Supplementary Figures and Legends

Intestinal colonisation patterns in breastfed and formula-fed infants during the first 12 weeks of life reveal sequential microbiota signatures

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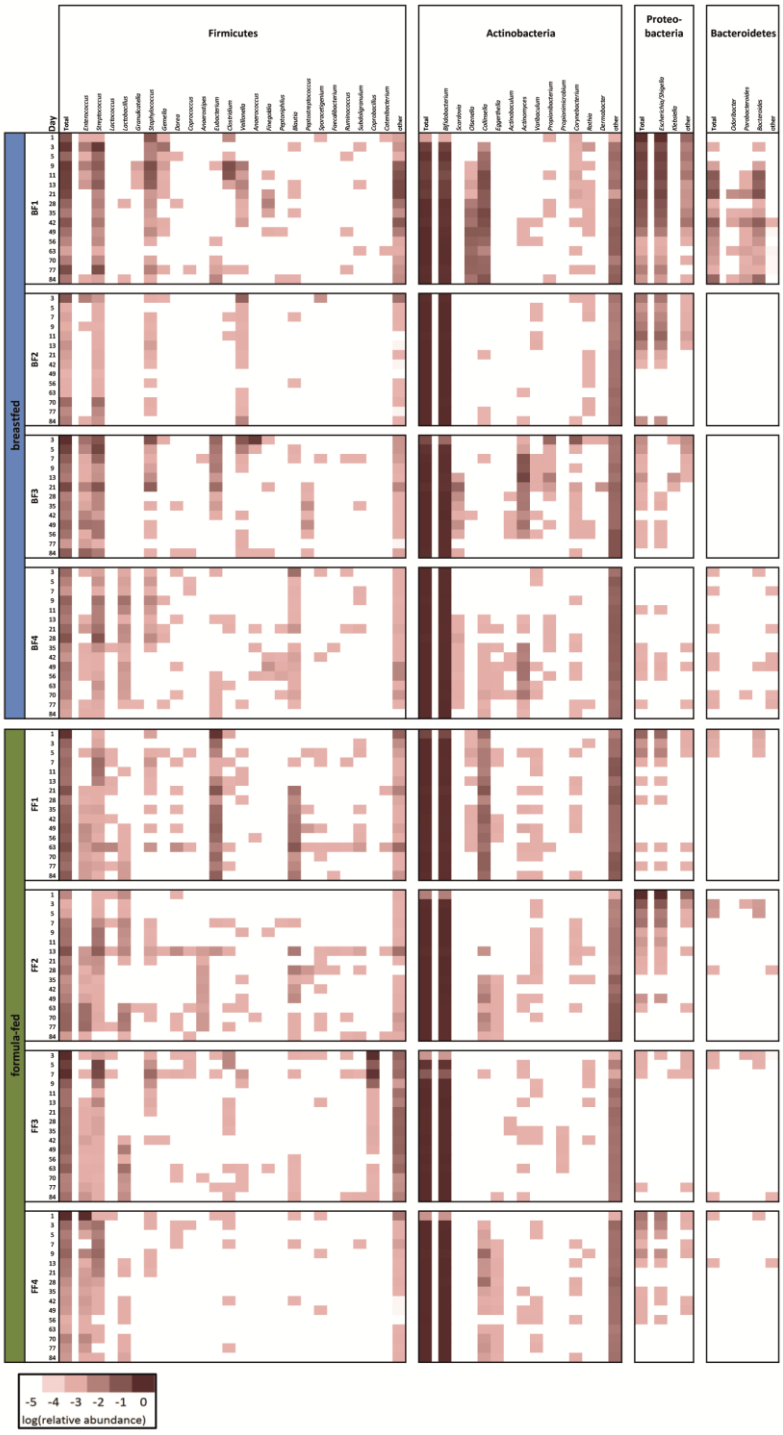
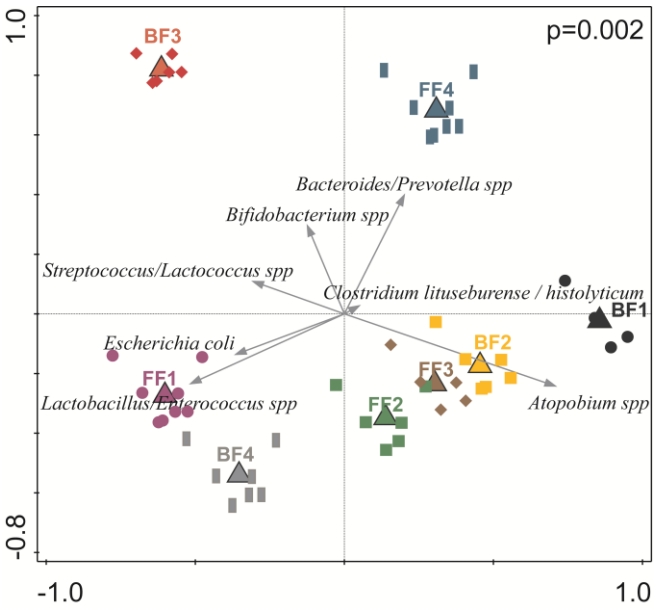


