

Supplementary Information

Strain-level profiling with picodroplet microfluidic cultivation reveals host-specific adaption of honeybee gut symbionts

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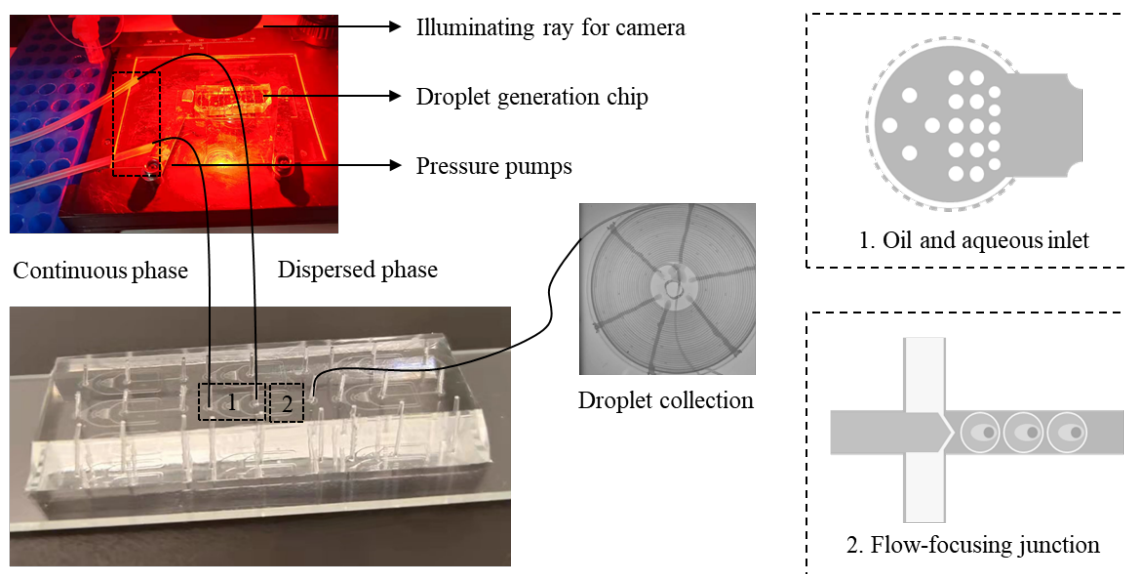


Figure S1. Devices for microfluidic droplet generation. The microfluidic droplets of bacterial cells were generated by the droplet entrapping microfluidic cell-sorter integrating with two pressure pumps. The chip consists of two inlets for continuous and aqueous phases and one outlet for collecting the water-in-oil emulsions. The two liquids confined within microfluidic channels are brought together at the flow-focusing junction, and the generated droplets were collected in a Teflon tube.

