Electronic Supplementary Material (ESI)

Supplementary material

16S rRNA gene sequencing by pyrosequencing

Barcoded primer designs were completed using a set of algorithms developed at JCVI. PCR reactions were performed in 50 µL mixtures containing 25 µL 2× rTaq Mix (TaKaRa), 1.0 µL of each primer, 1.0 µL DNA template, and 22 µL sterile water. The PCR cycling conditions were as follows: initial denaturation for 10 min at 94°C, followed by 28 cycles of 30 s at 94°C, 30 s at 50°C, and 50 s at 72°C, and a final extension step at 72°C for 5 min. Negative controls were performed to verify the lack of amplification without DNA template.

The final PCR product was quantified on a Qubit fluorometer (Life Technology, Grand Island, NY, United States) and the quality of the amplicon regions was assessed using a Fragment Analyzer (Advanced Analytics, Ames, IA, United States) to confirm amplicon regions and sizes. DNA samples were pooled in equal amounts according to concentration. Pooled samples were then size-selected on a 2% agarose gel (Life Technologies, Grand Island, NY, United States) and extracted using the Qiagen gel purification kit (Qiagen, Valencia, CA, United States).

DNA amplicon libraries were prepared using the TruSeq DNA LT Sample Preparation Kit (Illumina, catalog no. FC-121-4001) and were sequenced on the MiSeq Illumina platform using the MiSeq v3 Reagent Kit (600 cycles-PE, Illumina, catalog no. MS-102-3001) according to standard protocols.

Prediction of functional profiling by PICRUSt2

The functional profiles from 16S rRNA data were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) v.2.1.3-b software, which predicts gene family abundance. Using the ASV table (also called a feature table) from the bacterial community analysis (100% AV clustering; DADA2) and representative sequences, predicted functional profiles were obtained using the PICRUSt2 script with default options (picrust2_pipeline.py). We then inferred the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway abundances from the predicted KEGG ORTHOLOGY (KO) abundances with the "–no_regroup" option (pathway_pipeline.py). Moreover, we also obtained the EC table and pathway table based on the metabolic pathway database (MetaCyc). Based on the ko number, the KEGG hierarchy was downloaded, and the format was altered. PICRUSt2-related statistical analyses were performed using STAMP software (version 2.1.3).

Supplementary Figures

Fig. S1 The effects of two *Lactobacillus* strains on URT microbiome biodiversity.
Fig. S2 The effects of two *Lactobacillus* strains on the key URT taxa.
Fig. S3 Absolute abundance of KEGG pathways at Level 2.

