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Supplemental information

Microbiome assembly on *Drosophila* body surfaces benefits the flies to combat fungal infections

Song Hong, Yanlei Sun, Dapeng Sun, and Chengshu Wang

Supplemental information

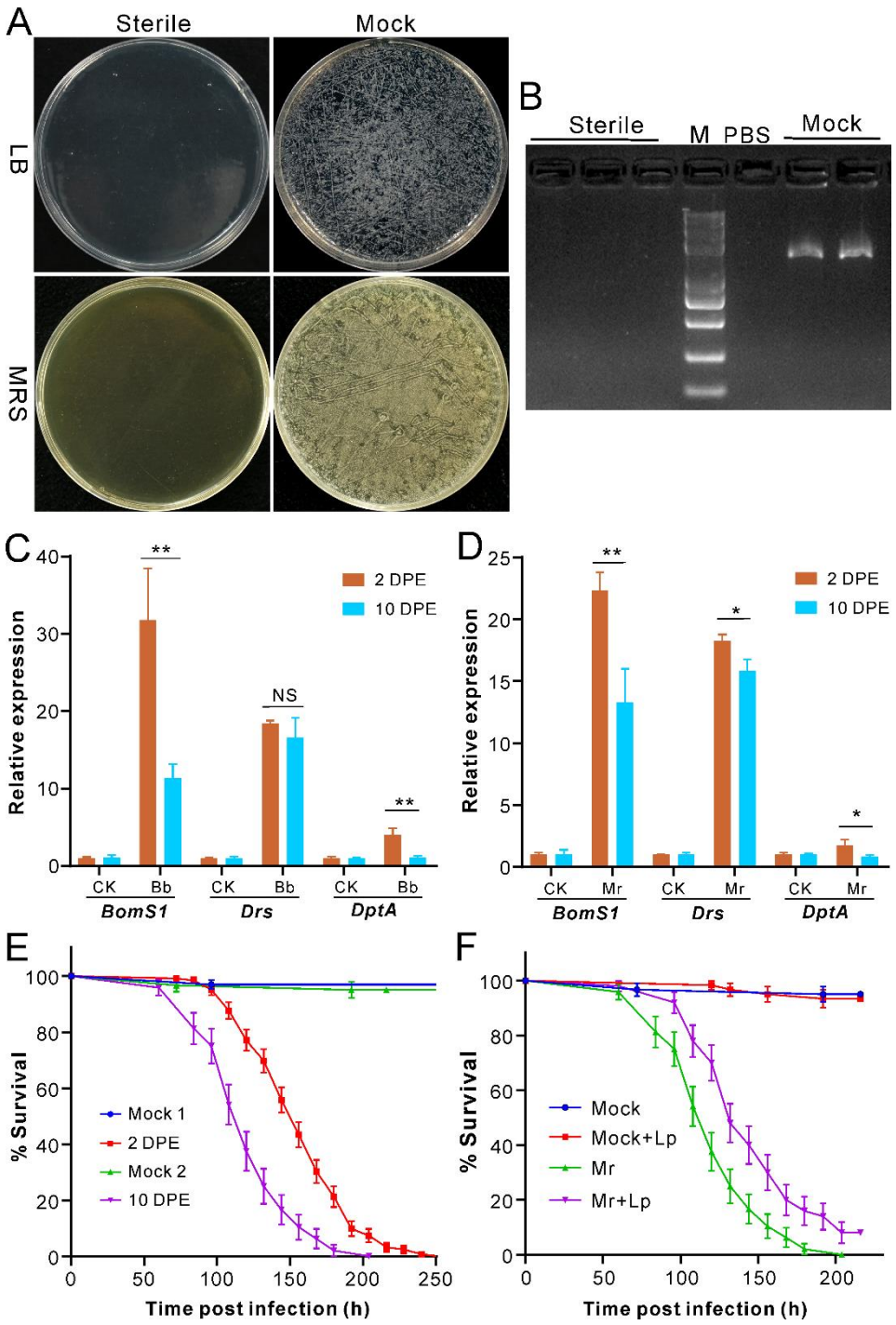

Figure S1. Topical infections of different ages of sterile flies, Related to Figure 1.

