

Supporting information

Supplementary Fig. S1-6

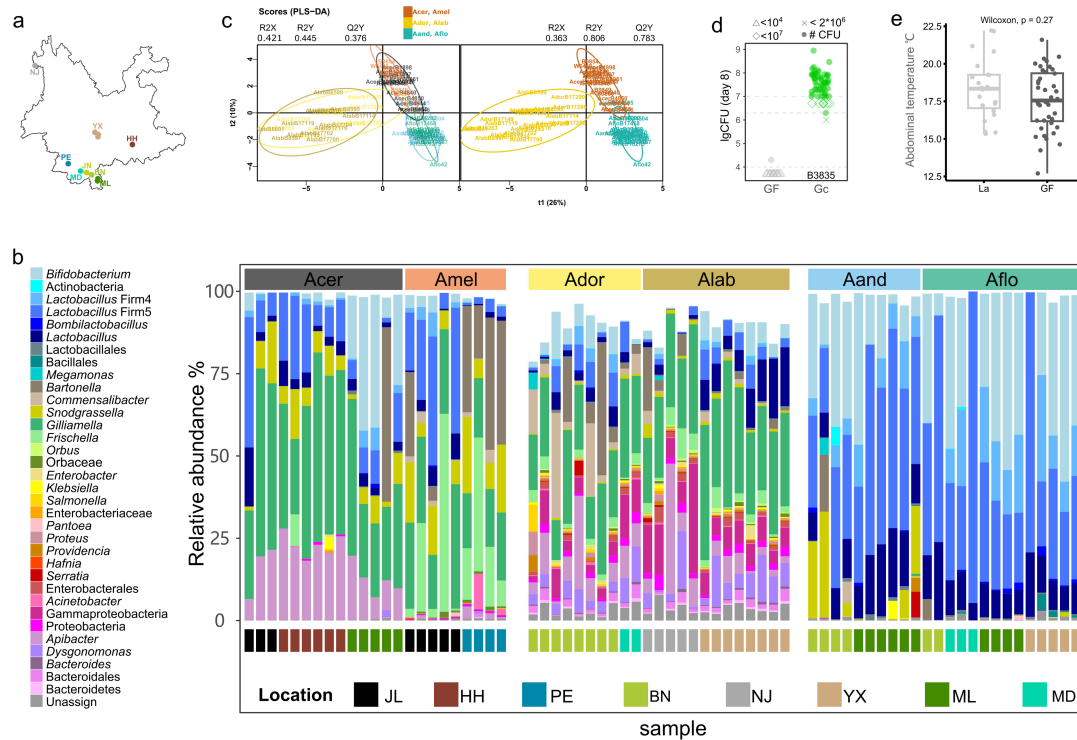


Fig. S1. Gut microbiota of six *Apis* honeybee species and *Gilliamella* gnotobiotic colonization for *in vivo* assays.

(a) Honeybees and colonies were sampled in multiple counties in Yunnan Province, China. (b) Bacterial community composition across six honeybee species. JL, Jiling; JN, Jinuo; HH, Honghe; PE, Pu'er; BN, Banna; NJ, Nujiang; YX, Yuxi; ML, Mengla; MD, Mandian. (c) Partial least-squares discrimination analysis (PLS-DA) of gut microbiota. The left and right plots show the same data, colored by honeybee species and subgenus, respectively. R2X and R2Y denote the proportions of variance explained in X and Y matrices; Q2Y indicates the model's predictive ability, with higher Q2Y reflecting stronger discrimination among subgenera. Acer, *A. cerana*; Amel, *A. mellifera*; Ador, *A. dorsata*; Alab, *A. laboriosa*; Aand, *A. andreniformis*; Aflo, *A. florea*. (d) Colony-forming unit count on day 8 post-inoculation. (e) Abdominal temperature comparison after 30 minutes' cold exposure. La, bees colonized by *Lactobacillus* strain B24606 that was isolated from *A. andreniformis*; GF, germ-free bees.

