SDC-Methods

**Bacterial community DNA in stool**

Genomic DNA was extracted from stool samples by a bead-beating method (29) incorporated in a Stool DNA kit protocol (Qiagen Inc., Valencia, CA, USA) for efficient and uniform recovery of the Gram positive bacterial DNA, along with other bacteria in stool. Since extended bead-beating resulted in DNA sheering, the technique was optimally effective at 5 minutes with quality DNA yield (Qubit standards). In brief, a 200-mg aliquot of each sample was suspended (while frozen) in 1 ml of ASL-buffer and incubated at 70°C for 5 min. To the resulting homogenate, 100 mg of 0.1 mm zirconia beads (Biospec Products Inc., Bartlesville, OK, USA) was added to lyse all bacterial cells by mechanical disruption with extensive bead beating for 5 min at room temperature. The above lysate (supernatant) was processed further using inhibitex-capsules and proteinase buffer to eliminate PCR inhibitors in stool. DNA was then column purified as per manufacturer’s protocol (Qiagen Inc., Valencia, CA, USA). DNA samples from 8 synbiotic treated and 3 control infants at two time-points (Day-7 and Day-60) were subjected to 16*S* rRNA gene amplification and DNA sequencing on a 454/Roche instrument to examine the probiotic mediated alterations in gut bacterial diversity and their relative abundance.

**Sample preparation for 454-analysis**

DNA purified from the stool samples was subjected to 16*S* rRNA gene amplicon sequencing using universal primer pairs [(forward-27f: AGAGTTTGATCMTGGCTCAG) and (reverse- 518r: WTTACCGCGGCTGCTGG)] known to amplify the relevant loci from all bacteria (except Archaea) (30). The reverse primer with a unique barcode was used to tag PCR amplicons from respective samples. A master DNA pool comprising equimolar ratios of purified PCR-products was sequenced using the Roche 454-Pyrosequencer.

**References:**

29. Li F, Hullar MA, Lampe JW. Optimization of terminal restriction fragment polymorphism (TRFLP) analysis of human gut microbiota. *J Microbiol Methods* 2007; 68:303-11.

30. Okubo T, Ikeda S, Yamashita A, et al. Pyrosequence read length of 16S rRNA gene affects phylogenetic assignment of plant-associated bacteria. *Microbes Environ* 2012; 27:204-8.