(A) Plating verification of the obtained axenic flies. The 10-DPE sterile flies and those conventionally reared (Mock) were homogenized in sterile PBS buffer and plated on LB medium (up panels) for two days and MRS medium for three days.

(B) PCR verification of the obtained aexnic flies. The 10-DPE sterile flies (five per sample) and those conventionally reared (Mock) were washed with sterile PBS buffer, and the buffer was directly used for PCR checks with the universal primers.

(C, D) qRT-PCR analysis of the antimicrobial gene expressions after topical infection of the 2- (C) and 10-DPE (D) axenic female flies with *B. bassiana* and *M. robertsii* for 48 hrs. The respective flies treated with 0.05% Tween 20 were used as mock controls. Values are mean ± SD.

(E) Survival of the different ages of axenic flies against the topical infection of *M. robertsii*. Mocks 1 and 2 represent the 2- and 10-DPE sterile flies treated with 0.05% Tween 20, respectively.

(F) Survival of the 10-DPE axenic flies infected by *M. robertsii* with and without the addition of *L. plantarum* (Lp) cells. The 10-DPE sterile flies treated with 0.05% Tween 20 and Lp cells were included as mock controls. Plotted values are mean ± SEM.

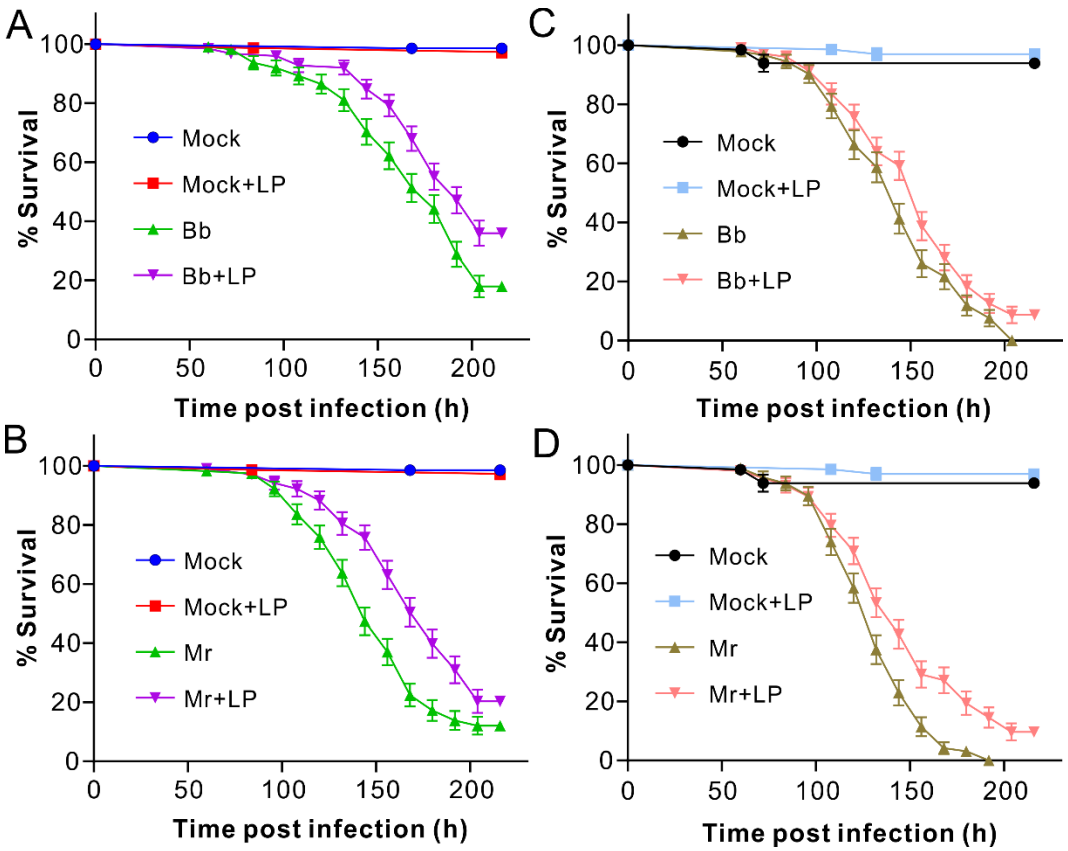

Figure S2. Repeated gnotobiotic assay of fly survivals, Related to Figure 2.