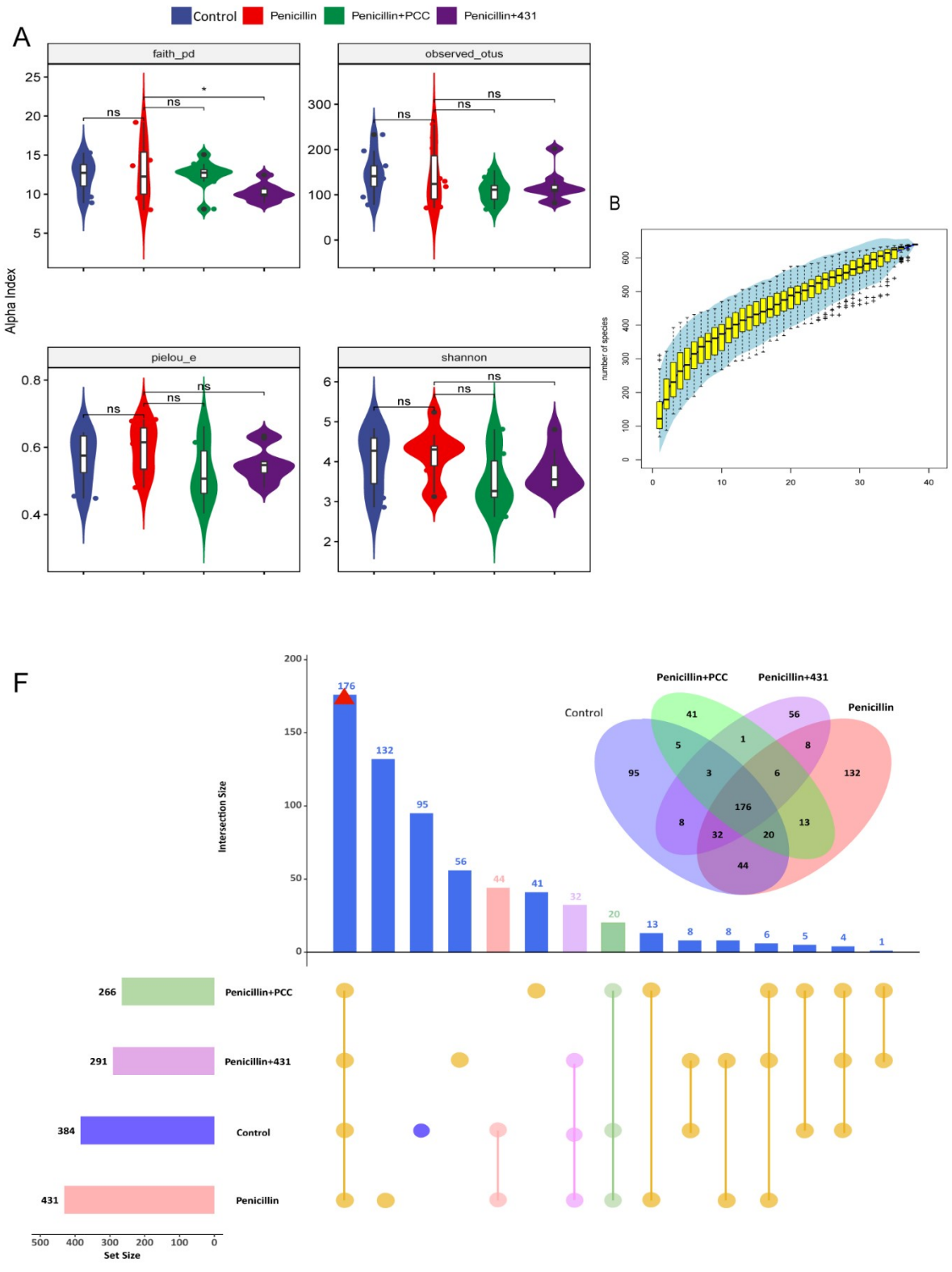

Fig. S1 The effects of two probiotic strains on URT microbiome biodiversity.
(A) The two strains altered the URT microbiome alpha diversity index, faith PD, observed OTUs, Pielou evenness, and Shannon index. ns represents no significant differences, asterisks represent P-values as calculated by Wilcoxon test (*P<0.05) and global test by the Kruskal-Wallis test.
(B) Accumulation curves of all samples revealed the richness.
(C) Venn diagram of the total number of URT samples shared among four groups.

