

Supplementary methods

Data analysis

Raw sequences were processed using a modified version of the pipeline proposed by Logares ¹, described above. Reads were first analyzed for error correction using the algorithms based on Hamming graphs and Bayesian subclustering (BAYES HAMMER tool) ² implemented in SPAdes v3.5.0 ³. Then a quality check was performed using `fastq_filter` in USEARCH-v10 ⁴. Reads that passed the quality control were analyzed using UNOISE2 ⁵ to define operational taxonomic units (OTUs) with no clustering (zero-radius OTUs [zOTUs]) ⁶. The zOTU table was created with the function `otutab` in USEARCH-v10 ⁷. OTUs identified as contaminants from the sequencing controls were also removed. Finally, taxonomy assignment of zOTUs was done by BLAST ⁸, using the SILVA database (SSU Ref 132 NR 99) as a reference. zOTUs ≤ 10 reads were removed.

References

1. Logares R. ramalok/amplicon_processing: Workflow for Analysing MiSeq Amplicons based on Uparse. 2017 DOI: <http://doi.org/10.5281/zenodo.259579>
2. Nikolenko SI, Korobeynikov AI, Alekseyev MA. BayesHammer: Bayesian clustering for error correction in single-cell sequencing. *BMC Genomics*. 2013;14(Suppl 1):S7.
3. Nurk S, Bankevich A, Antipov D, et al. Assembling Single-Cell Genomes and Mini-Metagenomes From Chimeric MDA Products. *Journal of Computational Biology*. 2013 Oct;20(10):714–37.
4. Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics*. 2015 Nov;31(21):3476–82.
5. Edgar RC. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*. 2016 DOI: <https://doi.org/10.1101/081257>
6. Edgar RC. Updating the 97% identity threshold for 16S ribosomal RNA OTUs. *Bioinformatics*. 2018 Jul;34(14):2371–5.
7. Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics*. 2015 Nov;31(21):3476–82.
8. Altschul SF, Gish W, Miller W, et al. Basic local alignment search tool. *Journal of Molecular Biology*. 1990 Oct;215(3):403–10.

Supplementary material.

Supplementary S1.