(A, B) Survival of the 2-DPE axenic flies infected by *B. bassiana* (Bb, A) and *M. robertsii* (Mr, B) with and without the addition of *L. plantarum* (Lp) cells.

(C, D) Survival of the 2-DPE axenic flies untreated and pre-treated with the Lp cells for topical infection of *B. bassiana* (C) and *M. robertsii* (D). The sterile flies were treated with the cells of Lp for 24 hours prior to the fungal topical infections.

The germ-free flies only treated with 0.05% Tween 20 and Lp cells were included as mock controls. Plotted values are mean ± SEM. More than 70 flies were used for each treatment. The log-rank test results of survival difference between the treatments of the fungal spores and fungal spores plus Lp cells are at: Panel A, $\chi^2 = 11.29$, $P = 0.0008$; Panel B, $\chi^2 = 13.67$, $P = 0.0002$; Panel C, $\chi^2 = 5.46$, $P = 0.019$; Panel D, $\chi^2 = 16.63$, $P < 0.0001$.

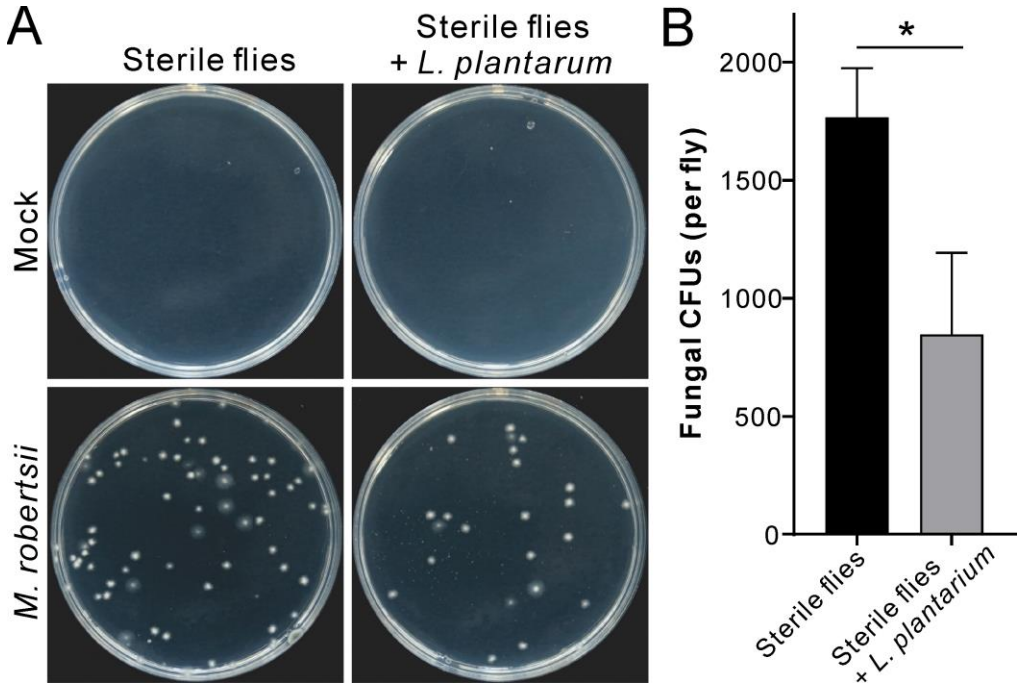

Figure S3. Quantification of fungal loads in 10-DPE flies after different treatments, Related to Figure 2.

(A) Fungal colony formation on PDA medium for three days. The 10-DPE sterile female flies after treatments for 96 hours were homogenized and plated on PDA amended the antibiotic mixes for three days.