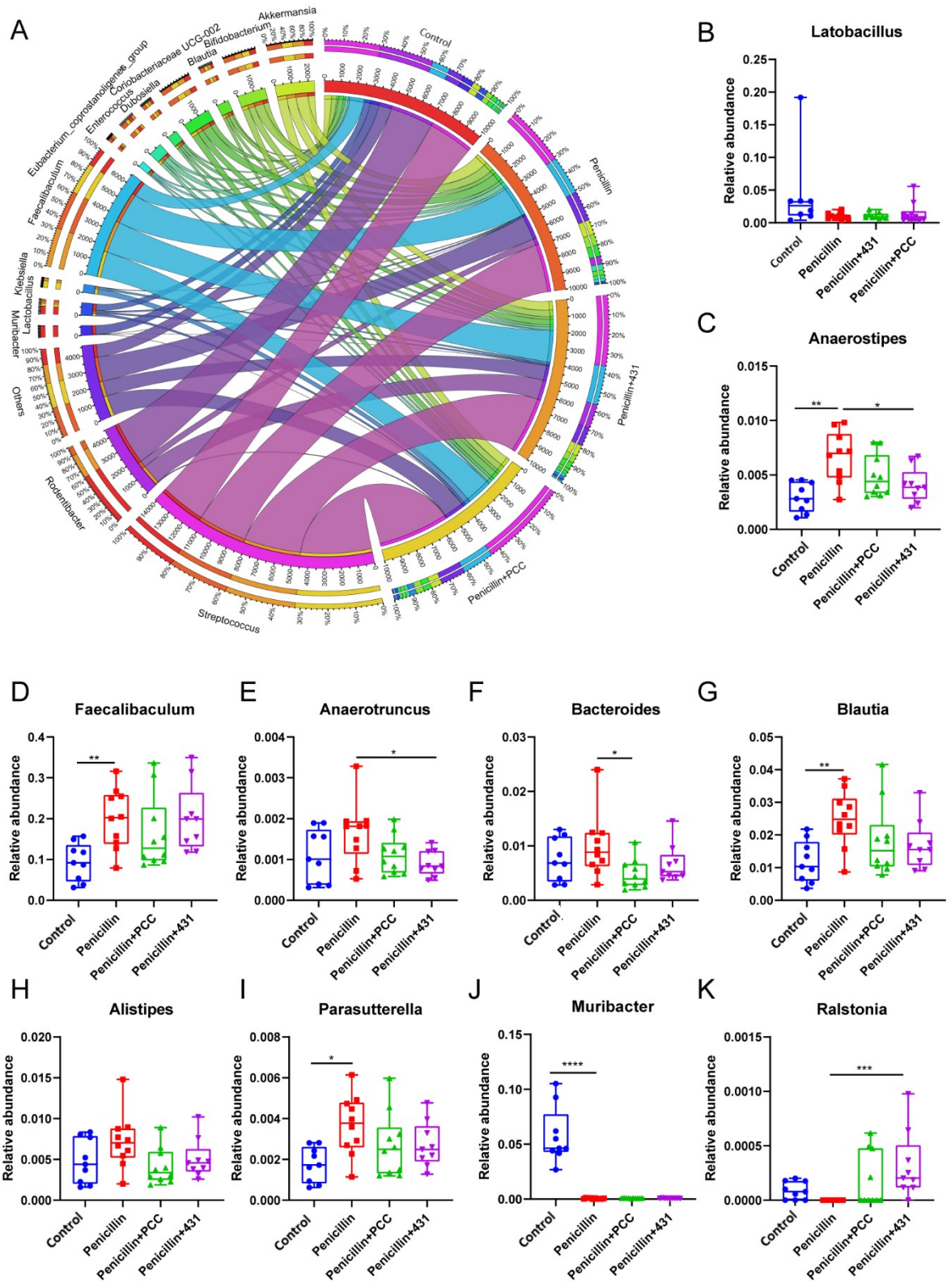

Fig. S2 The effects of two *Lactobacillus* strains on the key taxa of URT microbiome

(A) Circos of groups and genera: a visual circle diagram describing the correspondence between groups and genera. This graph indicates that *Lactobacillus* strains alter the URT microbiome at the genus level.

(B-K) *Lactobacillus* strains alter the URT microbiome at the genus level, and alters genera such as *Lactobacillus*, *Faecalibaculum*, *Anaerotuncus*, *Bacteroides*, *Blautia*, *Alistipes*, *Parasutterella*, *Muribacter*, and *Ralstonia*.