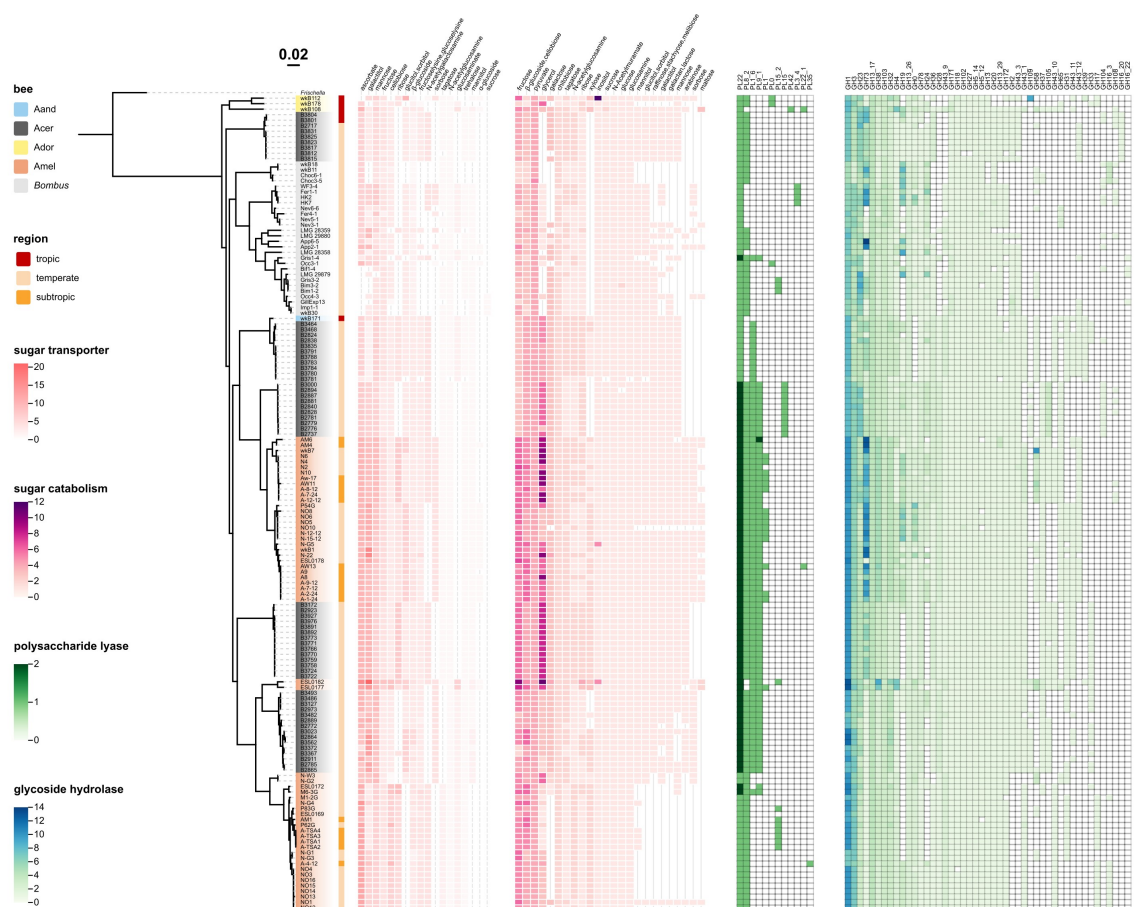


Fig. S2. Saccharide metabolic gene repertoires in *Gilliamella*.

A maximum-likelihood phylogenetic tree of *Gilliamella* strains, with tip colors indicating host bee species and adjacent labels denoting the geographic region for the hosts. From left to right, the heatmaps denote the number of genes for sugar transporters, sugar catabolism, polysaccharide lyases (PL) and glycoside hydrolases (GH).

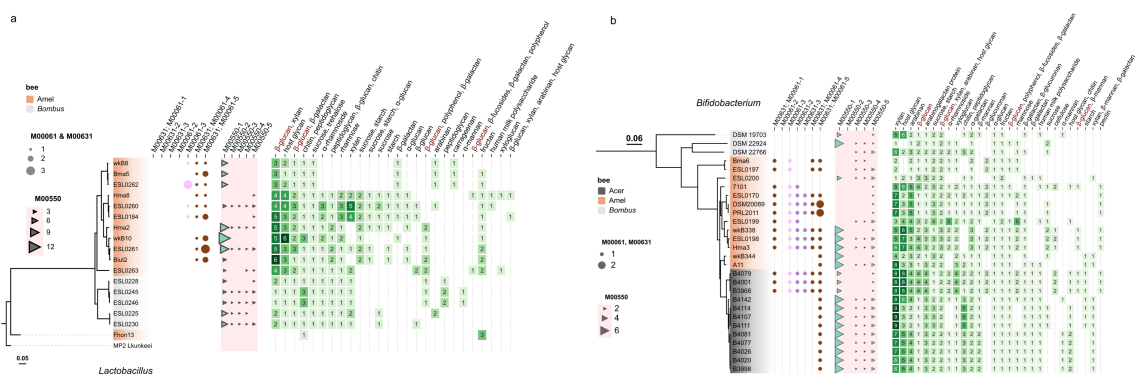


Fig. S3. Carbohydrate-degradation gene repertoires in (a) *Lactobacillus* and (b) *Bifidobacterium*.