(B) Comparison of the fungal colony formation units (CFUs) after treating the 10-DPE sterile flies with *M. robertsii* spores with and without the addition of *L. plantarum* cells. Values are mean ± SD. The two-tailed Student's *t*-test was conducted: *, $P < 0.05$.

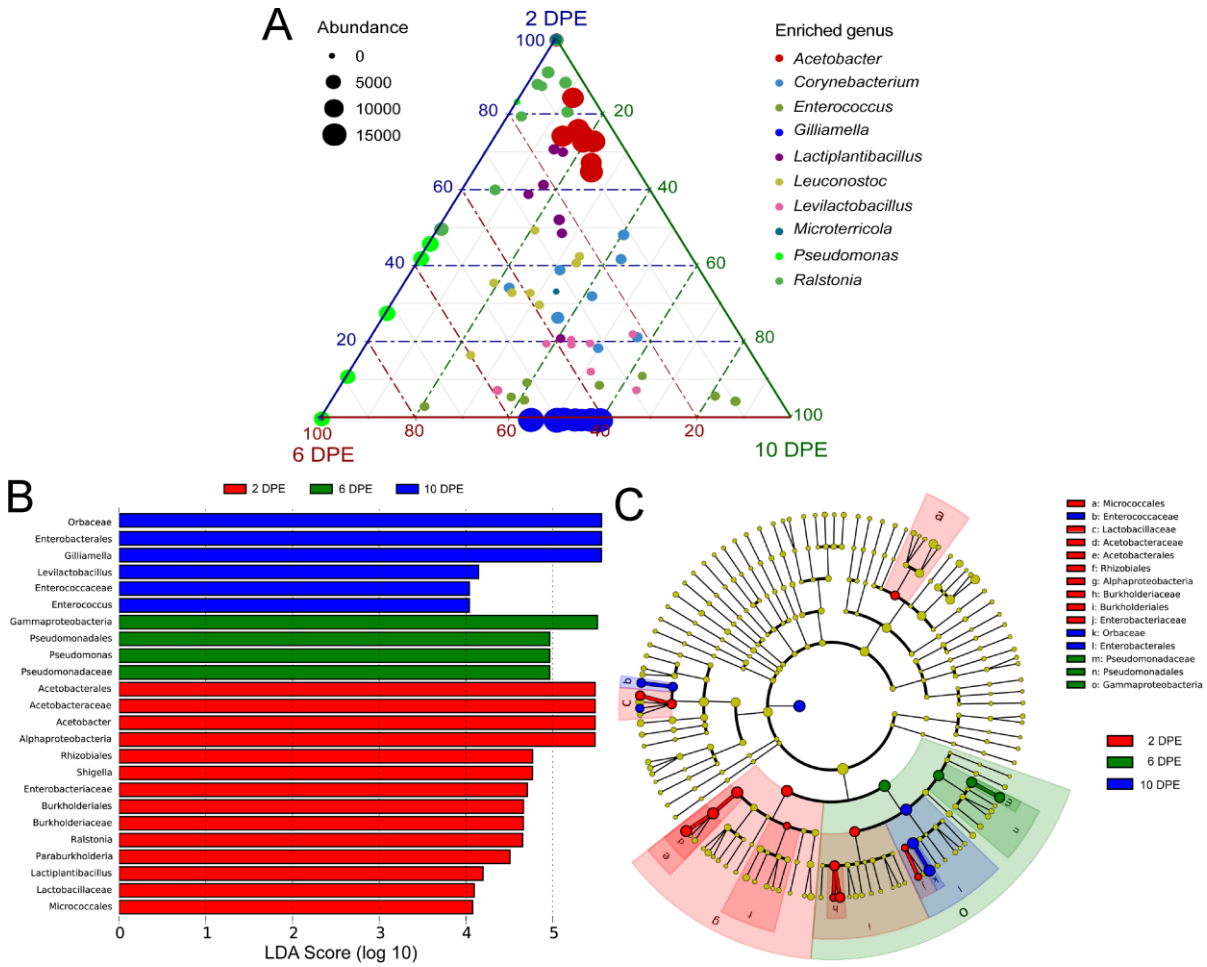

Figure S4. Ternary plotting and LEfSe analysis of the predominant bacterial lineages of the age-associated *Drosophila* surface microbiomes, Related to Figure 5.

