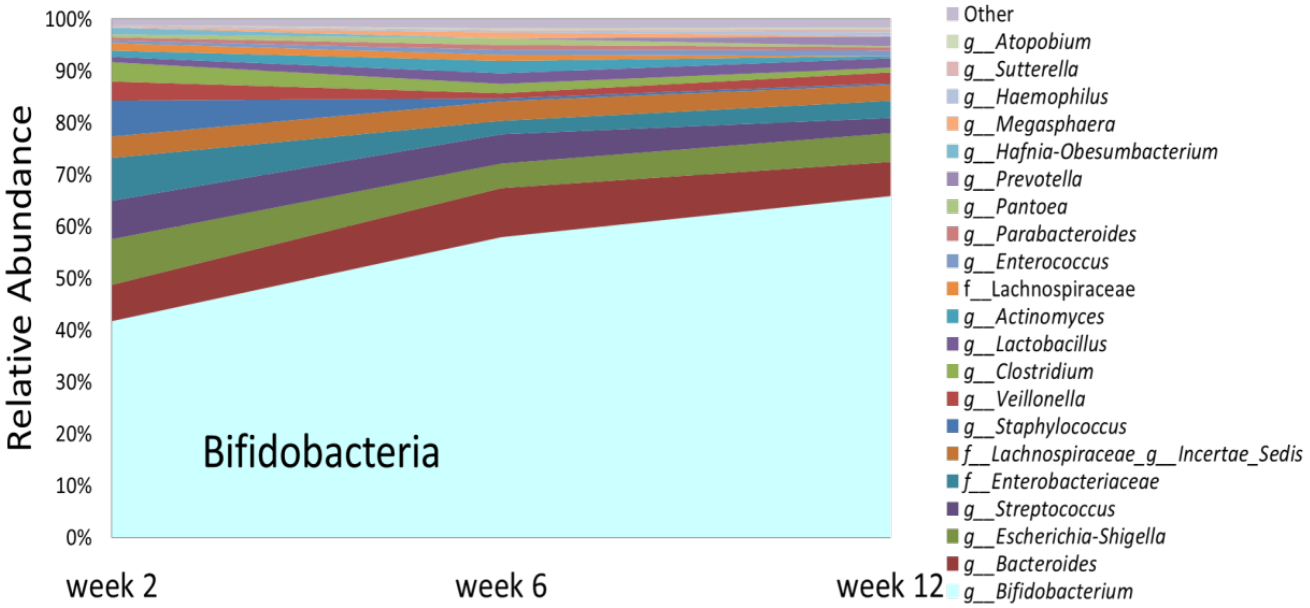
Correlation analyses show only a weak link between milk HMOs and *Bifidobacterium*



- Microbiota composition changes with age
- Increase in relative abundance of *Bifidobacterium*, decrease in *Staphylococcus*
- Multivariate analysis indicates that different factors play a role at different ages, including HMOs:
  - At 2 weeks - place and mode of delivery and LNFPIII were significant
  - At 6 weeks - gender, mode of delivery, 3SL and LSTc
  - At 12 weeks - gender and LNH

HMO utilisation is strongly linked with bifidobacteria

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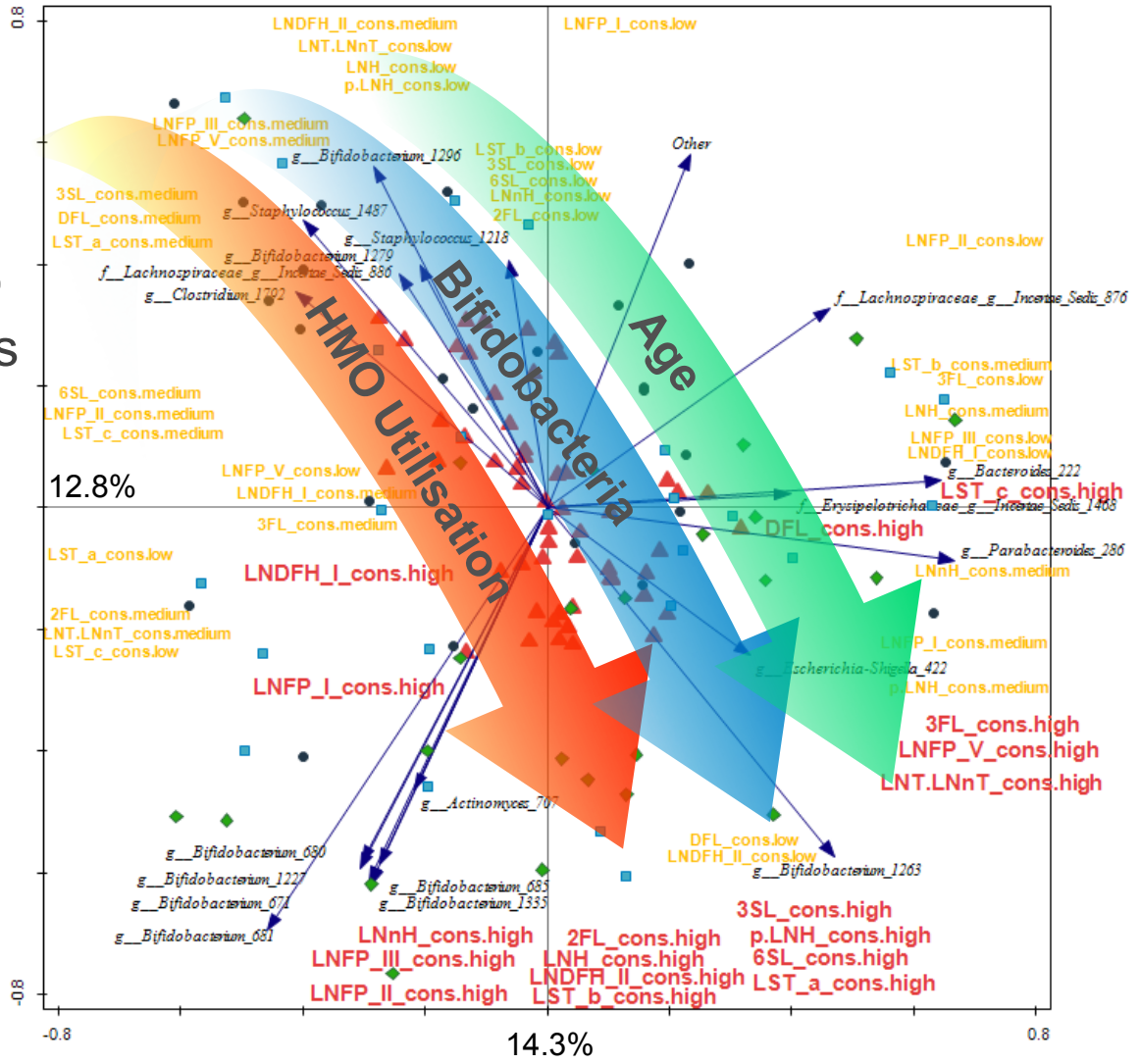
- Utilisation of the major HMO types in breastmilk increases with infant age
- High utilisation linked with high abundance of bifidobacteria



HMO utilisation linked with specific species (OTUs)