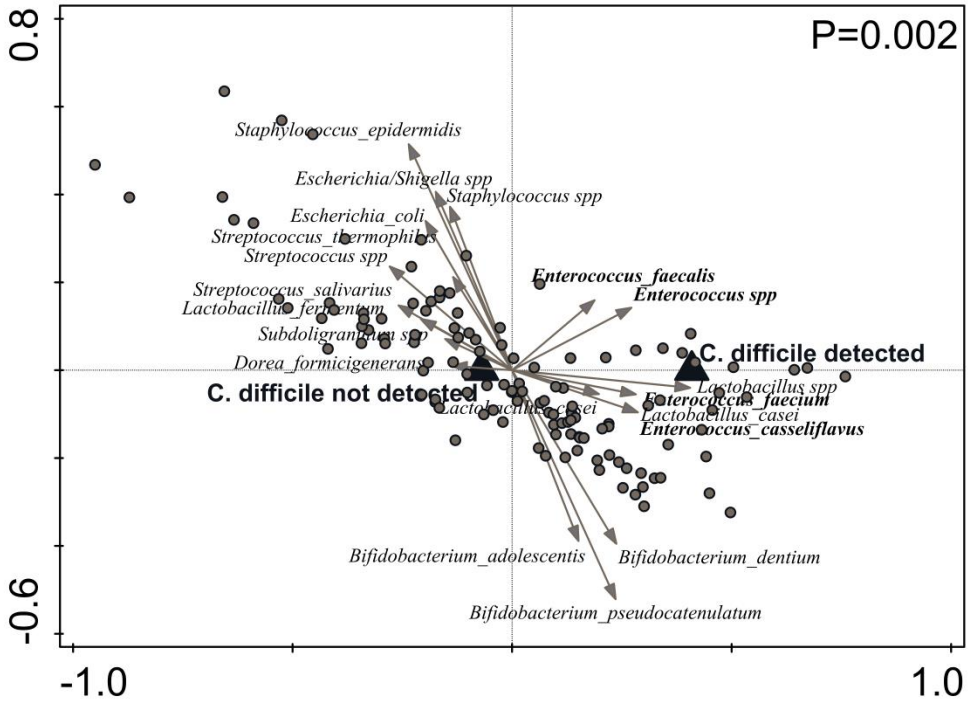
Supplementary Figure S1. Heatmap of all detected genera in the feces of breast and formula-fed infants from day 1 till 12 weeks

Overall microbiota composition at the genus level as determined by 16S profiling in 4 breast- and 4 formula-fed infants over time. Values are presented as log10 values of the relative abundance.