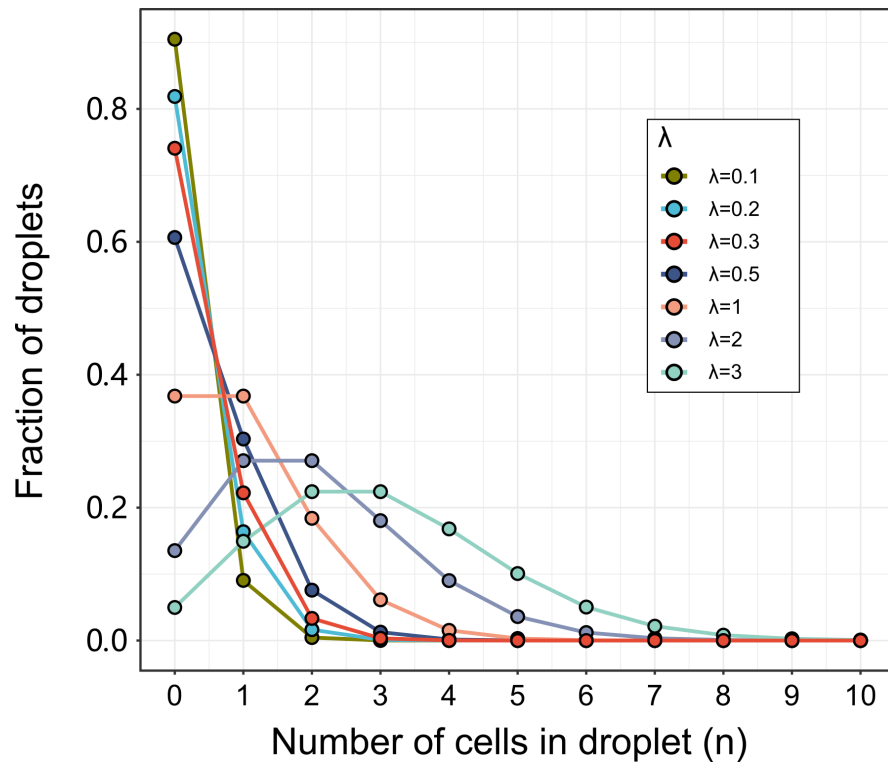


Figure S2. Poisson distribution curve. When the average number of cells per droplet (λ) is determined, the distribution of the number of bacteria in the droplet conforms to the Poisson distribution.



Figure S3. The generated droplets are introduced into Teflon tubes for incubation.

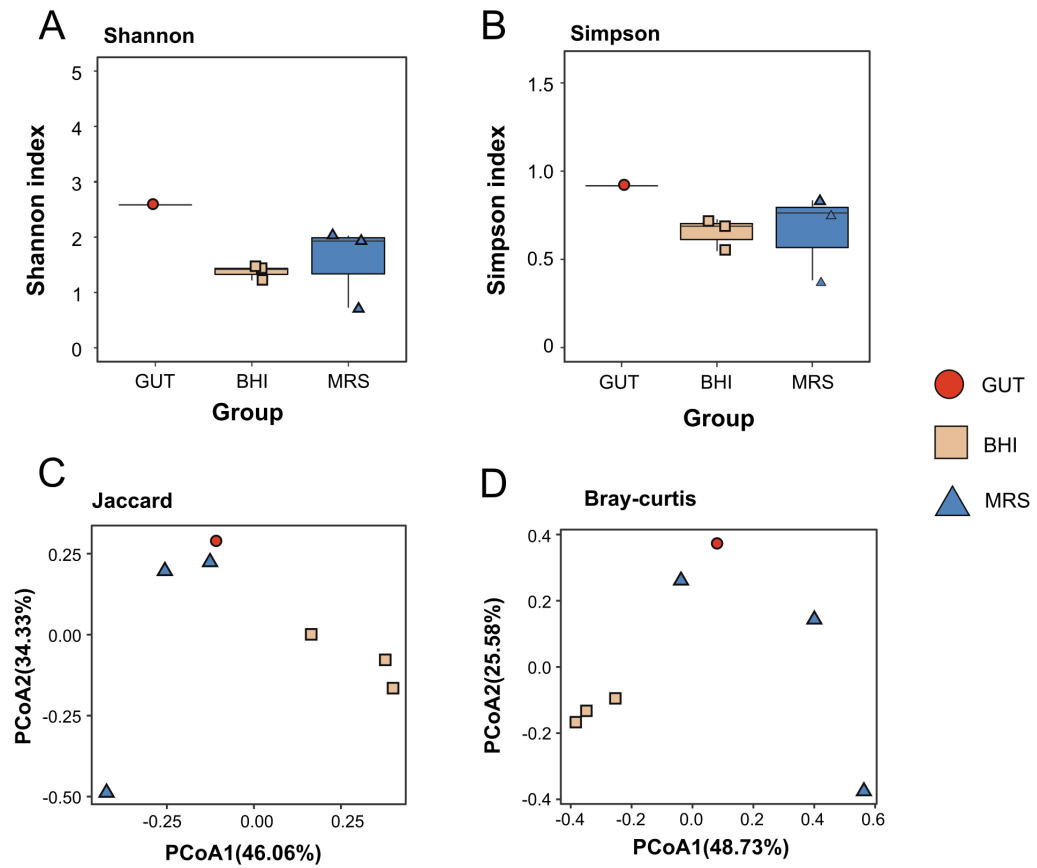


Figure S4. (A-B) The Shannon **(A)** and Simpson **(B)** diversity metrics of the data sets. **(C-D)** Microbiome compositions in different developmental stages are plotted on Jaccard **(C)** and Bray-Curtis **(D)** PCoA graphs.