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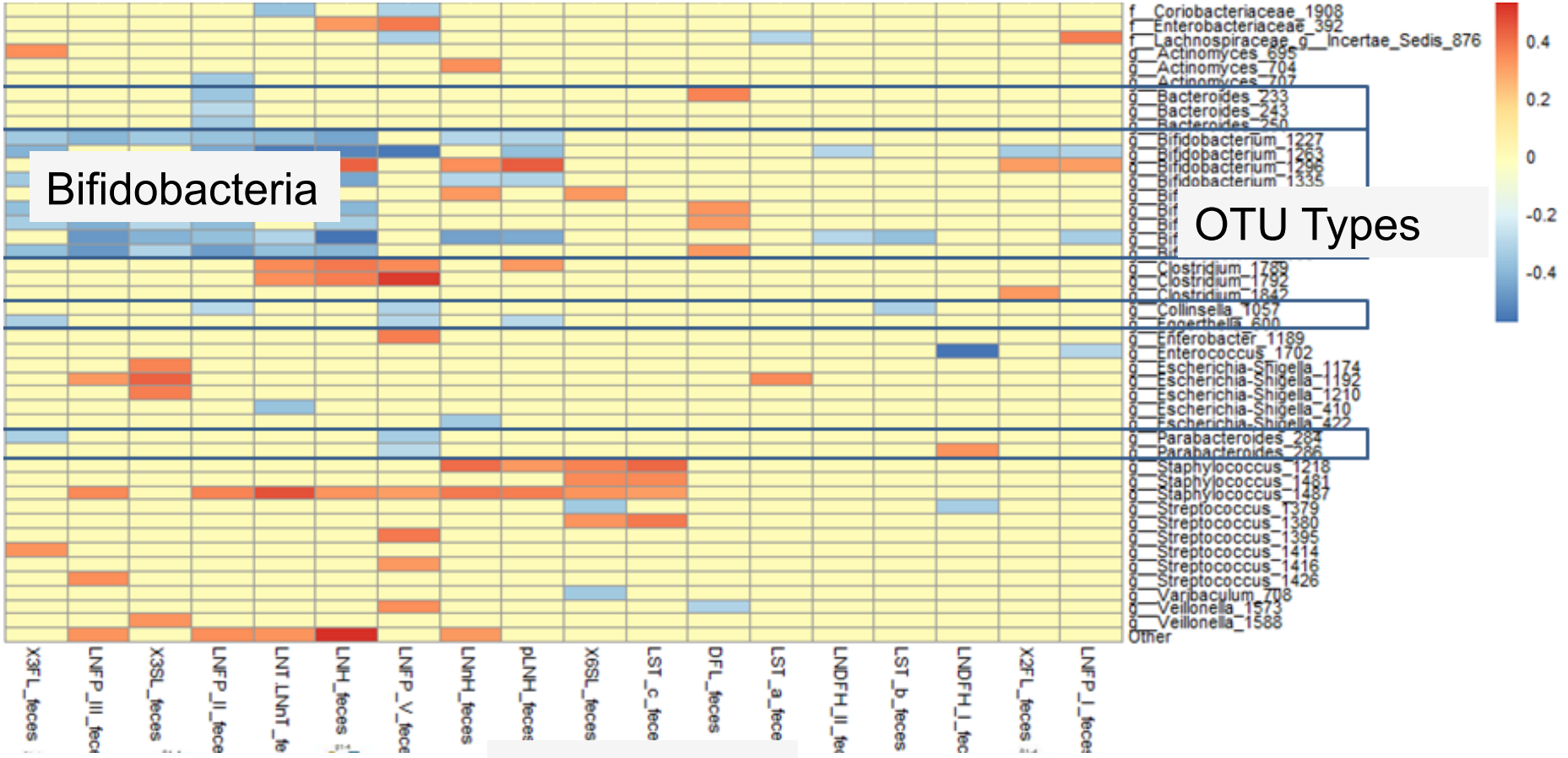
Correlation analyses suggest specialisation of species/strains to degrade specific HMOs *in-vivo*



Infant microbiota projects

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Three manuscripts in preparation

The effect of prebiotic fortified infant formulas on microbiota composition and dynamics in early life.

Klaudyna Borewicz[1], Maria Suarez-Diez[2], Christine Hechler[3], Roseriet Beijers[3], Carolina de Weerth[3], Ilja Arts[4], John Penders[4], Carel Thijs[4], Cordula Linder[5], Hauke Smidt[1]

[1]Laboratory of Microbiology, Wageningen University & Research, St
The Netherlands

[2]Laboratory of Systems and Synthetic Biology, Wageningen & Rese
WE, Wageningen, The Netherlands

[3]Department of Developmental Psychology, Behavioral Science Inst

The association between infant fecal microbiota composition and the degradation of human milk oligosaccharides in one month old, healthy breastfed infants.

Klaudyna Borewicz[1*§], Fangjie Gu[2*], Edoardo Saccenti[3], Ilja Arts[4,5], John Penders[4,6], Carel Thijs[4], Sander S. van Leeuwen[7], Cordula Linder[8], Henk Schols[2], Hauke Smidt[1]

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ory of Systems and Synthetic Biology, Wageningen University & Research, Stippeneng 4, 6708WE, Wageningen, erlands

ent of Epidemiology Maastricht University, CAPHRI School for Public Health and Primary Care, Maastricht, The nds (street address?)

School for Cardiovascular Diseases, and Maastricht Center for Systems Biology (MaCSBio), Maastricht, The nds (street address?)

ent of Medical Microbiology, Maastricht University Medical Center, NUTRIM School for Nutrition, Toxicology bolism, Maastricht and CAPHRI School for Public Health and Primary Care, Maastricht, The Netherlands (street

al Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, gh 7, 9747AG Groningen, The Netherlands

dCampina Domo, Bronland 20, 6708WH Wageningen, The Netherlands

The association between infant fecal microbiota composition and the degradation of human milk oligosaccharides in healthy breastfed infants at two, six and twelve weeks of age.

Klaudyna Borewicz[1*§], Fangjie Gu[2*], Edoardo Saccenti[3], Christine Hechler[4], Roseriet Beijers[4], Carolina de Weerth[4], Sander S. van Leeuwen[5], Henk Schols[2], Hauke Smidt[1]

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[4]Department of Developmental Psychology, Behavioral Science Institute, Radboud University Nijmegen, Postbus 9104, 6500 HE Nijmegen, The Netherlands

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* These authors contributed equally to this work

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Bifidobacteria - our friendly bacteria through infancy and beyond

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Bifidobacteria RA decreases with age, and their species composition change

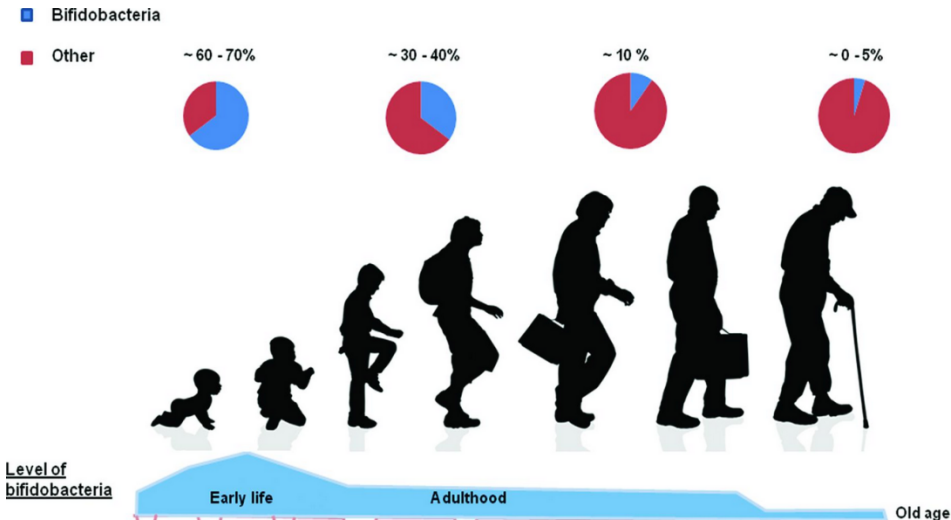
Increase or decrease of different species of bifidobacteria were associated with different diets and some diseases

Bifidobacteria inhibit enteropathogens through the production of organic acids, antibacterial peptides, quorum-sensing inhibitors, or immune stimulation

They have genetic adaptations that give them competitive advantage to utilize milk oligosacharides (HMOs) and complex carbohydrates (eg. starch and its derivatives)

Different *Bifidobacterium* species specialize for specific carbohydrates, and cooperate with other bifidobacterial and other species in trophic webs