Supplementary Figures and Legends

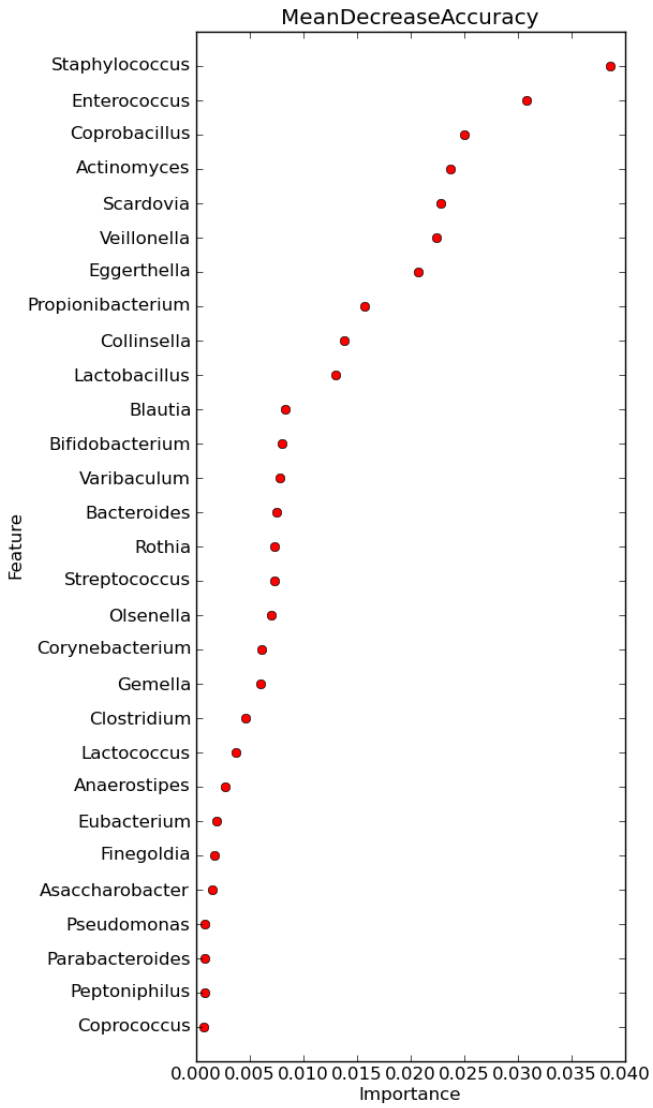


Supplementary Figure S2. Triplot of partial RDA based on the relative abundance of 8 taxa targeted by FISH probes of the variable individual after removing the effects of time and type of feeding. Constrained explanatory variables are indicated by triangles: BF1-4 represents infants being breastfed and FF1-4 represents infants being formula-fed. The arrows indicate the 7 targeted phylogenetic groups typically for the early life microbiome. Upper right shows the p-value of Monte Carlo Permutation testing.



Supplementary Figure S3. Triplot of partial RDA based on the relative abundance of detected species in relation to the detection of *C. difficile* by qPCR after removing the effects of individual. Constrained explanatory variables are indicated by triangles: *C. difficile* detection Yes/No. The arrows indicate species which had at least 1.8% of their variation explained by the first canonical axis. Upper right shows the p-value of Monte Carlo Permutation testing.

Supplementary Figures and Legends



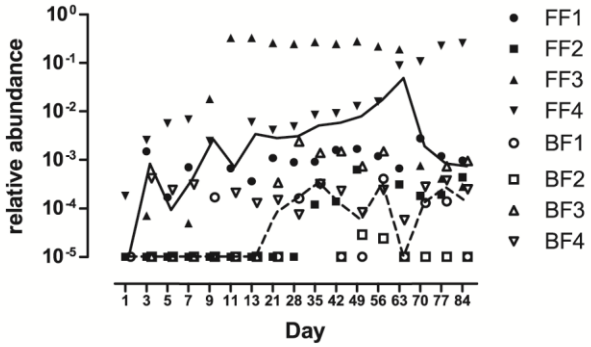
Supplementary Figure S4. Random Forest analysis on feeding regime.
In this Random Forest analysis it is depicted to what extent each genus is important for correctly predicting feeding type (breastfeeding or formula-feeding) based on relative abundance on the genus level determined through 16S rDNA sequencing. *Staphylococcus* was most predictive for breastfeeding. In our analysis, we considered a genus to be highly predictive if its importance score was at least 0.001.