Maximum-likelihood phylogenetic trees with tip colors indicating the host bees. Adjacent heatmaps (left to right) show the number of genes for the glucuronate degradation module (KEGG M00061), the galacturonate degradation module (M00631), the ascorbate degradation module (M00550), and the enzymes degrading varied polysaccharide substrates.

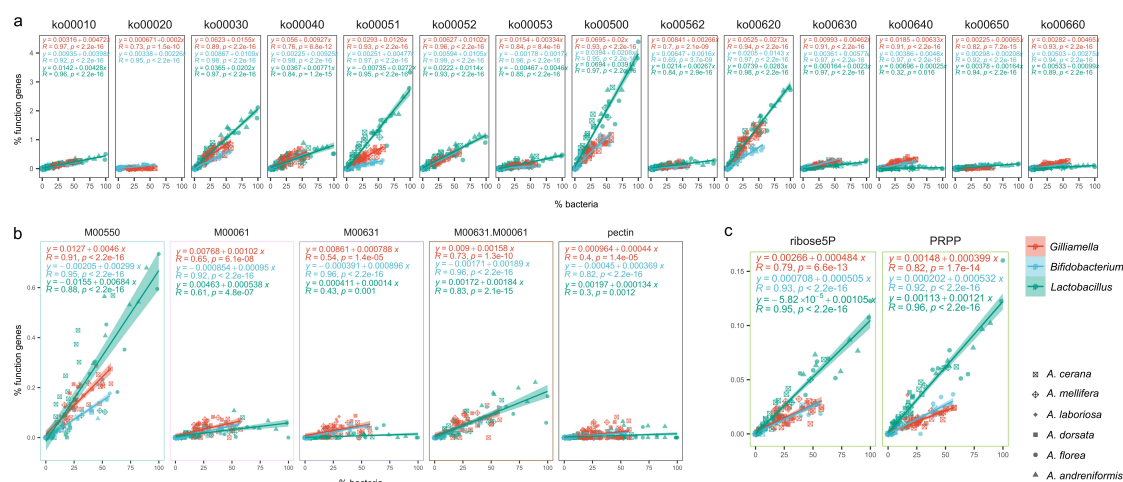


Fig. S4. Correlation of carbohydrate metabolism and bacterial abundance for the three core bacteria revealed from the gut metagenomes of six *Apis* honeybee species.

Pearson correlation of bacterium abundance associated with its function proportion of (a) carbohydrate metabolism pathway, (b) degradation modules, and (c) 5-phosphoribosyl diphosphate (PRPP) generation. Each point represents one gut metagenome sample.

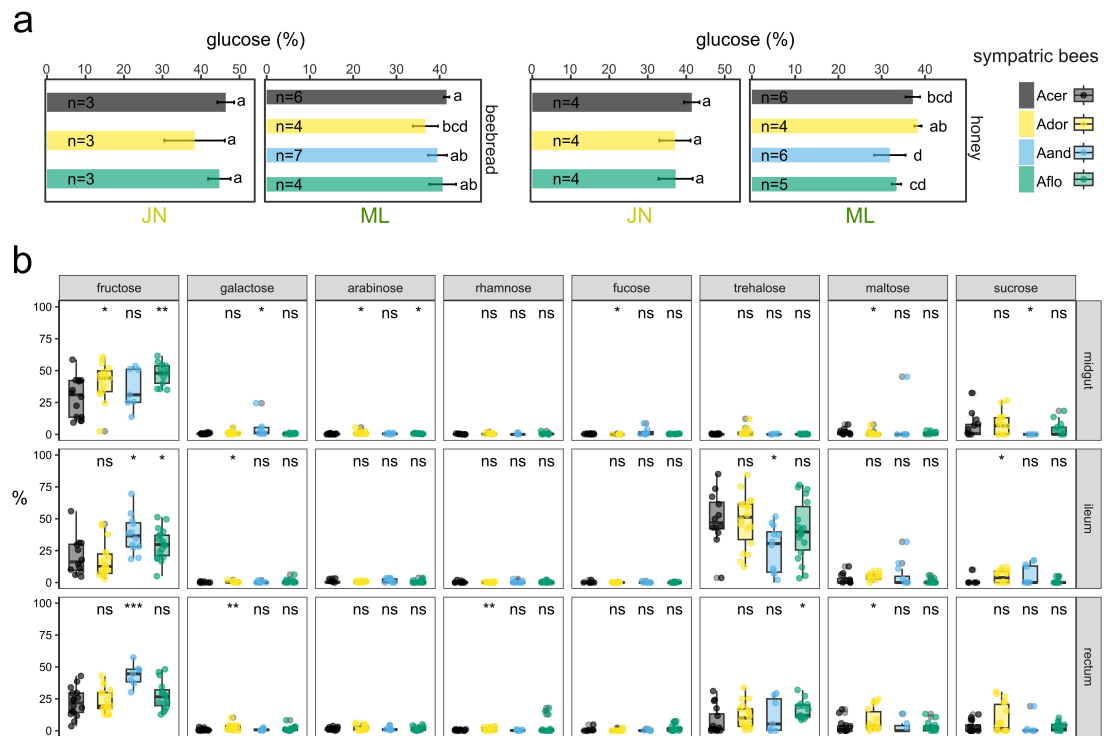


Fig. S5. Saccharide metabolomics for bee diets and gut contents.

