Prebiotics in Infant Formulas Promote Gut Microbiota Similar to that of Breastfed Infants

Klaudyna Borewicz[1], Maria Suarez-Diez[2], Fangjie Gu[3], Christine Hechler[4], Ilja Arts[5], John Penders[5], Carel Thijs[5], Carolina de Weerth[4], Henk Schols[3], Hauke Smidt[1]

Introduction

- Gut microbiota of young infants is highly variable and unstable
- Breastmilk provides the most optimal nutrition for an infant and its intestinal microbes
- Infants receiving breastmilk have a different gut microbial profile than those receiving infant formulas
- Modern infant formulas are fortified with prebiotics, such as FOS and GOS to mimic the bifidogenic effect of the human milk
- The effects of added prebiotics on the infant gut microbial composition and colonisation are still largely unknown

Here we define the existence of three unique patterns - "enterotypes" - in faecal microbial communities of infants. We show that these enterotypes relate to feeding mode, infant age and that they influence how babies digest different oligosaccharides present in breastmilk (HMOs). Finally, we compare gut microbiota profiles of breastfed infants to those receiving standard and prebiotic fortified infant formulas.

Materials and Methods

- Fecal samples from healthy infants in two cohort studies:
  - KOALA Cohort (2003): 242 infants, 1 month old: 124 breastfed (BF), 103 formulafed (FF), and 15 mixfed (MF)
  - BINGO Cohort (2015): 77 infants, 2, 6, 12 weeks old: 157 samples from BF, 25 samples FF, and 23 samples from MF
- Microbiota composition measured with HiSeq sequencing of V4 region of the 16S rRNA gene
- Milk and fecal HMO measured with HPAEC, NMR, HPSEC

Results

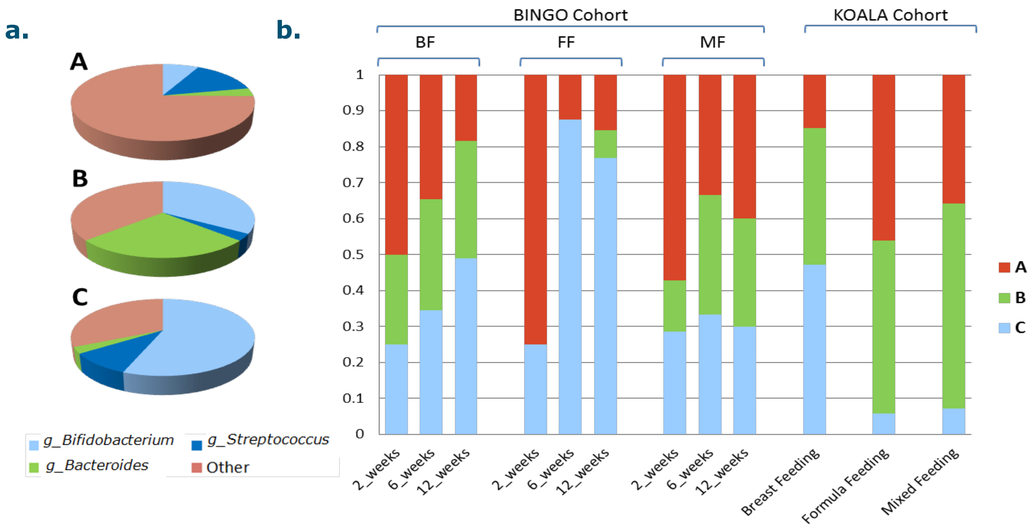

Figure 1. Infant Enterotypes and Cluster Distribution.
a. Simplified cluster composition: clustering performed on relative abundance of genus level taxa, using DMM. Cluster A –Mixed cluster, composed of many taxa, with a low abundance of *Bifidobacterium*; Cluster B – defined by a high abundance of *Bacteroides* and *Bifidobacterium*; Cluster C – defined by a high abundance of *Bifidobacterium*; b. Cluster distribution within age and feeding categories: BF infants gradually progress towards cluster C. Infants receiving modern infant formulas (BINGO cohort) belong mostly to *Bifidobacterium* dominated cluster, while FF infants in KOALA cohort receiving traditional formulas in the mixed or *Bacteroides* dominated microbial profiles (KOALA cohort)