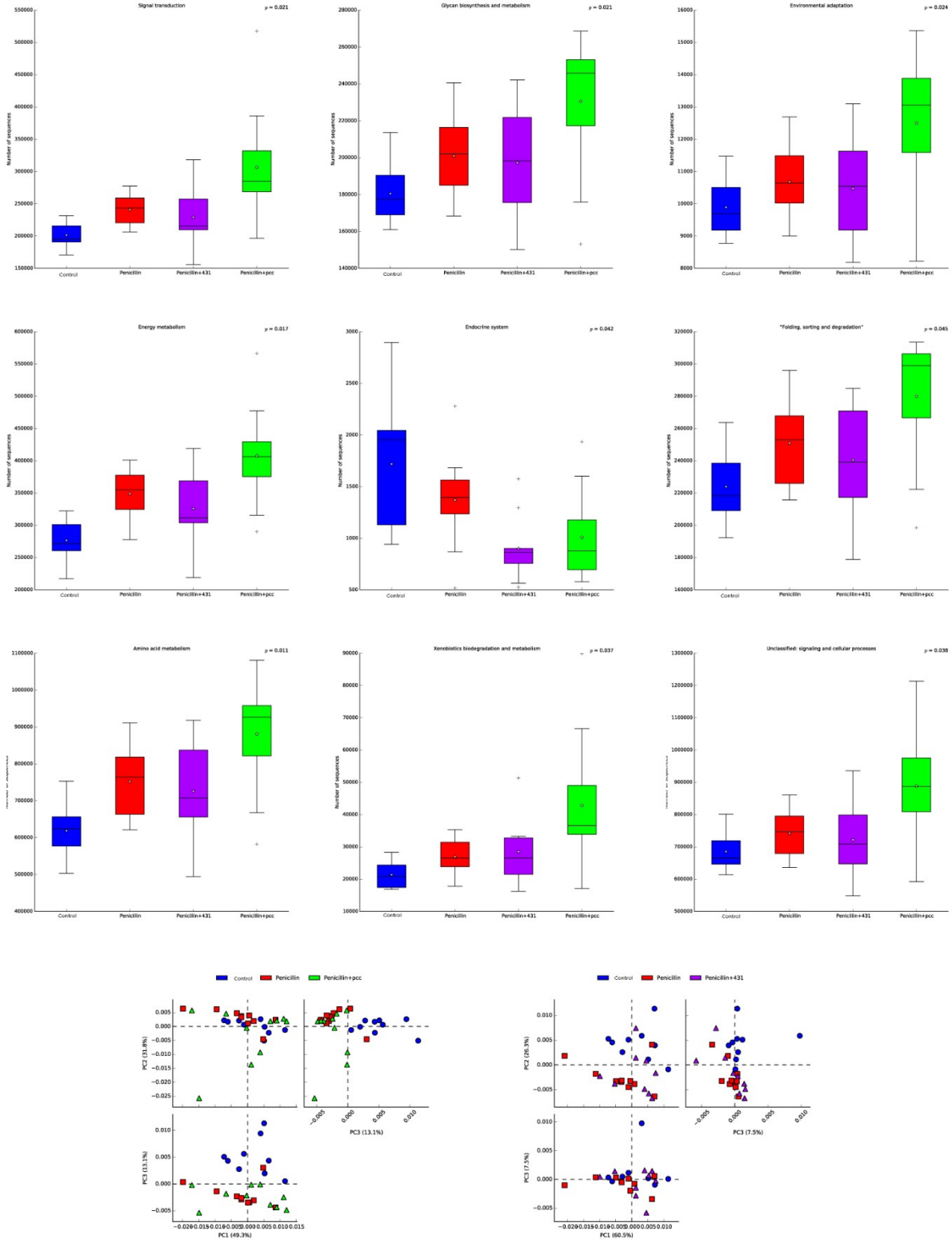

Fig S3. Functional prediction. (A-I) Absolute abundance of the KEGG pathways at Level 2 by PICRUSt2; (J-K) PCA of the administration of the two strains; boxplot represents the KEGG pathway differences among groups at Level 2.