Bifidobacteria can be supplemented as probiotics, or stimulated with prebiotics



Prebiotic potential of Isomalto/malto-polysaccharides

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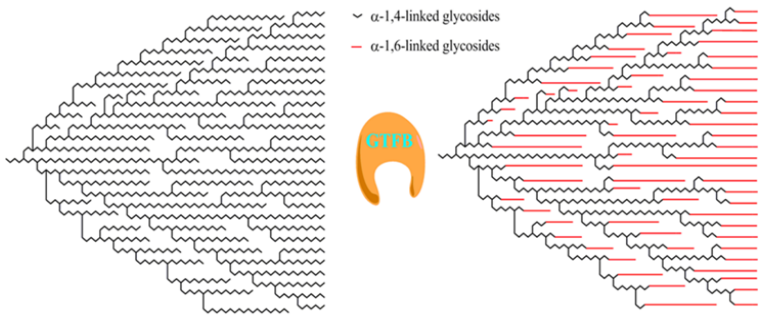
- Isomalto/malto-polysaccharides (IMMPs) are modified starch
- Chemical and biological properties of different IMMPs depend on the starting starch material, and the ratios of the α1-4 and α1-6 bonds

Starch
α1-4 bonds are degraded by host α-amylases in saliva and pancreatic juice



Modified Starch
α1-6 bonds are degraded by microbiota in the colon

- Presence of **α1-6 bonds** gives substrates their prebiotic properties

Prebiotic potential of Isomalto/malto-polysaccharides

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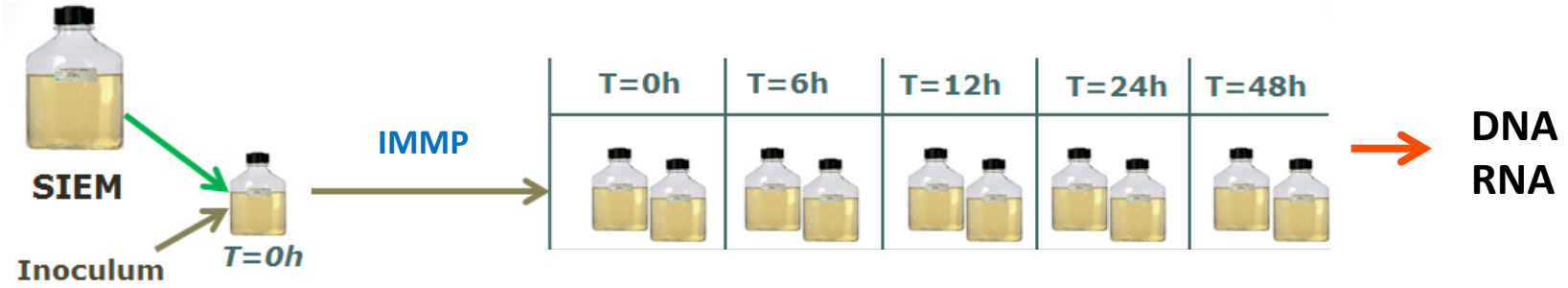
- We tested 4 IMMPs from different starch sources and with different α1-6 bonds content for their effect on fecal microbiota *in-vitro*

IMMP - 94 %
IMMP - 96%
IMMP - 27 %
IMMP - dig27%



Different IMMPs differentially modulate microbial composition

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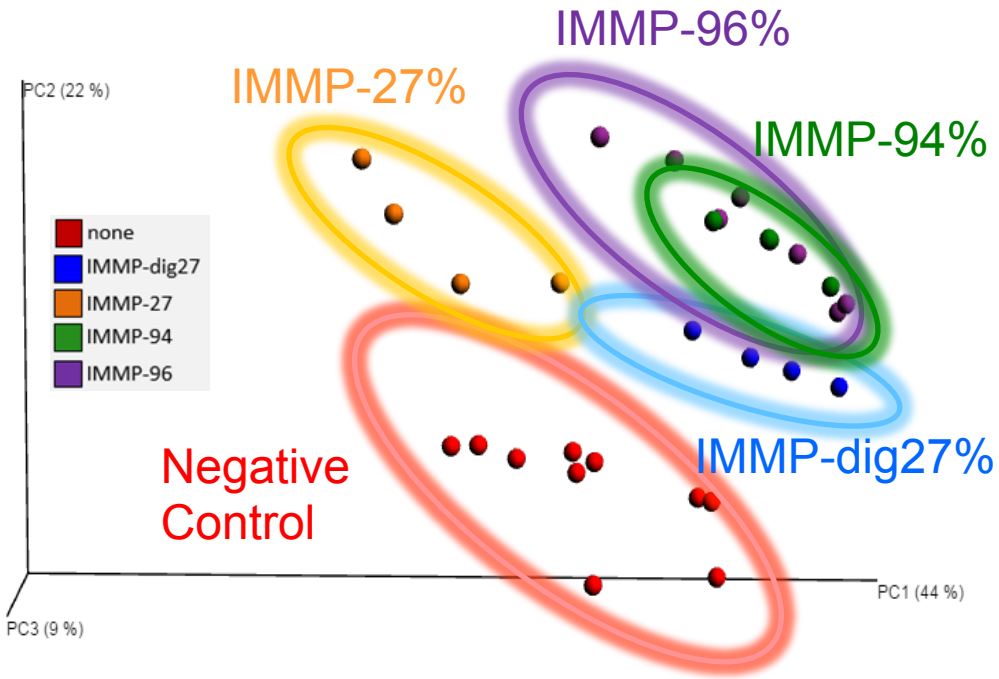
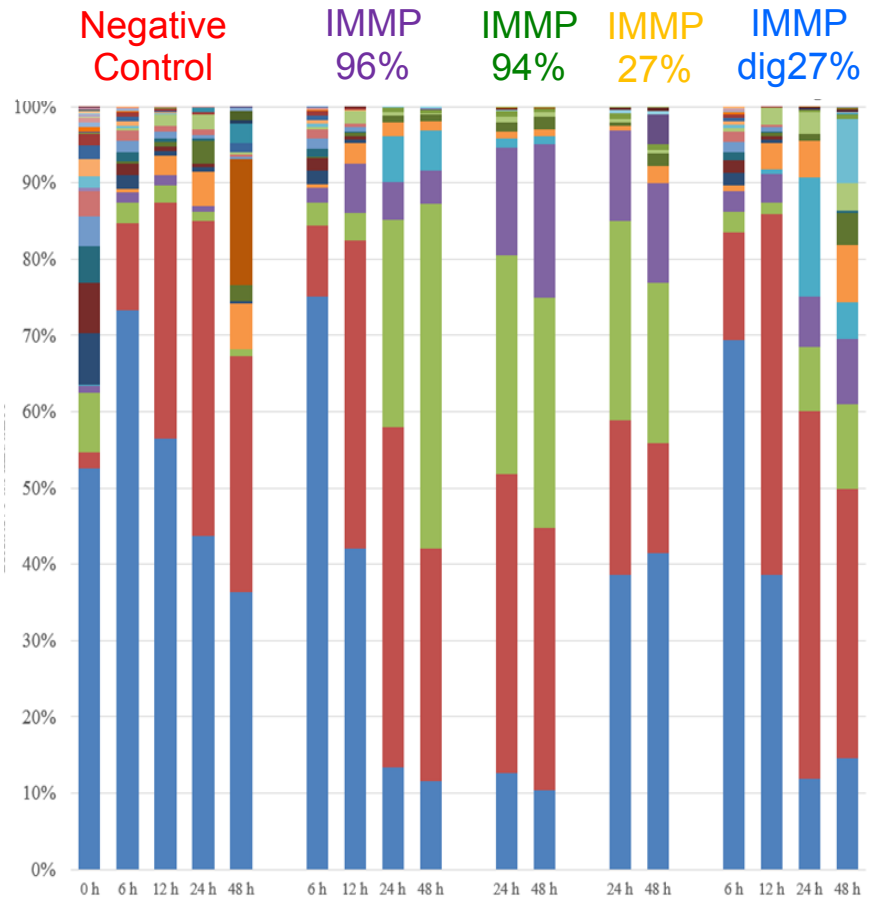


