Supplemental Material

For: Specific strains of honeybee gut *Lactobacillus* stimulate host immune system to protect against pathogenic *Hafnia alvei*

Haoyu Lang, Huijuan Duan, Jieni Wang, Wenhao Zhang, Jun Guo, Xue Zhang, Xiaosong Hu, Hao Zheng,

**Contents:**

| Figure/S | Page |
|----------|------|
| S1       | 2    |
| S2       | 3    |
| S3       | 4    |
| S4       | 5    |
| Table S1 | 6    |
| Table S2 | 7    |
| Table S3 | 8    |
| Supplementary References | 9 |
**FIG S1** *B. apousia* and *B. apis* strains did not protect against *H. alvei* infection in honeybee gut, and scheme for engineered *H. alvei* SMH01 mutant constitutively expressing the green fluorescent protein. (A) Absolute abundance of *H. alvei* of different treatment groups 5 days post-inoculation with *H. alvei*. Bees treated with *B. apousia* W8102 and *B. apis* W8152 do not affect the colonization of *H. alvei*. (B) Wild-type *H. alvei*. (C) Green fluorescent mutant *H. alvei*. (D) The GFP gene and the kanamycin resistance gene were knocked-in the chromosome downstream of the *lacZ* gene.
**FIG S2** *Gilliamella* and *Lactobacillus* strains did not protect against *H. alvei* infection in the honeybee gut. (A-D) The liquid cultures or cell-free supernatant of *G. apicola* W8136, *G. apis* W8123, *L. melliventris*, and *L. apis* W8172 did not inhibit the growth of *H. alvei in vitro.*
FIG S3 KEGG analysis of the gut epithelial of honeybees mono-colonized with *Gilliamella*. Representative enriched KEGG pathways upregulated in the *G. apicola* W8136 group, compared to W8123.
**FIG S4** KEGG analysis of the gut epithelial of honeybees mono-colonized with *L. melliventris* W8171. Representative enriched KEGG pathways up- (A) and down-regulated (B) in the *L. melliventris* W8171 group, compared to MF.
| Gene                | Reference | Forward               | Reverse                                      |
|---------------------|-----------|-----------------------|----------------------------------------------|
| Abaecin             | (1)       | TCGGATTGAATGGTCCCTGAC | ATCTTCGCCACTACTCGCCAC                       |
| Apidaecin           | (1)       | GTAGGTCGAGTAGCGGATCT  | TTTTGCTTTAGCAATTCCTTGTG                   |
| Cactus-1            | (1)       | CTATCGTGGAAGAACTCGGTAT| TCAGGAAGTGGTTCTGTTAT                        |
| Cactus-2            | (1)       | ATCAGACCGCTCTGCTAT    | TCGTCTTCGTCACTGTTAT                       |
| Dorsal              | (1)       | AGAGATGGAACGCAGAAAC   | TGACAGGATATAGGACGAGGA                     |
| Dredd               | (1)       | GCGTCATAAAAGAAAAGGATCA| TTTCGGATAATTGAGCAAC                      |
| Hymenoptaecin       | (1)       | GTCGTCCATCCCTGGACATT | TTTCCAAAACCTCGAATCC                      |
| PGRP-LC             | (1)       | TCCGTCAAGCCGTAATTTTTC | CGTTTGTCGAATCGAACAT                      |
| Relish              | (1)       | GGAGCTGATCCAAATCGAAC | AGTGGCATCCATCCATCATT                     |
| RPS18               | (1)       | AGGTGTGTGTCGTCTTAT    | CATTCCTCAAGCAGCCTAT                      |
| Toll                | (1)       | TAGAGTGCCGATTGTCAAG   | ATCGCAATTGGTCCCAAAC                      |
| Defensin-1          | (2)       | TGGGCGTGAACATCGTACAG | AATGGCAACTTAACCGAAC                      |
| Defensin-2          | (3)       | GCAACTACCGCCTTTATGTC  | GGGTAACGTCGCAGGTTTA                      |
| Lysozyme            | (3)       | ACACGGTTGTCACGCTGTC   | GTCCCGACGGTTTGAATCCCT                    |
| Actin               | (4)       | TGCCAACACTGTTTCCTT    | AGAATTGACCCACCAATCCA                    |
Table S2. AMPs used in this study.