Supplementary Figures and Legends

Supplementary Figure S5. Typical skin taxa from day 1 till 12 weeks of age.

Relative abundance of 17 skin genera as detected by 16S profiling in breastfed and formula-fed infants over the course of the study.

Supplementary Figures and Legends



Supplementary Figure S6. *Bifidobacterium dentium*, a taxon more abundant in formula-fed infants during the first weeks of life.

Relative abundance of *B. dentium* and close relatives as determined by 16S profiling, during the course of the study. Curves represent the median of the 2 feeding groups.

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Supplementary Tables

TABLE S1. Clinical characteristics of the infants included in this study.

	Breastfed infants (n=4)*	**Formula-fed infants (n=4)***
Birth weight (g) (mean and per individual)	3736 BF1: 3250 BF2: 4100 BF3: 3580 BF4: 4015	3328 FF1: 3000 FF2: 4225 FF3: 2960 FF4: 3125
Weight at 3 months (g) (mean and per individual)	6331 BF1: 6000 BF2: 7300 BF3: 6095 BF4: 5930	5909 FF1: 5445 FF2: 7240 FF3: 4820 FF4: 6130
Gender	BF1: M BF2: M BF3: M BF4: F	FF1: F FF2: M FF3: M FF4: F
Duration of gestation (mean and per individual)	39 weeks + 6 days BF1: 37 weeks + 5 days BF2: 41 weeks + 1 day BF3: 39 weeks	40 weeks + 2 days FF1: 40 weeks + 6 days FF2: 39 weeks + 5 days FF3: 41 weeks

Supplementary Tables

	BF4: 40 weeks	FF4: 39 weeks
Number of siblings (mean and per individual)	0.5 BF1: 1 BF2: 0 BF3: 0 BF4: 1	0.5 FF1: 1 FF2: 1 FF3: 1 FF4: 0
Medicin use - **Antibiotics** - **Antifungals**	 No Yes (BF2, week 2-3, treatment of Sprue with nystatin and miconazole)	 No No
Illness reported by parents	BF3: common cold >7 days	none

*No significant differences between the groups at the 0.05 level

Supplementary Tables

TABLE S2 Bacterial taxa targeted by qPCR in this study

Target	Target gene (E. coli position for 16S)	Technology	Positive controls	Negative controls	Primer/probe sequences (5'–3')	Annealing temperature	References
Bacteria (total)	16S rRNA gene	FAM-TAMRA	*L. lactis*. *B. longum.* *B. infantis*	Fungi	Fw: CGGTGAATACGTTCYCGG Rv: GGWTACCTTGTTACGACTT P: CTTGTACACACCGCCCGTC	56°C	(Suzuki, Taylor et al. 2000)
C. perfringens	16S rRNA gene (176-276)	FAM-TAMRA	*C. perfringens (DSM756)*	*C. difficile* (DSM1296)	Fw: CGCATAACGTTGAAAGATGG Rv: CCTTGGTAGGCCGTTACCC P: TCATCATTCAACCAAAGGAGCAATCC	55°C	(Wise and Siragusa 2005)
C. difficile	16S rRNA gene (57-227)	FAM-TAMRA	*C. difficile (DSM1296)*	*C. perfringens*	Fw: CAAGTTGAGCGATTTACTTCGGTAA Rv: CTAATCAGACGCGGGTCCAT P: CCTACCCTGTACACACGGATAACATACCGAAAG	60°C	(Magdesian and Leutenegger 2011)
K. pneumoniae	*Phoe (outer membrane phosphate porin)*	TaqMan FAM-BHQ	*K. pneumoniae*	*E. coli*.	Fw: CCTGGATCTGACCCTGCAGTA Rv: CCGTCGCCGTTCTGTTTC P: CAGGGTAAAAACGAAGGC	60°C	(Shannon, Lee et al. 2007)
S. pneumoniae	Alpha-fucosidase	TaqMan FAM-BHQ	*S. pneumoniae*	*E. coli*.	Not provided by manufacturer	60°C	Commercial assay–Genesig
H. parainfluenzae	16S-23S rRNA spacer	SYBR Green	*H. parainfluenzae* (DSM8978)	*E. coli.* *K. pneumoniae*	Fw: ACGAGAGACAATAAGTGTCCACACAGATT Rv: TTGCTTTTGTTCAATCAAGATTTT	59°C	(Giannino, Rappazzo et al. 2001)