PCoA based on weighted (relative abundance) Unifrac distances between observed communities



Increase in RA of *Bifidobacterium* and *Lactobacillus*

Influence of IMMPs on microbial activity

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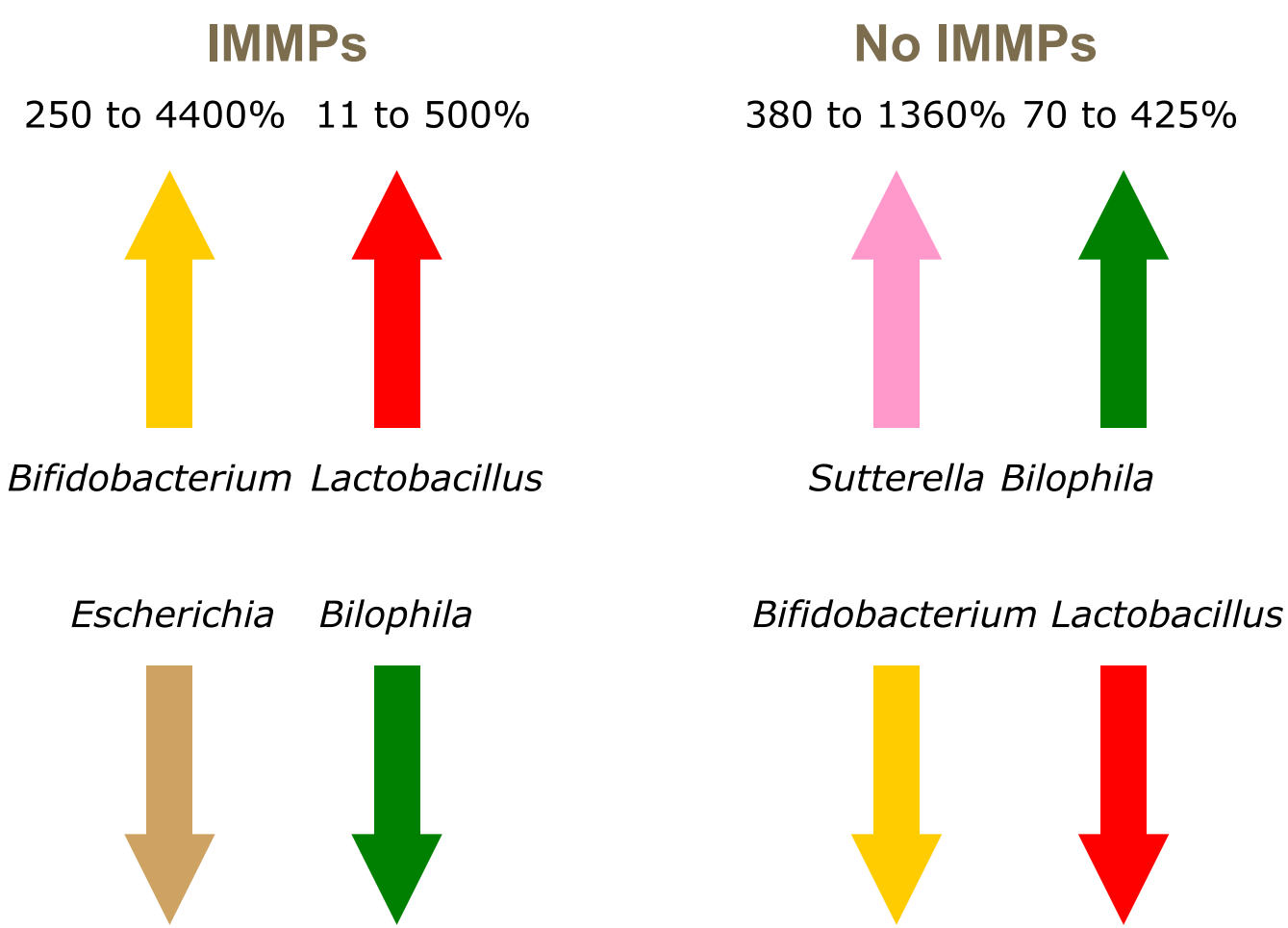

IMMP *in vitro* fermentation study

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Two manuscripts in preparation

Isomalto/malto-polysaccharides maintain normal gut functioning while promoting growth and activity of beneficial bacteria

Klaudyna Borewicz[1,*], Bastian Hornung[1,2,*,#], Fangjie Gu[3], Pieter H. van der Zaal[4], Henk Schols[3], Peter J. Schaap[2], Hauke Smidt[1]

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[3]Laboratory of Food Chemistry, Wageningen University, Bornse Weil

[4]Biobased Chemistry and Technology, Wageningen University, The

*These authors contributed equally to this work

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***In vitro* fermentation behaviour of isomalto/malto-polysaccharides using human faecal inoculum indicates prebiotic potential**

Fangjie Gu[1*], Klaudyna Borewicz[2*], Bernadette Richter[1], Pieter H. van der Zaal[3], Hauke Smidt[2], Piet Buwalda[3,4], Henk A Schols[1]

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* These authors contributed equally to this work

Testing the effects of IMMP97 *in vivo*

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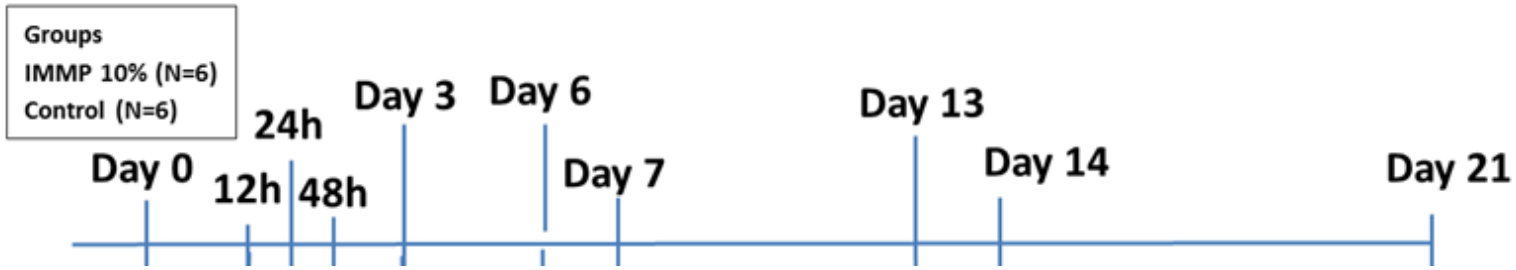
IMMP mouse study (SP1 and SP3)

3 week intervention study using adult mice to see the effect of 10% **IMMP97** supplementation in diet on various metabolic parameters (cholesterol metabolism, insulin resistance) and microbiota composition in feces, ileum, cecum and colon