(A) Ternary plot of the enriched bacterial genera detected on the body surface of the flies different days post eclosion (DPE).

(B) Identification of the most differentially abundant taxa of three microbiomes.

(C) Cladogram of significant changes at the taxonomic levels among three microbiomes. The size of the node represents the taxon abundance.

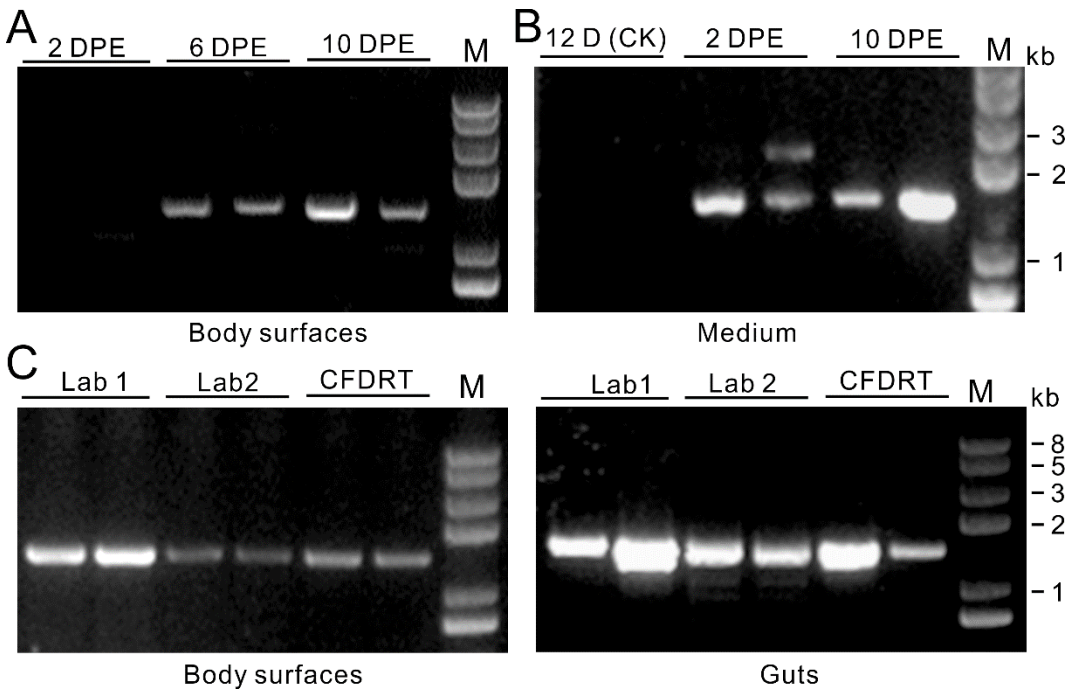

Figure S5. PCR verification of the *Gilliamella*-like bacterium detected in this study, Related to Figure 5.

(A) PCR verifications using the DNA templates extracted from the bacteria washed off from different age of flies. The bacteria washed from the 2-, 6- and 10 DPE (days post eclosion) flies were used for DNA extractions as the templates for PCR reactions.

(B) PCR verification using the DNA templates extracted from the medium with and without the rearing of flies. 12 D (CK), DNA template extracted from the medium kept in the same growth camber without flies for 12 days; 2 DPE, DNA template extracted from the medium after fly eclosion for two days; 10 DPE, DNA template extracted from the medium after fly eclosion for 10 days.

(C) PCR verification using the DNA templates extracted from the body surface and gut bacterial samples collected from the *Drosophila* W1118 line reared in different labs. Different labs: lab1, our own lab; lab2, Dr. Erjun Ling's lab, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences (CAS); CFDRT, Core Facility of Drosophila Resource and Technology, Shanghai Institute of Biochemistry and Cell Biology, CAS. The specific primers were designed from the 16S *rRNA* gene of *G. apcolia*.

