Appendix S1.

SUPPLEMENTARY METHODS

Quantitative PCR

Microbial gDNA was used as template for qPCR amplification with SYBR Green using the Bio-Rad CFX Connect apparatus (Bio-Rad, Hercules, CA, USA). One reaction contained 5 µl gDNA, 12.5 µl SYBR Green, 1.5 µl of each forward and reverse primer (10 µM) and 4.5 µl sterile water. Strain-specific quantitative PCR primers were designed manually on unique (i.e. strain-specific) protein-coding DNA sequences that were identified using a customized workflow of in-house built programming scripts (Table SI[1]). Briefly, this workflow determines unique genes by OrthoMCL (S1) on publicly-available reference genomes of the 5 bacterial strains used in this study. The genome sequences are segmented into DNA fragments of 40 nt with a sliding window of 10 nt. Subsequently, these 40 nt sequences were aligned to the 5 reference genomes by BLAST. Only sequences that aligned perfectly with the genome sequence they originated from, and did not align with the other 4 genomes, were retained. Primers were developed manually on the strain-specific 40-bp fragments. Cross-reactivity of candidate primers was: (*i*) validated by BLAST, and (*ii*) by an *in silico* PCR using Primer Prospector (S2) on the BLAST database of the 5 genomes. BRU 16S qPCR primers were adopted and applied as described in Nadkarni et al. (S3). qPCR primers (from Biolegio, Nijmegen, The Netherlands) were used to obtain Ct values. Colony-forming units (CFU)/ml were calculated from Ct values using calibration curves (Fig. S6[1]).

16S amplification prior to sequencing

Microbiota samples derived from skin of the lower back contained small amounts of microbial gDNA. The following universal primers were applied for amplification: forward primer (338F), 5'-ACTCCTACGGGAGGCAGCAG-3'; reverse primer (1061R), 5'-CRRCACGAGCTGACGAC -3'. The PCR mixture contained 5 µl microbial gDNA, 1 µl KOD DNA polymerase (1 U/µl; Novagen, Madison, WI, USA), 5 µl KOD-buffer (10×), 3 µl KOD $MgSO_4$ (25 mM), 5 µl KOD dNTP mixture (2 mM each), 1 µl of each forward and reverse primer (10 µM) and 29 µl sterile water. PCR conditions were: 2 min at 95°C, followed by 30 cycles of 20 s at 95°C, 10 s at 55°C, 15 s 70°C and ending at 70°C for 10 min. The amplicons were purified using the MSB Spin PCRapace kit (Invitek, Westburg, The Netherlands). The concentration of the purified DNA samples was determined with NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

16S metagenomic library preparation and Illumina sequencing

Illumina 16S metagenomic amplicon libraries were generated and sequenced at BaseClear BV (Leiden, The Netherlands). A pre-amplified full-length 16S PCR amplicon, as described above, was used as input for a 2-step PCR-based library preparation protocol targeting the V3-V4 regions of the 16S rRNA gene. The resultant libraries were checked on a Bioanalyzer (Agilent Technologies, Waldbronn, Germany) and quantified. The libraries were multiplexed, clustered and sequenced on an Illumina MiSeq with paired-end 250 cycles protocol and indexing. The sequencing run was analysed with the Illumina CASAVA pipeline (v1.8.3) with demultiplexing based on sample-specific barcodes. The raw sequencing data produced was processed removing the sequence reads of too low quality (only "passing filter" reads were selected) and discarding reads containing adaptor sequences or failing PhiX Control with an in-house filtering protocol. A quality assessment on the remaining reads was performed using the FASTQC quality control tool version 0.10.0.

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