Sample collection time points

IMMP97 *in vivo* mouse study

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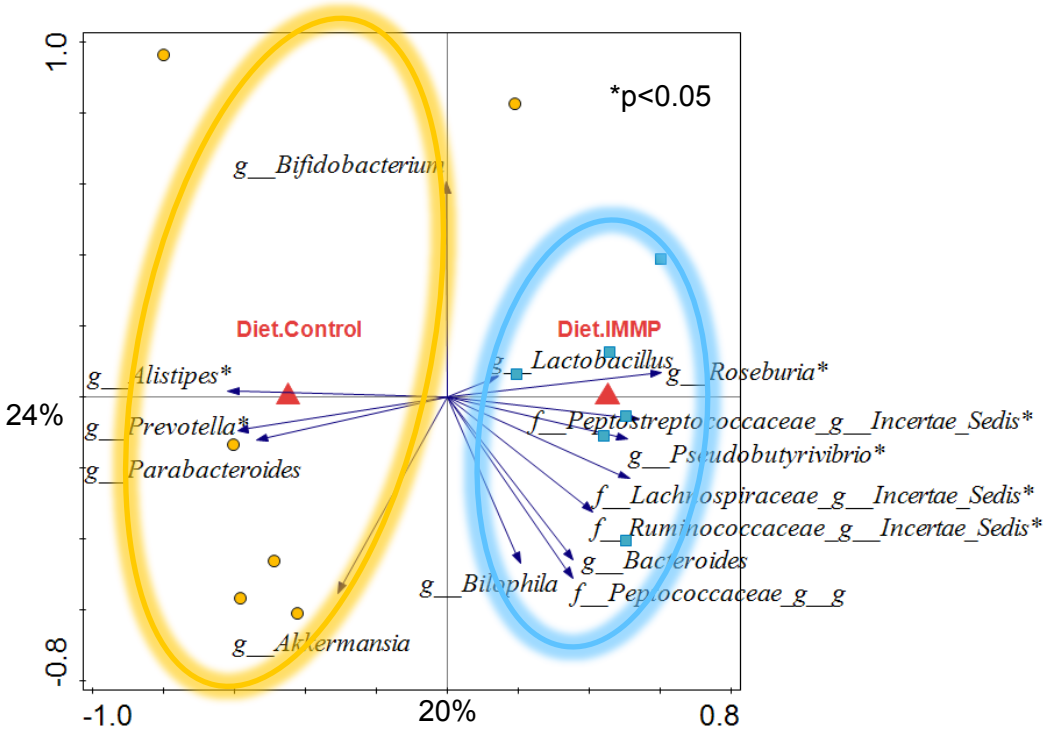
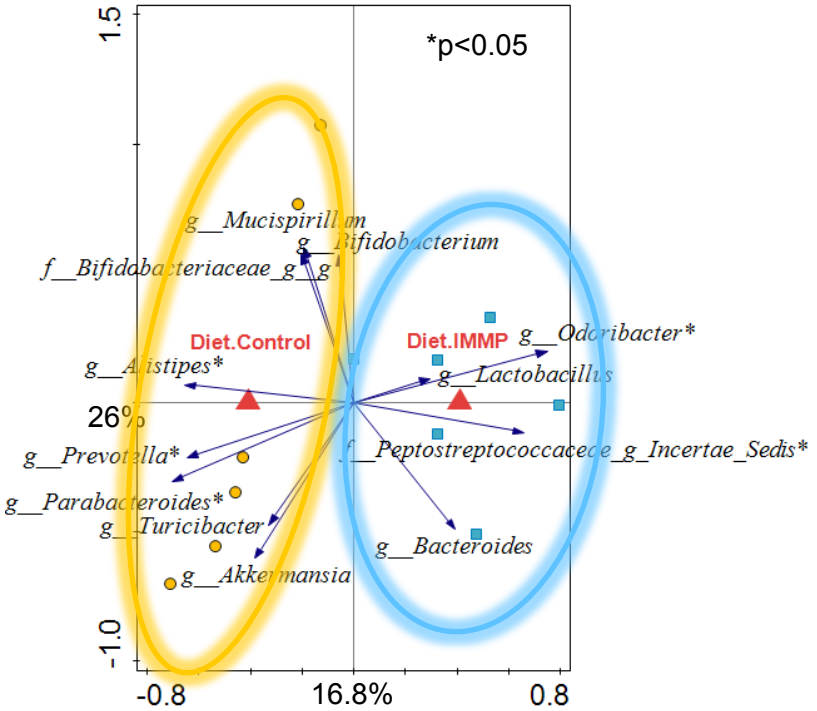
- IMMP diet significantly changed microbiota in cecum and colon, not ileum
- Changes in relative abundance of *Bifidobacterium* and *Lactobacillus* were not significant



Cecum



Colon

Metabolic effects of IMMP97 *in vivo*

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Dietary IMMP supplementation was associated with:

- ↑ Fecal output (associated with increased *Odoribacter*, decreased *Parabacteroides*)

- ↑ Fecal propionic acid (negatively correlated with *Odoribacter*) on day 14 and 21
- ↑ Fecal lactic acid (positively correlated with colonic *Odoribacter*) on day 14 and 21
-
- ↓ Fecal DiH-cholesterol (positively correlated with *Mucispirillum* and unclassified *Ruminococcaceae*)

- ↑ Plasma non-esterified fatty acids on day 21(positively correlated with *Bacteroides,* negativly with uncultured family in *Closridiales*)

The majority of processes relating to lipid metabolism occur primarily in the small intestine, where the microbial effect of IMMP was the weakest.

Results should be verified using a larger study population

Animal studies

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Two manuscripts in preparation

The effects of Isomalto/malto-polysaccharides (IMMPs) on mouse physiology and gut microbiota composition and function.

Klaudyna Borewicz[1*$], Rima Mistry[2*], Fangjie Gu[3*], Henk Schols[3], Uwe Tietge[2], Hauke Smidt[1]

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[2]UMGC, The Netherlands

[3]Laboratory of Food Chemistry, Wageningen Univ
6708 WG Wageningen, The Netherlands

*These authors contributed equally to this work

The combination long-chain inulin with *Lactobacillus acidophilus* W37 confers microbiota associated protective effects through weaning and against *Salmonella* Typhimurium in neonate piglets.

Klaudyna Borewicz (1), Prokopis Konstanti (1), Alexia F.P. Lépine (2, 3), Paul de Vos (2) and Hauke (1)

1 MIB WUR

2 Immunoendocrinology, Division of Medical Biology, Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands.

3 Food and Biobased Research, Wageningen University and Research, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands

Thank You!

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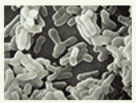
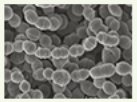
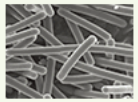
Hauke Smidt
Henk Schols
Maria Suarez, Edoardo Saccenti, Peter Schaap (WU-SSB), Carolina de Weerth (RU),
Collaborations with PhD students : Fangjie Gu (WU-FCH), Bastian Hornung (WU-SSB), Rima Mistry (UMGC), Christine Hechler (RU)

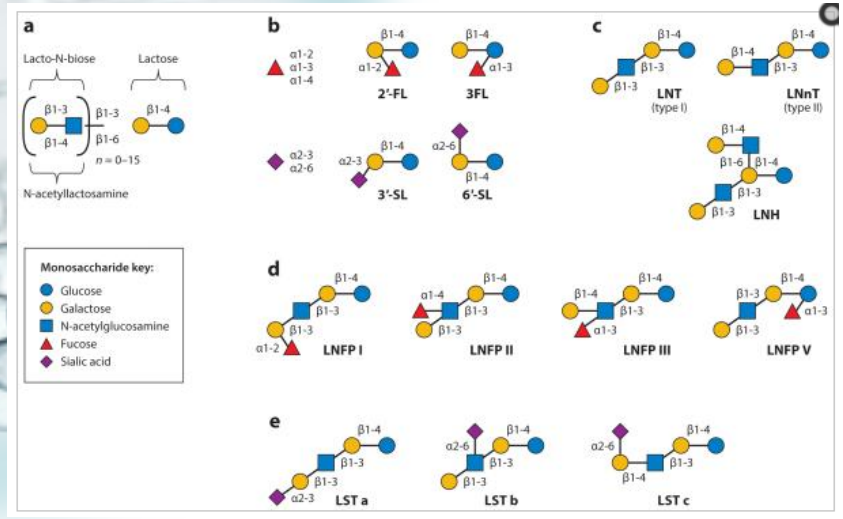
Partners:
University of Groningen, Radboud University, CCC, AVEBE, Nutreco, Hanzehogeschool Groningen, Winclove, Danone, FrieslandCampina, Sensus, Nutrica, Maastricht University

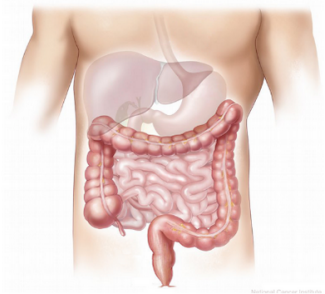
AVEBE
nutreco
NUTRICIA Advanced Medical Nutrition
Maastricht University
Radboud University
WINCLOVE PROBIOTICS
FrieslandCampina
SENSUS
TOPSECTOR AGRI&FOOD
TKI Agri&Food

Extra slides...