Table S1. Culture-dependent isolation of the *Drosophila* body-surface bacteria, Related to Figures 1 and 4.

Clones	Top hit by 16S rDNA Blast	Gram
Two days post eclosion		
FLY2D-1	*Lactiplantibacillus plantarum*	G+
FLY2D-2	*Leuconostoc mesenteroides*	G+
FLY2D-3	*Lactiplantibacillus plantarum*	G+
FLY2D-4	*Lactiplantibacillus brevis*	G+
FLY2D-5	*Lactiplantibacillus plantarum*	G+
FLY2D-6	*Leuconostoc pseudomesenteroides*	G+
FLY2D-7	*Lactiplantibacillus plantarum*	G+
FLY2D-8	*Lactiplantibacillus plantarum*	G+
FLY2D-9	*Lactiplantibacillus plantarum*	G+
FLY2D-10	*Lactiplantibacillus plantarum*	G+
FLY2D-11	*Lactiplantibacillus plantarum*	G+
FLY2D-12	*Lactiplantibacillus plantarum*	G+
FLY2D-13	*Lactiplantibacillus plantarum*	G+
FLY2D-14	*Lactiplantibacillus plantarum*	G+
FLY2D-15	*Lactiplantibacillus plantarum*	G+
FLY2D-16	*Lactiplantibacillus plantarum*	G+
FLY2D-17	*Acetobacter persici*	G-
FLY2D-18	*Acetobacter indonesiensis*	G-
FLY2D-19	*Lactiplantibacillus plantarum*	G-
FLY2D-20	*Corynebacterium nuruki*	G+
FLY2D-21	*Corynebacterium nuruki*	G+
FLY2D-22	*Lactiplantibacillus plantarum*	G+
FLY2D-23	*Acetobacter persici*	G-
FLY2D-24	*Leuconostoc mesenteroides*	G+
FLY2D-25	*Nocardioides panzhihuensis*	G+
FLY2D-26	*Lactiplantibacillus plantarum*	G+
FLY2D-27	*Leuconostoc mesenteroides*	G+
FLY2D-28	*Lactiplantibacillus plantarum*	G+
FLY2D-29	*Acetobacter persici*	G-
FLY2D-30	*Acetobacter tropicalis*	G-
FLY2D-32	*Acetobacter persici*	G-
FLY2D-32	*Corynebacterium nuruki*	G+
Ten days post eclosion		
FLY10D-1	*Corynebacterium nuruki*	G+
FLY10D-2	*Lactiplantibacillus plantarum*	G+
FLY10D-3	*Acetobacter tropicalis*	G-
FLY10D-4	*Lactiplantibacillus plantarum*	G+
FLY10D-5	*Corynebacterium nuruki*	G+
FLY10D-6	*Corynebacterium nuruki*	G+
FLY10D-7	*Enterococcus faecalis*	G+
FLY10D-8	*Corynebacterium nuruki*	G+
FLY10D-9	*Corynebacterium nuruki*	G+
FLY10D-10	*Corynebacterium nuruki*	G+
FLY10D-11	*Lactiplantibacillus plantarum*	G+
FLY10D-12	*Corynebacterium nuruki*	G+
FLY10D-13	*Microbacterium aurum*	G+
FLY10D-14	*Enterococcus faecalis*	G+
FLY10D-15	*Enterococcus faecalis*	G+
FLY10D-16	*Corynebacterium nuruki*	G+
FLY10D-17	*Acetobacter tropicalis*	G-
FLY10D-18	*Leuconostoc pseudomesenteroides*	G+
FLY10D-19	*Leuconostoc mesenteroides*	G+
FLY10D-20	*Lactiplantibacillus plantarum*	G+
FLY10D-21	*Lactiplantibacillus plantarum*	G+
FLY10D-22	*Lactiplantibacillus plantarum*	G+
FLY10D-23	*Lactiplantibacillus plantarum*	G+
FLY10D-24	*Acetobacter* sp.	G-