Results

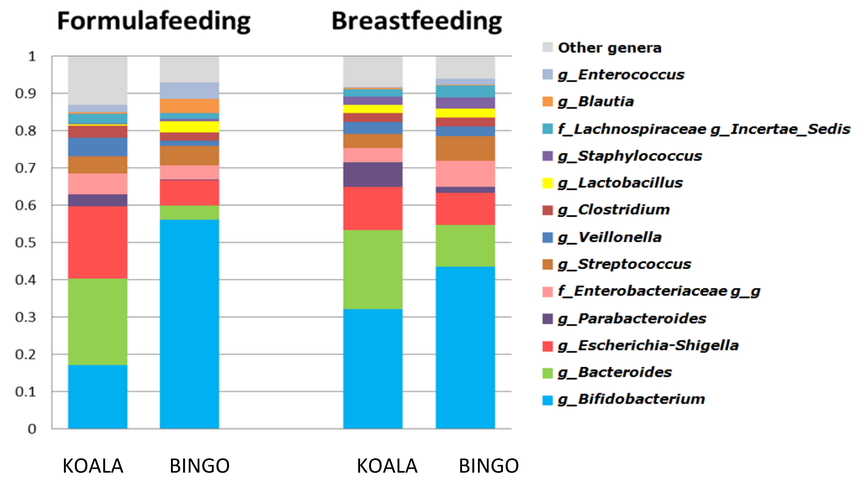

Figure 2. Comparison of Microbial Profiles between BINGO and KOALA Cohorts. Modern infant formulas used in BINGO cohort result in higher levels of genus *Bifidobacterium* and *Lactobacillus*, lower levels of *Bacteroides* and *Escherichia-Shigella* group than traditional formulas used in KOALA

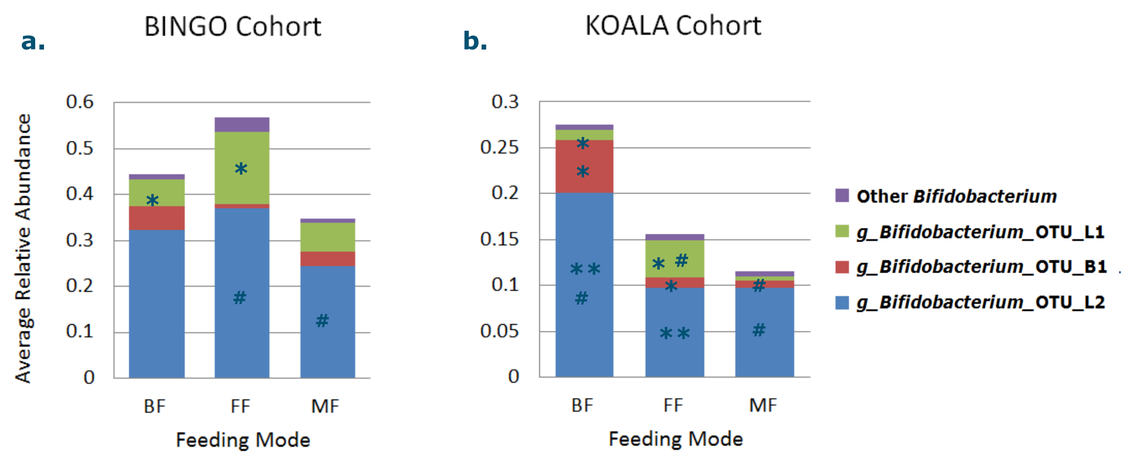

Figure 3. Relative Abundance of Bifidobacterial OTUs in Different Feeding Modes.
a. Differences of the main *Bifidobacterium* OTUs between FF and BF infants in BINGO cohort are not significant for OTUs L2 and B1 (FDR $p>0.05$), and significant for OTU L1 ($p<0.05$); b. There are significant differences in the main *Bifidobacterium* OTUs between BF and FF infants in KOALA cohort (OTU L2 FDR $p<0.05$; OTU L1 and B1 $p<0.05$). In both cohorts MF resulted in decrease of bifidobacteria, and there was a significant decrease for OTU L2 ($p<0.05$) between FF and MF in BINGO cohort and between BF and MF in KOALA cohort, and significant increase in OTU L1 in FF compared to MF in KOALA cohort.