Differences between bifidobacteria and lactic acid bacteria

	Bifidobacterium	Lactic acid bacteria	
Cell morphology	Rods, clubs, or branched rods	Cocci or rods	
			
	Bifidobacterium	Lactococcus	Lactobacillus
Habitat	Mainly human and animal intestines	In nature in general, milk and dairy products, human and animal intestines, fermented foods such as pickled vegetables	
Sensitivity to oxygen	Unable to live in the presence of oxygen (strict anaerobic)	Able to live in the presence of oxygen (facultative anaerobic)	
Main metabolites	Lactic acid Acetic acid	Lactic acid	





Credit: Arnavaz at French Wikipedia

NUTRECO piglet study

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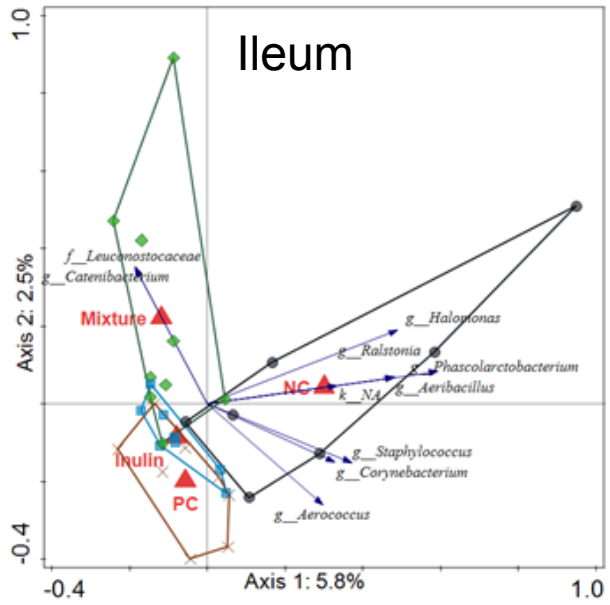
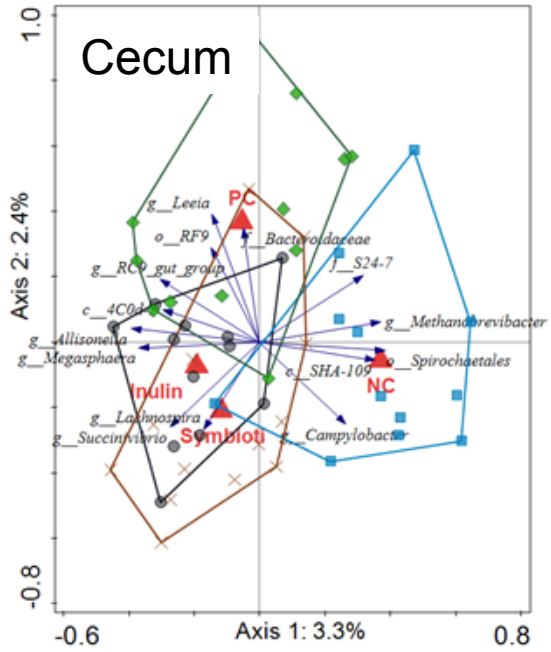
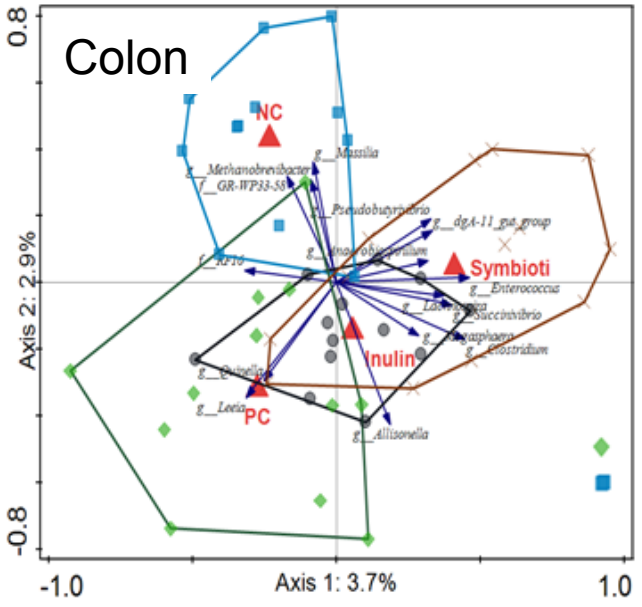
- Age (not diet) had significant effect on microbiota composition in feces
- The differences in microbial composition in ileum, cecum and colon between animals from different treatment groups was detectable, but not significant







Control + vaccine Control+ no vaccine Inulin Inulin+LW37 (symbiotic)

Lacobacilli make a small fraction of adult fecal microbiota

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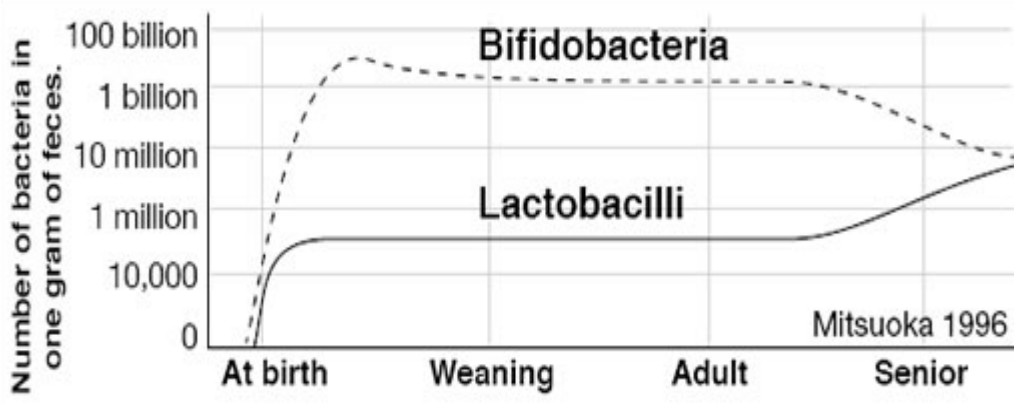
Lactobacilli (~ 17sp) are low abundance in the gut - 0.01% of the total cultivable human fecal microbiota and <1% small intestinal microbiota

They are highly unstable and most are “hitchhiking” through the gut from fermented food, the oral cavity, or more proximal parts of the GIT

Strain taken as probiotics don’t persist in the host, but get outcompeted by other bacteria

They activate immune system - there is no indication that colonization is required for the health benefits of these strains



Animal characteristics, hepatic and plasma parameters

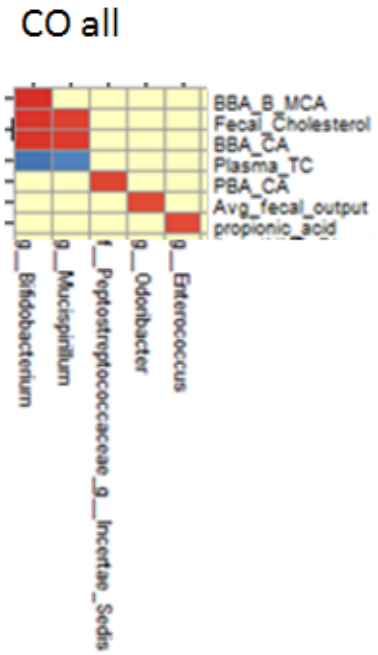
CARBOHYDRATE COMPETENCE CENTER

FDR p<0.05, CorrTh 0.5

- IMMP increased the fecal output mass and decreased fecal dihydrocholosterol (DiH-Chol)
  - IMMP increased plasma nonesterified fatty acid (PNFA) and cholic acid (PBA-CA)
- IMMP increased levels of lactate and propionate in feces