| AMPs          | Sequence                                                                 | Length | Reference |
|---------------|--------------------------------------------------------------------------|--------|-----------|
| abaecin       | YVPLPNIPQPGRPPTFGQGPFNPKIKWPQGY                                           | 34     | (5)       |
| apidaecin-1a  | GNNRPVYIPQPRPHPI                                                          | 18     | (6)       |
| apidaecin-1b  | GNNRPVYIPQPRPHPI                                                          | 18     | (6)       |
| defensin-1    | VTCDLLSFKGVNDACANCLSLGKAGGHCEKVCRKTSFKDLWDKRF                             | 52     | (7)       |
| defensin-2    | VTCDVLSWQKLSINHSCAIRCLAQRKGGSCRVGCRK                                      | 43     | (7)       |
| hymenoptaecin | QERGSIVIQGTKRNPSDLQRYDKNGMTGDAYGGVIRPGQRTRQHA GFEFKEYKNGFIRGSEQVRGGLSPYG | 93     | (8)       |
|               | GINGGFRF                                                                |        |           |
**Table S3.** Bacterial isolates used in this study.

| Strain                     | Collection_site | Isolated medium    | NCBI accession number               |
|----------------------------|-----------------|--------------------|-------------------------------------|
| *Hafnia alvei* SMH01       | China: Jilin    | Heart infusion agar| OK206815 (16S rRNA sequence)        |
| *Bartonella apis* W8152   | China: Jilin    | Heart infusion agar| GCF_016100395                       |
| *Bifidobacterium asteroids* W8102 | China: Jilin | Heart infusion agar| GCF_007559275                       |
| *Gilliamella apis* W8123   | China: Jilin    | Heart infusion agar| GCF_016101085                       |
| *Gilliamella apicola* W8136| China: Jilin    | Heart infusion agar| GCF_016101285                       |
| *Lactobacillus melliventris* W8171 | China: Jilin  | MRS                | GCF_016102065                       |
| *Lactobacillus apis* W8172 | China: Jilin    | MRS                | GCF_016102055                       |
Supplementary References

1. Horak R D, Leonard S P, Moran N A. Symbionts shape host innate immunity in honeybees. Proc Biol Sci. 2020; 287(1933):20201184.

2. Daisley B A, Pitek A P, Chmiel J A, Al K F, Chernyshova A M, Faragalla K M, et al. Novel probiotic approach to counter Paenibacillus larvae infection in honey bees. ISME J. 2020; 14(2):476-491.

3. Daisley B A, Pitek A P, Chmiel J A, Gibbons S, Chernyshova A M, Al K F, et al. Lactobacillus spp. attenuate antibiotic-induced immune and microbiota dysregulation in honey bees. Commun Biol. 2020; 3(1):534.

4. Kesnerova L, Emery O, Troilo M, Liberti J, Erkosar B, Engel P. Gut microbiota structure differs between honeybees in winter and summer. ISME J. 2020; 14(3):801-814.

5. Casteels P, Ampe C, Riviere L, Van Damme J, Elicone C, Fleming M, et al. Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (Apis mellifera). Eur J Biochem. 1990; 187(2):381-386.

6. Welch N G, Li W, Hossain M A, Separovic F, O'Brien-Simpson N M, Wade J D. (Re)Defining the proline-rich antimicrobial peptide family and the Identification of putative new members. Front Chem. 2020; 8:607769.

7. Klaudiny J, Albert S, Bachanova K, Kopernicky J, Simuth J. Two structurally different defensin genes, one of them encoding a novel defensin isoform, are expressed in honeybee Apis mellifera. Insect Biochem Mol Biol. 2005; 35(1):11-22.

8. Casteels P, Ampe C, Jacobs F, Tempst P. Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (Apis mellifera). J Biol Chem. 1993; 268(10):7044-7054.