Supplementary materials

Supplementary method

Sample collection

We enrolled 19 healthy pregnant women who vaginally delivered healthy newborns at full term. For each mother, we sampled the fecal samples, gut microbiota, and breast milk. Each infant was sampled for feces from paper diapers at 42 days postpartum. All infants were exclusively breastfed. Before milk sample collection, the maternal gland was sterilized with 75% alcohol, and the first drops were discarded to minimize contamination [1]. Spot infant fecal samples were collected from paper diapers on a super clean bench. Maternal fecal samples were stored in a plastic box. Maternal and infant fecal and breastmilk specimens were stored in plastic containers at 4 °C in home refrigerators until they were brought to the study clinic no more than 24 h after collection. At the study clinic, samples were frozen at -80 °C until analysis.

For each type of sample, sIgA coated microbiota was enriched by streptavidin magnetic particles. All samples were characterized by 16S rRNA gene amplicon sequencing of the V3-V4 region.

DNA extraction

Bacterial genomic DNA was extracted using a reaped bead-beating method using an Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech, Shanghai, China) with a minor modification as previously reported [2]. Briefly, cell lysis was achieved by bead beating with zirconium beads (0.1 g, 0.7 mm: 0.3 g, 0.15 mm) on an oscillator at 6,000 rpm with two circulations (30 s per circulation; Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France) in the presence of 4% (w/v) sodium dodecyl sulphate, 500 mM NaCl, and 50 mM ethylenediaminetetraacetic acid. Ammonium acetate was used to precipitate and remove impurities, and sodium dodecyl sulphate in addition to isopropanol precipitation was used for nucleic acid recovery. RNA and proteins were removed or degraded using RNase and Proteinase K, respectively, followed using an Ezup Column Bacteria Genomic DNA Purification Kit. Genomic DNA concentration was determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and DNA integrity was determined by electrophoresis on 1% agarose gels. The DNA concentration for all samples was standardised to 10 ng/μL.

16S rRNA gene sequencing

Microbial profiles were analysed by 16S rDNA sequencing at GENEWIZ, Inc.

(Suzhou, China). To maximise the effective length of the MiSeq 250PE and 300PE sequencing reads, a region of approximately 469 bp encompassing the V3 and V4 hypervariable regions of the 16S rRNA gene was targeted for sequencing. The PCR primers used to amplify V3 and V4 hypervariable regions were as follows: forward 5'-CCT ACG GRR BGC ASC AGK VRV GAA T -3' and reverse 5'- GGA CTA CNV GGG TWT CTA ATC C -3'. In addition, an indexed linker was added to the end of the 16S rDNA PCR product for next-generation sequencing (Illumina, San Diego, CA, USA). First-round PCR products were used as templates for the second round of PCR amplicon enrichment (94 °C for 3 min, followed by 24 cycles at 94 °C for 5 s, 57 °C for 90 s, and 72 °C for 10 s, and a final extension at 72 °C for 5 min). PCR reactions were performed in triplicate using a 25 μL mixture containing 2.5 μL of TransStart Buffer, 2 μL of dNTPs, 1 μL of each primer, and 20 ng of template DNA. DNA library concentration was validated using a Qubit 3.0 Fluorometer. The libraries were quantified to 10 nM, and subsequently multiplexed and loaded on an Illumina MiSeq instrument according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Sequencing was performed using PE250/300 paired-end; image analysis and base calling were performed using the MiSeq Control Software embedded in the MiSeq instrument.

The 16s rRNA sequencing resulted in 21,318 ASVs, of which 20,817 (97.65) was assigned to genus level using Kraken2 based on the MiniKraken2_v1_8GB database. There were 10,072 ASVs classified to species level (52.75%).

Reference

1 R. Albesharat, M. A. Ehrmann, M. Korakli, S. Yazaji and R. F. Vogel, *Syst. Appl. Microbiol.*, 2011, **34**, 148–155.

2 M. Ding, C. Qi, Z. Yang, S. Jiang, Y. Bi, J. Lai and J. Sun, *Food Funct.*, 2019, **10**, 554–564.