- There was a negative association between Plasma Total Cholesterol and Fecal Cholesterol
- Fecal Cholesterol levels positively correlated with abundance of *Bifidobacterium , Mucispirillum, f_Erysipelotrichaceae g_g,* (IL) o_Anaeroplasmatales g_g (CE) and o_Clostridiales g_g (CE-Cont)
- PBA-CA is positively correlated with *f_Peptococcaceae g_Incertae_Sedis*
- Propionate correlates with Enterococcus and lactate with *Bifidobacterium* and Lactobacilli (IL/CE/CO-Cont)

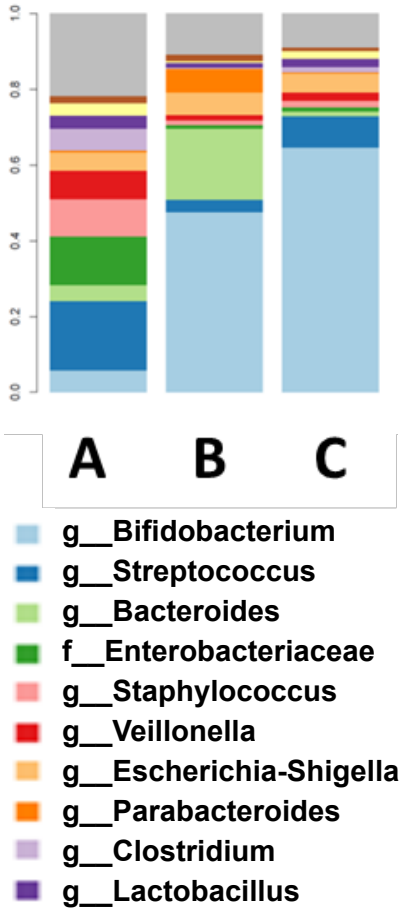
- In the group receiving the IMMP these associations are not significant (p>0.01)

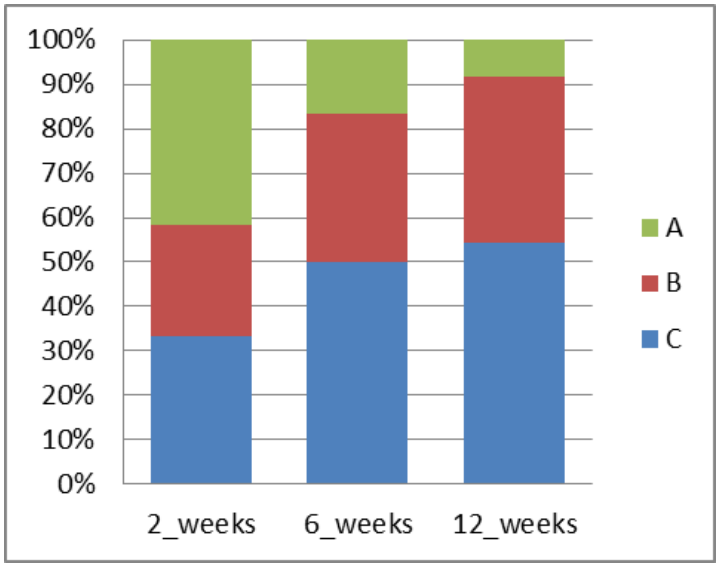


CARBOHYDRATE COMPETENCE CENTER

Microbial clusters

Samples: Clustering analyses: 210 fecal samples from BINGO cohort.
Time course: 24 BF infants for which data was available at 2,6, and 12 weeks of age
Methods: % of infants within each cluster at each timepoint





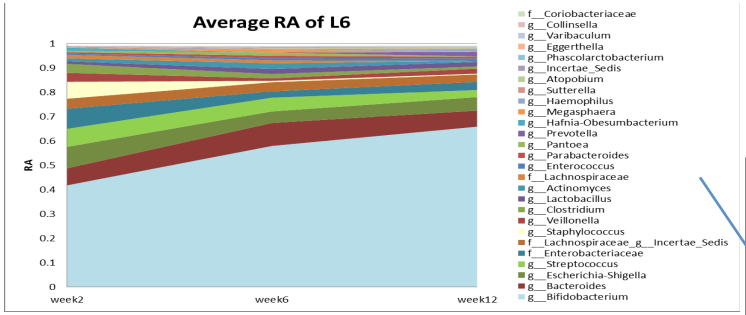
Microbial profile of infants transitions into a cluster type characterized by high bacteroides/ bifidobacteria abundance

CARBOHYDRATE COMPETENCE CENTER

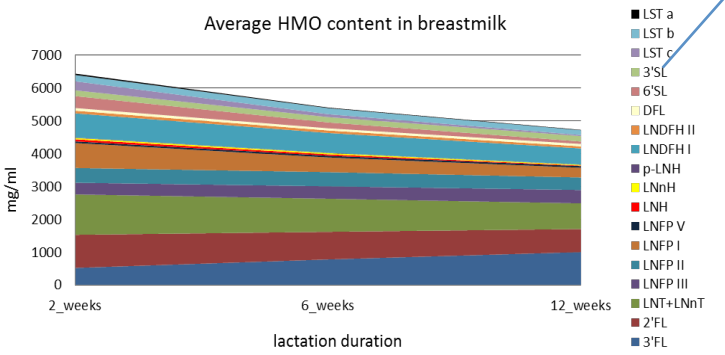
3. Do HMOs play a role in promoting Bifidobacteria in the gut?

Samples: 24 BF infants for which data was available at 2, 6, and 12 weeks of age. Methods: XY plotting

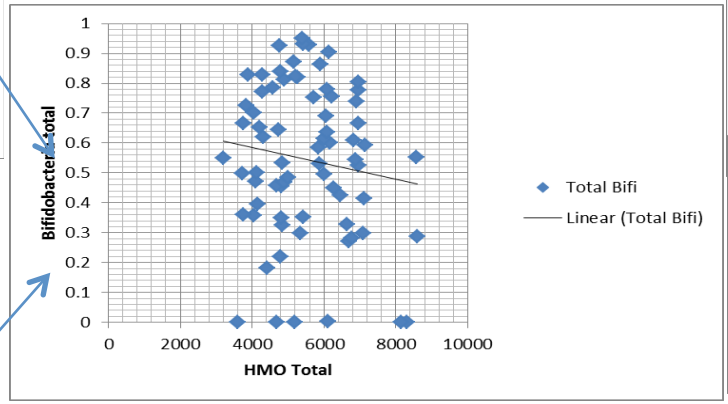
Bifidobacteria RA increase in time

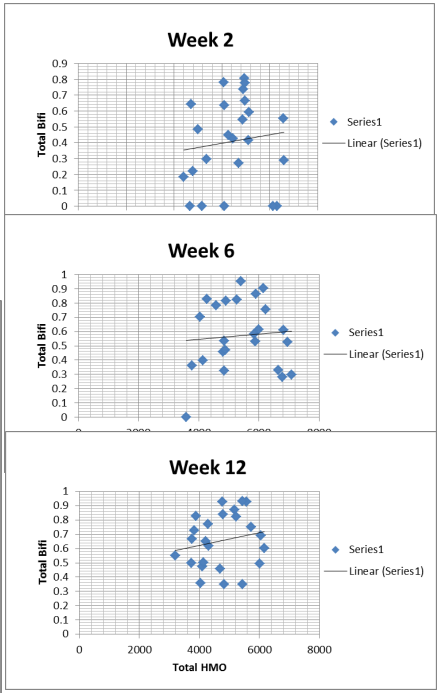


HMOs in milk decrea se in time



Negative correlation between HMOs and Bifidobacteria





High initial concentrations of HMOs may be sufficient to kick-start bifidobacteria, which are further sustained by slightly declining concentrations of HMOs