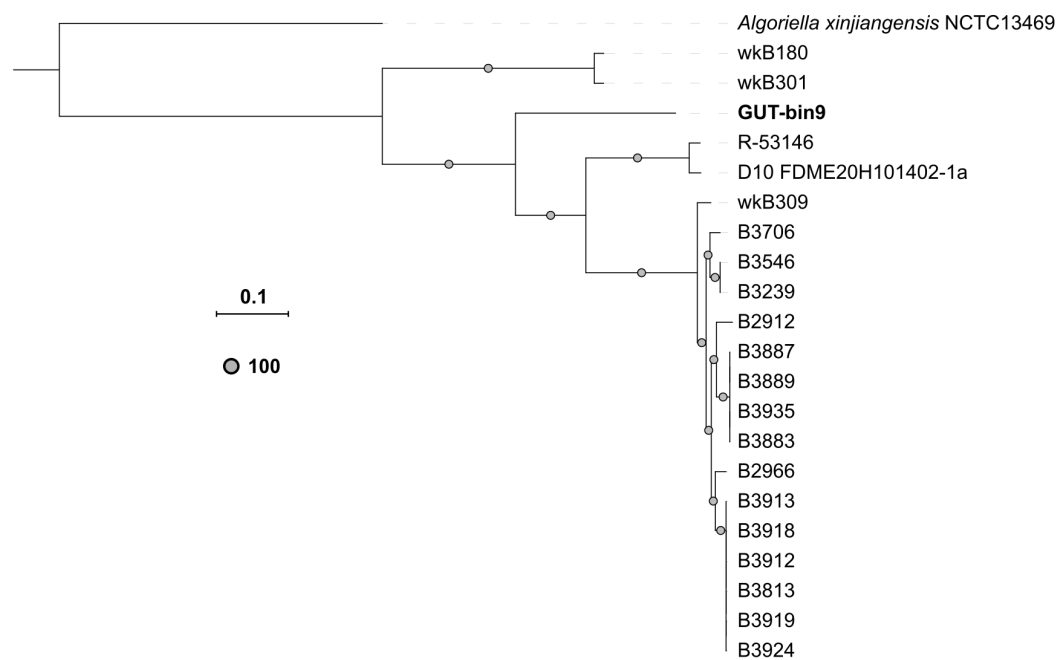


Figure S5. Whole-genome phylogenetic tree based on MAGs and representative isolates' genomes from *Apibacter*.