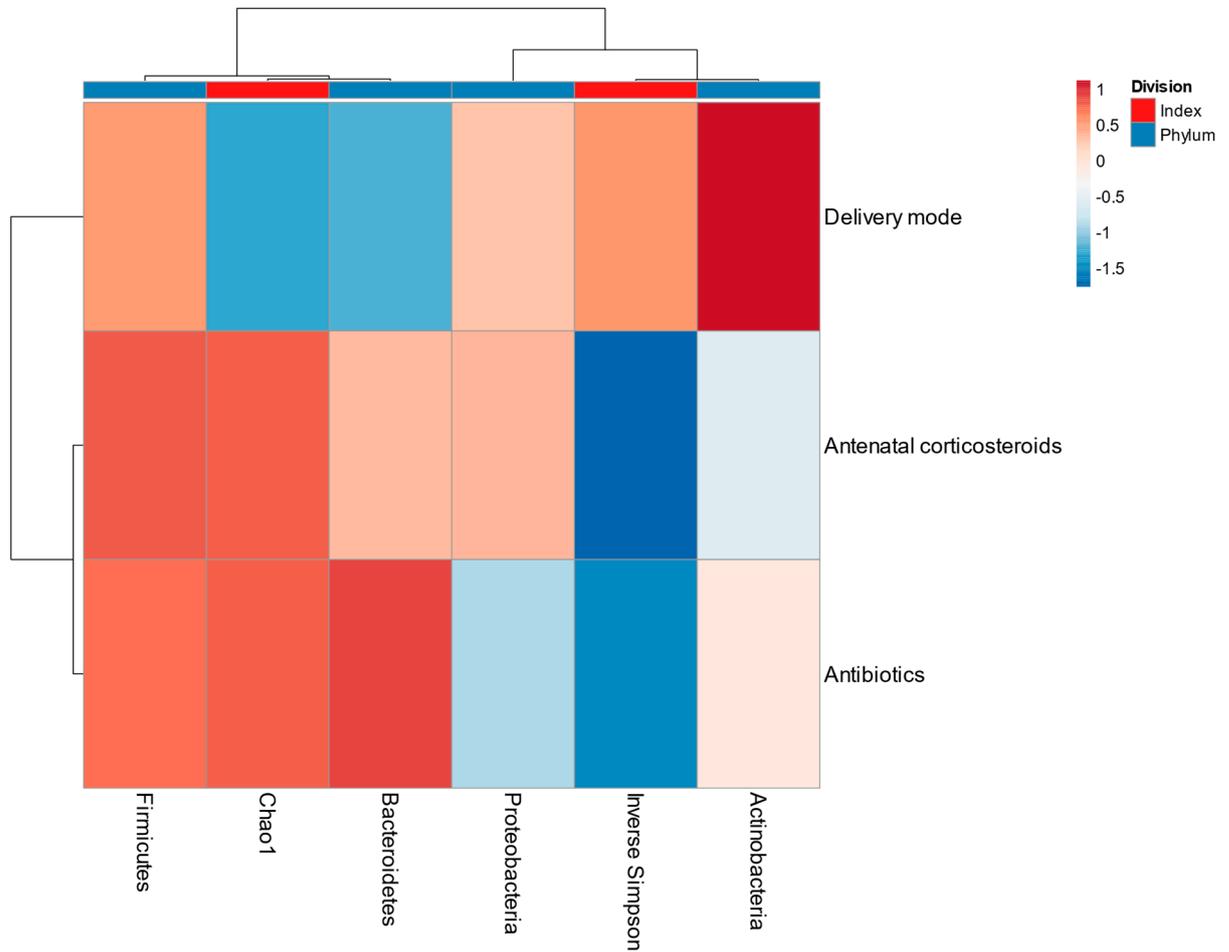


Figure S1. Clinical data association with milk microbiota composition at phylum and alpha diversity indices. Delivery mode: Cesarean section/Vaginal. Prenatal administration of antibiotics and corticoids: Yes/No. β coefficients of univariate associations with linear regression are visualized for taxa abundance and alpha diversity index value. * $p < 0.05$, ** $p < 0.001$.

Table S1. Regression coefficients and significances of clinical data association with milk microbiota at phylum, genera levels, and alpha diversity indices.

	β	p value
Delivery mode vs. Actinobacteria phylum	0.124	0.484
Delivery mode vs. Bacteroidetes phylum	-0.154	0.384
Delivery mode vs. Firmicutes phylum	0.052	0.771
Delivery mode vs. Proteobacteria phylum	0.021	0.904
Delivery mode vs. Chao1 index	-0.167	0.351
Delivery mode vs. Inverse Simpson index	0.057	0.749
Delivery mode vs. Shannon index	-0.005	0.976
Antenatal Corticosteroids vs. Actinobacteria phylum	-0.079	0.659
Antenatal Corticosteroids vs. Bacteroidetes phylum	0.063	0.663
Antenatal Corticosteroids vs. Firmicutes phylum	0.137	0.441
Antenatal Corticosteroids vs. Proteobacteria phylum	0.065	0.716
Antenatal Corticosteroids vs. Chao1 index	0.133	0.455
Antenatal Corticosteroids vs. Inverse Simpson index	-0.259	0.125
Antenatal Corticosteroids vs. Shannon index	-0.064	0.718
Antibiotics vs. Actinobacteria phylum	-0.004	0.984
Antibiotics vs. Bacteroidetes phylum	0.171	0.334
Antibiotics vs. Firmicutes phylum	0.139	0.538
Antibiotics vs. Proteobacteria phylum	-0.154	0.383
Antibiotics vs. Chao1 index	0.152	0.391
Antibiotics vs. Inverse Simpson index	-0.262	0.134
Antibiotics vs. Shannon index	-0.121	0.495

Table S2. Clinical data of the mother-child pair

Parameter	Preterm	Term	P value
Number of samples	24	12	Not applicable
Number of mothers	12	12	Not applicable
Days postpartum, 1st sample	8 ± 3	8 ± 3	0,799 ²
Days postpartum, 2nd sample	22 ± 9	Not applicable	Not applicable
Relation vaginal/Caesarean section	3:09	4:08	Not applicable
% Caesarean section	75 (9:12)	67 (8:12)	Not applicable
Gestational age (weeks)	33,4 ± 1,2	38,3 ± 1,2	0,000 ¹
Neonatal weight (g) at birth	1968 ± 308	2925 ± 484	0,000 ¹
Neonate Sex (M/F)	6:06	5:07	Not applicable
Age (years) of mothers	25,7 ± 7,5	29,1 ± 8,2	0,300 ¹
Allergies of mothers (Yes / No)	5:07	4:07	Not applicable
Diabetes before pregnancy (Yes / No)	0:12	1:11	Not applicable
Hypo / Hyper Thyroidism (Yes / No)	2:10	1:11	Not applicable
Anemia during pregnancy (Yes / No)	2:10	2:10	Not applicable
Gestational diabetes (Yes / No)	2:10	2:10	Not applicable
Antenatal corticosteroids (Yes / No)	11:01	2:10	Not applicable
Antibiotics (Yes / No)	10:02	7:05	Not applicable
Active smoker during pregnancy (Yes / No)	2:10	2:10	Not applicable
Passive smoker during pregnancy (Yes / No)	5:07	3:09	Not applicable

¹ Student's T-test for independent samples for parametric data

² Mann-Whitney U test for non-parametric data

* p <0.05, significant