Microbiota vs. metabolites at d=21

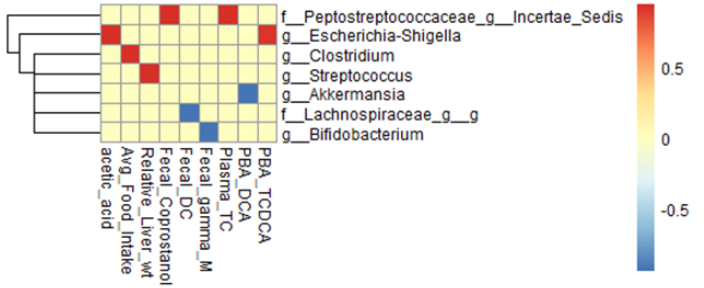
CARBOHYDRATE COMPETENCE CENTER

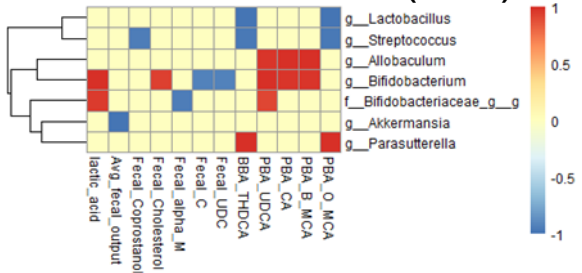
CorrTh=0.9, p<0.05

IMMP (n=6)

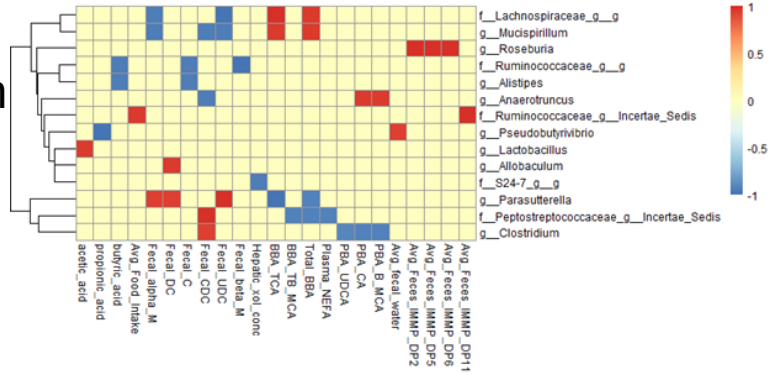
Control (n=6)

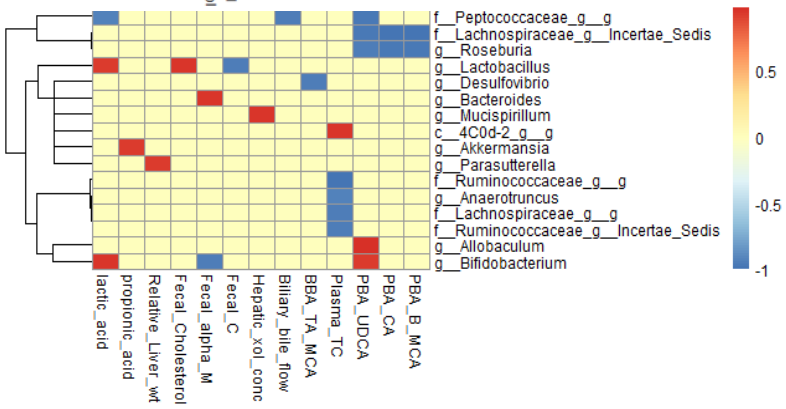
Ileum



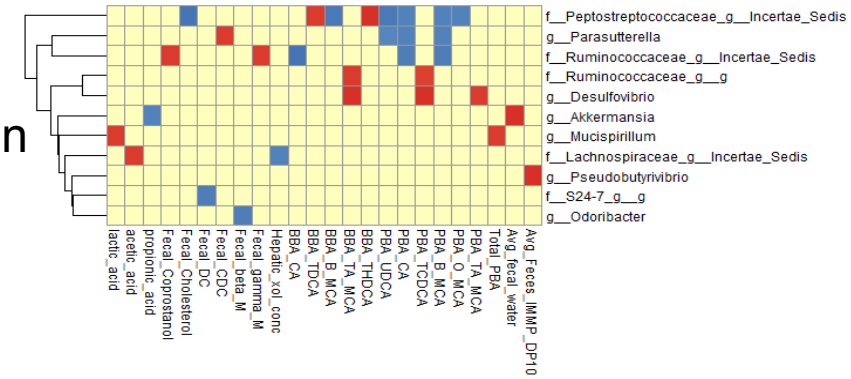


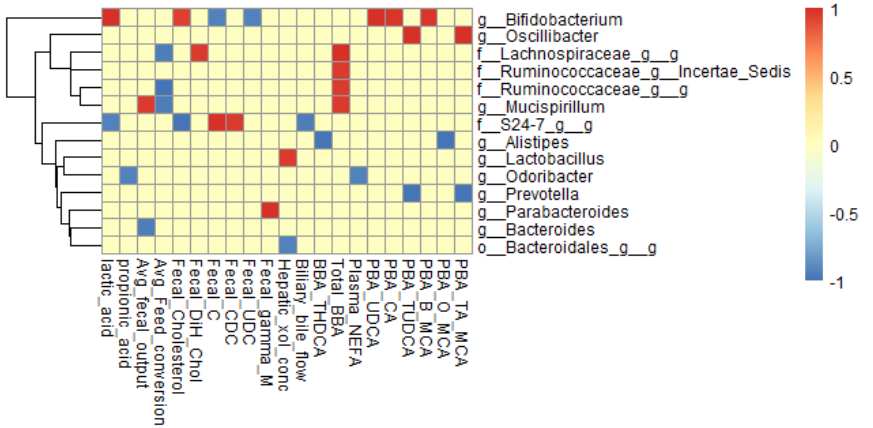
Cecum



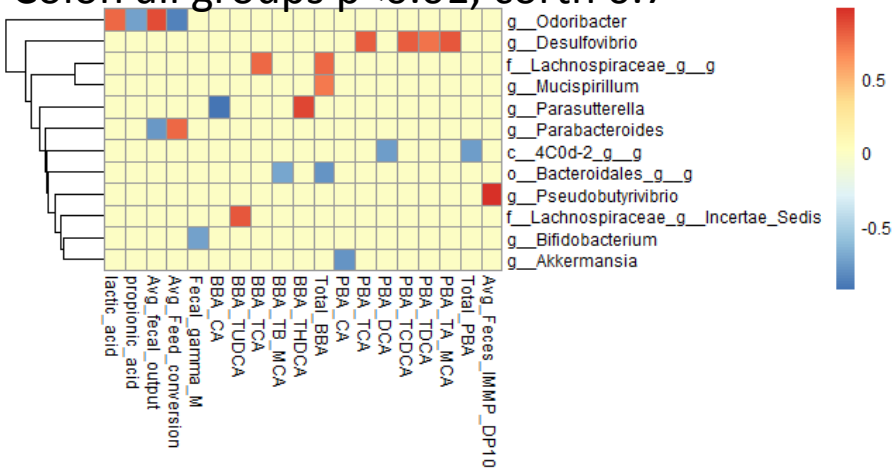


Colon

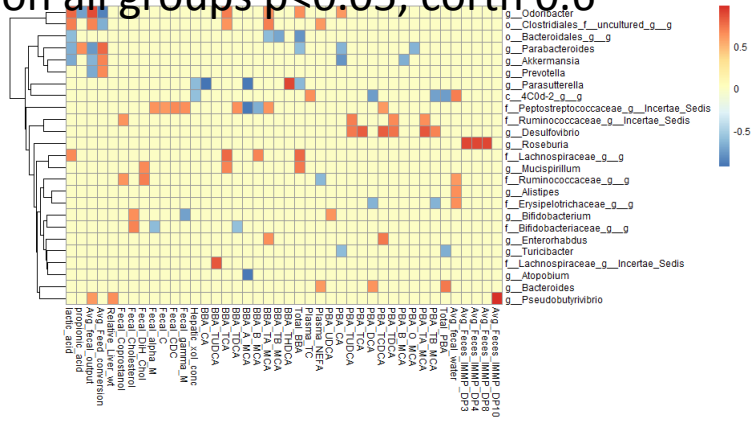




Colon all groups p<0.01, corth 0.7



Colon all groups p<0.05, corth 0.6



Cecum all groups p<0.05, corth 0.6