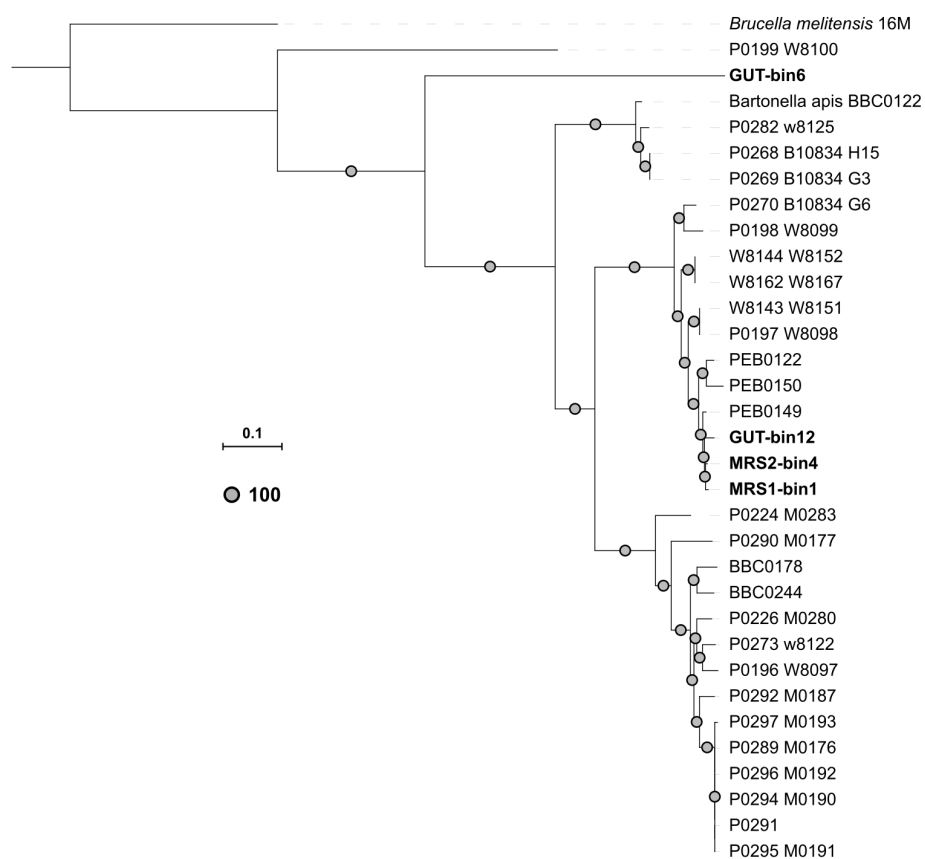


Figure S6. Whole-genome phylogenetic tree based on MAGs and representative isolates' genomes from *Bartonella*.

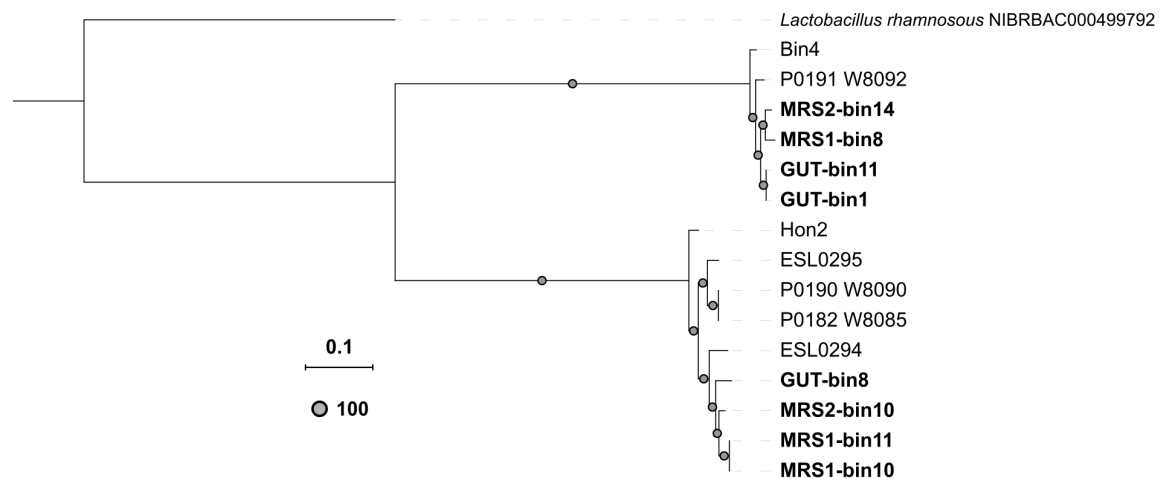


Figure S7. Whole-genome phylogenetic tree based on MAGs and representative isolates' genomes from *Lactobacillus* Firm4.



Figure S8. Whole-genome phylogenetic tree based on MAGs and representative isolates' genomes from *Lactobacillus kunkeei*.

