

MATERIALS AND METHODS

DNA Extraction

Total bacterial genomic DNA samples were extracted using the Metagenomic DNA was extracted from all Samples using the **PowerMax (stool/soil) DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA)**, following the manufacturer's instructions, and stored at -20°C prior to further analysis. The quantity and quality of extracted DNAs were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

16S rDNA Amplicon Pyrosequencing

PCR amplification of the bacterial 16S rRNA genes **V4 region** was performed using the forward primer **515F (5'-GTGCCAGCMGCCGCGGTAA-3')** and the reverse primer **806R (5'-GGACTACHVGGGTWTCTAAT-3')**. Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 25 μl of Phusion High-Fidelity PCR Master Mix, 3 μl (10 μM) of each Forward and Reverse primer, 10 μl of DNA Template, and 6 μl of ddH₂O. Thermal cycling consisted of initial denaturation at 98°C for 30 s, followed by 25 cycles consisting of denaturation at 98°C for 15 s, annealing at 58°C for 15 s, and extension at 72°C for 15 s, with a final extension of 1 min at 72°C . PCR amplicons were purified with Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2×300 bp sequencing was performed using the Illumina HiSeq4000 platform at GUHE Info technology Co., Ltd (Shanghai, China).

Sequence Analysis

The Quantitative Insights Into Microbial Ecology (QIIME, v1.9.0) pipeline was employed to process the sequencing data, as previously described (Caporaso, Kuczynski *et al.* 2010). Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. The low-quality sequences were filtered through following criteria (Gill, Pop *et al.* 2006, Chen and Jiang 2014): sequences that had a length of <150 bp, sequences that had average Phred scores of <20 , sequences that contained ambiguous bases, and sequences that contained mononucleotide repeats of >8 bp. Paired-end reads were assembled using FLASH (Magoc and Salzberg 2011). Operational taxonomic unit (OTU) picking using Vsearch v1.11.1, included dereplication(--derep_fulllength), cluster(--cluster_fast, --id 0.97), detection of chimeras(--uchime_ref) (Rognes 2016). A representative sequence was selected from each OTU using default parameters. OTU taxonomic classification was conducted by VSEARCH searching the representative sequences set against the **SILVA128 database (Quast *et al.* 2013)**.

An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of these OTUs. OTUs containing less than 0.001% of total sequences across all samples were discarded. To minimize the difference of sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under the 90% of the minimum sequencing depth for further analysis.

Bioinformatics and Statistical Analysis

Sequence data analyses were mainly performed using QIIME and R packages (v3.2.0). OTU-level alpha diversity indices, such as Chao1 richness estimator, ACE metric (Abundance-based Coverage Estimator), PD_whole_tree, Shannon diversity index, and Simpson index, were calculated using the OTU table in QIIME. OTU-level ranked abundance curves were generated to compare the richness

and evenness of OTUs among samples. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using UniFrac distance metrics (Lozupone and Knight 2005, Lozupone, Hamady *et al.* 2007) and visualized via principal coordinate analysis (PCoA), nonmetric multidimensional scaling (NMDS) (Ramette 2007).

Differences in the Unifrac distances for pairwise comparisons among groups were determined using Student's t-test and the Monte Carlo permutation test with 1000 permutations, and visualized through the box-and-whiskers plots. Principal component analysis (PCA) was also conducted based on the genus-level compositional profiles (Ramette 2007). The significance of differentiation of microbiota structure among groups was assessed by PERMANOVA (Permutational multivariate analysis of variance) (McArdle and Anderson 2001) using R package "vegan". The taxonomy compositions and abundances were visualized using MEGAN (Huson, Mitra *et al.* 2011) and GraPhlAn (Asnicar, Weingart *et al.* 2015). Venn diagram was generated to visualize the shared and unique OTUs among samples or groups using R package "VennDiagram", based on the occurrence of OTUs across samples/groups regardless of their relative abundance (Zaura, Keijser *et al.* 2009). Taxa abundances at the phylum, class, order, family, genus and species levels were statistically compared among samples or groups by Kruskal.test from R stats package. LEfSe (Linear discriminant analysis effect size) was performed to detect differentially abundant taxa across groups using the default parameters (Segata, Izard *et al.* 2011). Random forest analysis was applied to discriminating the samples from different groups using the R package "randomForest" with 1,000 trees and all default settings (Breiman 2001, Liaw and Wiener 2002). The generalization error was estimated using 10-fold cross-validation. The expected "baseline" error was also included, which was obtained by a classifier that simply predicts the most common category label. Co-occurrence analysis was performed by calculating Spearman's rank correlations between predominant taxa. Correlations with $|\text{RHO}| > 0.6$ and $P < 0.01$ were visualized as co-occurrence network using Cytoscape (Shannon, Markiel *et al.* 2003). Microbial functions were predicted by PICRUSt (Phylogenetic investigation of communities by reconstruction of unobserved states), based on high-quality sequences (Langille, Zaneveld *et al.* 2013). The output file was further analysed using Statistical Analysis of Metagenomic Profiles (STAMP) software package v2.1.3 (Parks *et al.*, 2014). The β diversity on taxonomy and functions based on Meta-Storms distances were generated by Parallel-META 3 (version 3.3.2) (Jing *et al.*, 2017).

Data Access

All raw sequences were deposited in the NCBI Sequence Read Archive under accession number SRP*****.

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