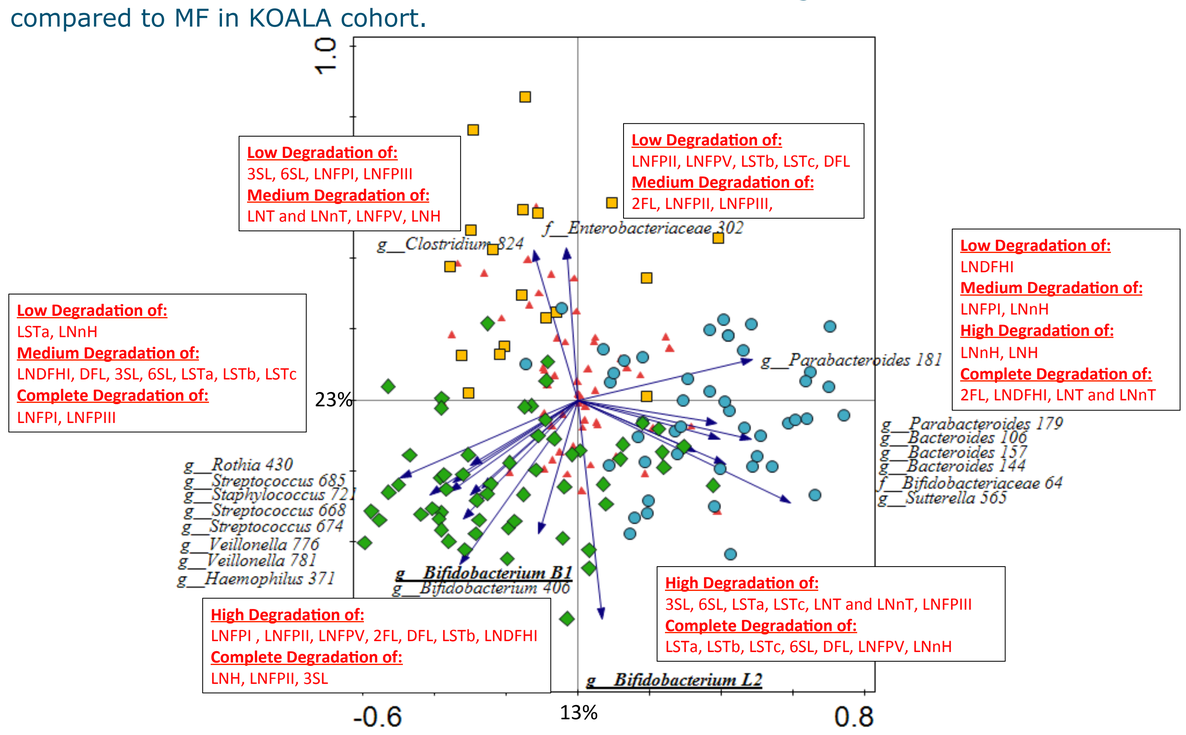

Figure 4. RDA of Infant Fecal Microbiota OTUs of BF Infants and the Level of Breastmilk Oligosaccharides (HMOs) Degradation in the KOALA Study. 19 best fitting bacterial OTUs are displayed. Samples colour-coded by enterotype. High RA abundance of selected OTUs from *Bifidobacterium* and *Bacteroides* correlate with high levels of degradation of main groups of HMOs found in breastmilk. This finding supports the importance of presence of bifidobacteria for optimal utilisation of food in infant nutrition.

Conclusions

- Bifidobacteria-rich gut ecosystem is characteristic for healthy BF infants
- Higher levels of bifidobacteria are essential for degradation of HMOs, which are the third main component of human milk
- Modern infant formulas result in higher fecal bifidobacteria abundance, and species pattern more similar to that found in BF infants

Laboratory of Microbiology,
Stippeneng 4, 6708 WE Wageningen
Contact: klaudyna.borewicz@wur.nl
www.wageningenUR.nl/mib www.wageningenUR.nl/SSB

Affiliations

1 Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands **2** Laboratory of Systems and Synthetic Biology, Wageningen University, Wageningen, The Netherlands **3** Laboratory of Food Chemistry, Wageningen University, Wageningen, The Netherlands, **4** Department of Psychology, Radboud University, Nijmegen, The Netherlands, **5** Maastricht University, Maastricht, The Netherlands

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