Supplementary Tables

TABLE S3 – Oligonucleotide probes and hybridization conditions used in FISH analysis of fecal bacteria

Probe	Target bacterial group	Sequence (5'–3')	Hybridization Conditions (°C)	References
EUB338	Total bacterial count	GCTGCCTCCCGTAGGAGT	50	(Amann, Binder et al. 1990)
Ato291	*Atopobium* spp.	GGTCGGTCTCTCAACCC	50	(Harmsen, Wildeboer-Veloo et al. 2000)
Bif164	*Bifidobacterium* spp.	CATCCGGCATTACCACCC	50	(Langendijk, Schut et al. 1995)
Bac303	*Bacteroides/Prevotella* spp.	CCAATGTGGGGGACCTT	45	(Manz, Amann et al. 1996)
CLis135	*Clostridium lituseburense*	GTTATCCGTGTGTACAGGG	50	(Franks, Harmsen et al. 1998)
CHis150	*Clostridium histolyticum*	TTATGCGGTATTAATCT(C/T)CCTTT	50	(Franks, Harmsen et al. 1998)
Lac158	*Lactobacillus/Enterococcus* spp.	GGTATTAGCA(C/T)CTGTTTCCA	50	(Harmsen, Wildeboer-Veloo et al. 2000)
Strc493	*Streptococcus/Lactococcus* spp.	GTTAGCCGTCCCTTTCTGG	50	(Franks, Harmsen et al. 1998)
Ecol1513	*Escherichia coli*	CACCGTAGTGCCTCGTCATCA	37	(Poulsen, Lan et al. 1994)

Supplementary Tables

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Supplementary materials and methods

FISH Microscopy

Fecal samples were processed for analysis of microbiota using FISH (Franks 1998). Briefly, fecal aliquots were diluted 1:10 (w/v) in PBS (NaCl (8 g/l), KCl (0·2 g/l), $Na_2HPO_4 \cdot 2H_2O$ (1·44 g/l), KH_2PO_4 (0·24 g/l), pH 7·4) and fixed in 4% paraformaldehyde in PBS for at least 4 h. Washed cells were resuspended in PBS–ethanol solution (1:1, v/v) and stored at -80 C until analysis. Fluorescent *in situ* hybridization was used to quantify specific bacterial groups as well as the total bacterial counts in the fecal samples, multiple slides with 1 cm^2 wells were prepared for cell counting. Per well, 10 µl of diluted sample was spread. After drying, the cells were fixed to the glass surface with 96% ethanol for 10 min. In the present study hybridization was performed with an extended set of 16S rRNA-targeted probes (summarized in **Supplementary Table 3**). The probe set used for bacterial groups covers approximately 88% of the total number of bacteria which hybridize with the EUB338 probe in healthy volunteers (Franks et al 1998). The probes were manufactured by Eurogentec (Seraing, Belgium) and were 5'-labelled with either fluorescein isothiocyanate (FITC) or Cy3. The samples were hybridized overnight at 37°C or 50°C (see **Supplementary Table 3**) in hybridization buffer [0·9M NaCl, 20mM Tris-HCl (pH 7·2), 0·1% SDS (w/v)] containing 9 ng labelled probe per slide. The slides were washed for 20 min in wash buffer [0·9 M-NaCl, 20mM-Tris-HCl (pH 7·2)], rinsed briefly in Milli-Q water and dried using compressed air. Total cells were enumerated after staining with 40,6-diamidino-2-phenylindole (DAPI). Slides were mounted in Vectashield (Vector Labs, Burlingame, CA, USA) to minimize fading of the fluorescent signal. The fluorescent cells in the samples were counted automatically with a Leica DMRA2 epifluorescence microscope using a modified version of the Leica QWin software (Leica, Wetzlar, Germany).

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