(a) Glucose proportion in the honeybee diets shows no significant difference. (b) Sugar proportions in the guts of four sympatric honeybees show no significant difference between cold-adapted *A. cerana* (Acer) and tropical bees *A. dorsata* (Ador), *A. andreniformis* (Aand) and *A. florea* (Aflo). ns: no significant difference, *P-value < 0.05, **P-value < 0.01, ***P-value < 0.001 by Mann-Whitney U test.

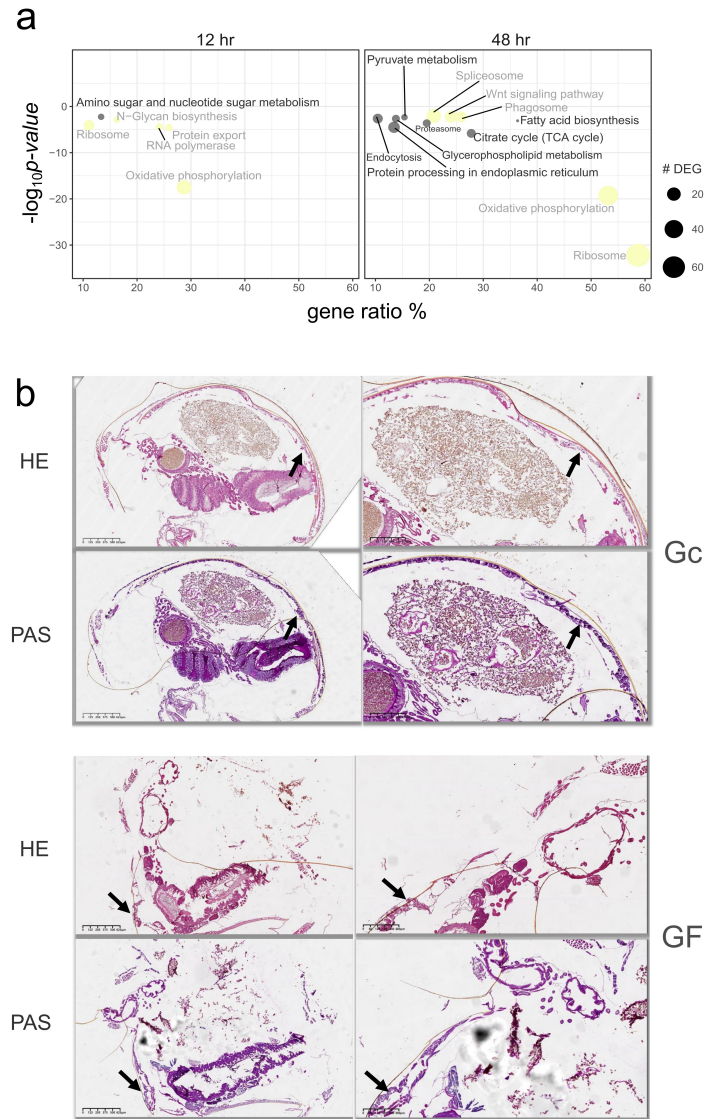


Fig. S6. The host transcriptome and lipogenesis are affected by *Gilliamella* inoculation.

(a) Pathway enrichment of differentially expressed genes (DEGs) in *Gilliamella*-inoculated *A. mellifera*, highlighting the up-regulated carbohydrate utilization. The transcriptome was examined at 12- and 48-hours post-inoculation. (b) Histological analysis of fat bodies. HE (hematoxylin and eosin) and PAS (Periodic-Acid Schiff) were used for cell and glycogen staining, respectively. The black arrows denote fat body cells, which formed a cell layer attached to the exoskeleton in *Gilliamella*-colonized (Gc) *A. mellifera*, but were sporadically found in germ-free (GF) ones.

Supplementary Data 1. Excel file containing the sample information and source data

Supplementary Data 2. Excel file containing the source data for saccharide examination

Supplementary Movie 1. Cold resistance assay, related to Fig. 2.

Scripts and intermediate files related to transcriptome analysis (pathway enrichment) are available at <https://github.com/Sustainable-to-bee-Lab/honeybee-Gilliamella-metabolic-synergy>.