Microbiome profiling

NGS library preparations and Illumina MiSeq sequencing were conducted by GENEWIZ, Inc. (Suzhou, China). For the library preparation, a library sequence of the V3 and V4 regions of 16S rRNA was constructed using a 10ng DNA aliquot isolated from each digestive juice sample. The DNA obtained from digestive juice was used to amplify the V3-V4 region of 16S rRNA genes. After purification, the amplicons were equally combined and subjected to a sequencing library preparation for 454 GS FLX pyrosequencing. A total of 2545641 sequences were subjected to quality control standards. Sequences had to meet the following criteria[1]: (1) no Ns in the trimmed sequence, (2) an exact match to the 5' primer, (3) Lucy's identified region of poor quality at the 0.002 threshold did not extend beyond the 5' primer. The 5' primer was trimmed from the sequences before analysis. Any sequences that did not meet a length requirement from 180 to 280 bases after trimming were discarded. The remaining 2039953 sequences were subjected to the next analysis.

Microbiome bioinformatics analysis was performed using the Quantitative Insights Into Microbial Ecology (QIIME) software package, version 1.9[1], 35791 OTUs at the similarity level of 97% were obtained. The most abundant sequence of each OTU was selected as the representative sequence and assigned taxonomy using RDP classification software with a bootstrap cutoff of 50%. The abundance data of representative sequences were normalized for each sample and log-transformed. We calculated the relative abundance from phylum level to genus level for each collection method. α-diversity indices (Chao 1 index and Shannon index) and β-diversity Bray-Curtis distances were calculated using the R GUniFrac package[2].

Metabolomics profiling

The sample extracts were analyzed using an LC-ESI-MS/MS system[3] by Wuhan Metware Biotechnology Co., Ltd. The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm*100 mm); column temperature, 40℃; flow rate, 0.4 mL/min; injection volume, 2μL; solvent system, water (0.04% acetic acid): acetonitrile (0.04% acetic acid); gradient program, 95:5 V/V at 0 min, 5:95 V/V at 11.0 min, 5:95 V/V at 12.0 min, 95:5 V/V at 12.1 min, 95:5 V/V at 14.0 min.

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: source temperature 500℃; ion spray voltage (IS) 5500 V (positive), -4500 V (negative); ion source gas I (GSI), gas II (GSII), curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

Statistical analysis

We performed t-tests to compare the differences in the microbial α-diversity indices (Shannon index and Chao-1 index) across two juice collection methods. PERMANOVA with Bray-Curtis dissimilarity and principal-coordinate analysis (PCoA) were carried out for the

microbial β-diversity analyses. Square-root transformation was conducted for the relative abundance of taxonomic units before analysis[4]. For untargeted metabolomics, metabolite values were first normalized using Quantile normalization for each method and then Log10 transformation was performed before analyses. All data in this study were represented as mean ± SEM. Differences between two groups were determined by Student's two-tailed t-test. When more than two groups were examined, one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test for multiple comparison. The non-parametric ANOSIM test was used for independent variables. Post hoc tests were run only if F achieved $P<0.05$ and there was no significant variance inhomogeneity. P value < 0.05 was considered significant. All statistical analyses were performed with SPSS software, version 19.0.

Reference

1. Wang W, Zhao J, Gui W, et al. Tauroursodeoxycholic acid inhibits intestinal inflammation and barrier disruption in mice with non-alcoholic fatty liver disease. *Br J Pharmacol.* 2018;175(3):469-484.
2. Zhai B, Ola M, Rolling T, et al. High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. *Nat Med.* 2020;26(1):59-64.
3. Ortmayr K, Dubuis S, Zampieri M. Metabolic profiling of cancer cells reveals genome-wide crosstalk between transcriptional regulators and metabolism. *Nat Commun.* 2019;10(1):1841.
4. Nakatsu G, Li X, Zhou H, et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun.* 2015;6:8727.