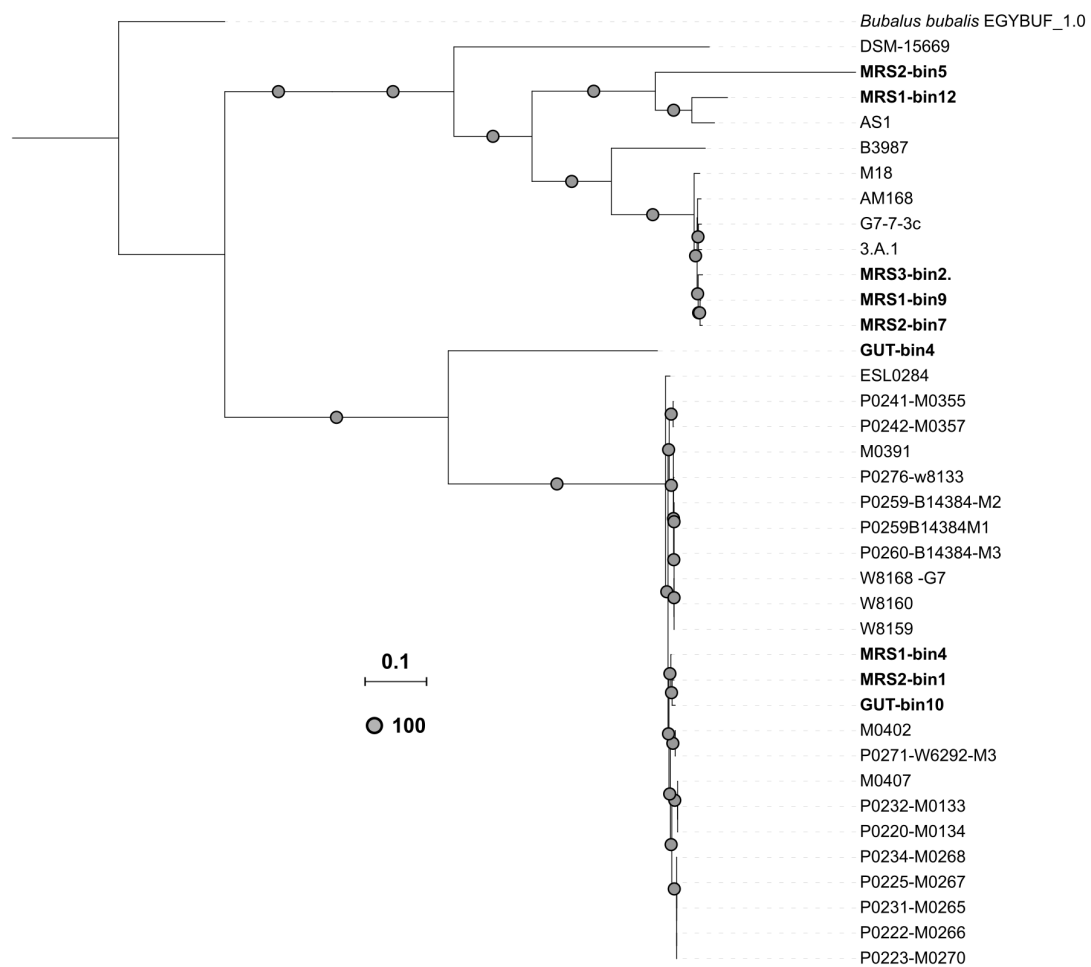


Figure S9. Whole-genome phylogenetic tree based on MAGs and representative isolates' genomes from *Acetobacteraceae*.

Table S1. The bacterial concentration is required to generate a given volume of droplets.

Diameter of droplets (μm)	The volume of droplets (pL)	Bacterial concentration (CFU/mL)
10	0.523	5.736×10^8
15	1.766	1.699×10^8
20	4.533	6.618×10^7
25	8.177	3.669×10^7
30	14.13	2.123×10^7
40	33.493	8.957×10^6

Additional files:

Additional file 1: Dataset S1. Information of metagenome-assembled genomes.

Additional file 2: Dataset S2. Reports for homologous gene cluster analysis of the novel cluster from *Bifidobacterium*.

Additional file 3: Dataset S3. Reports for homologous gene cluster analysis of *Lactobacillus panisapium*.

Additional file 4: Video S1. Generation of microfluidic droplets.

Additional file 5: Video S2. Morphologies of gut bacteria in droplets after cultivation.