

Supplementary Information

***Lactobacillus* spp. attenuate antibiotic-induced immune and microbiota dysregulation in honey bees**

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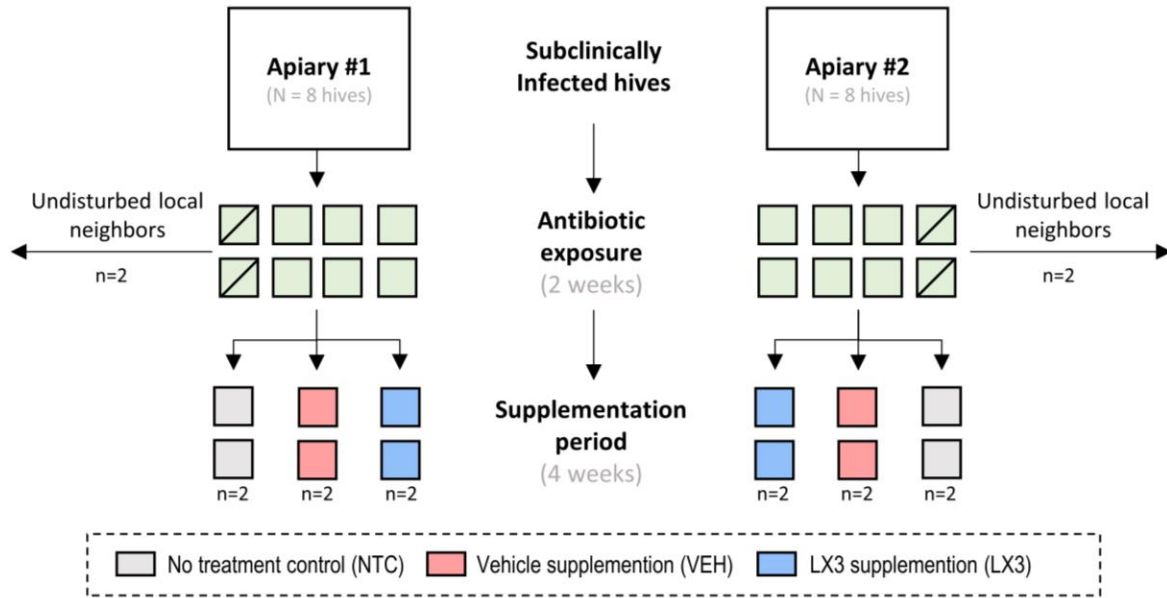
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This PDF file includes:

Supplementary Figures 1-4
Supplementary Tables 1-2

Other Supplementary Materials for this manuscript include the following:

Supplementary Datasets 1-3



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31 **Supplementary Figure 1. Schematic diagram of experimental overview.** Experimental apiaries were chosen on the basis of their inclusion
 32 within a boundary assessed to have a recent increase of AFB incidence as denoted through provincial apiary inspection by the Ontario Ministry of
 33 Agriculture and Food and Ministry of Rural Affairs. All hives received standard treatment with oxytetracycline hydrochloride (OTC) for two-
 34 weeks. Two hives in each apiary were then left to freely interact with experimental hives in an effort to emulate realistic buffering conditions of
 35 undisturbed local neighboring colonies. The remaining hives were longitudinally monitored for an additional four weeks while receiving either no
 36 treatment as a negative control (NTC), pollen patty supplementation as a vehicle control(VEH), or pollen patty with LX3 supplementation (LX3).

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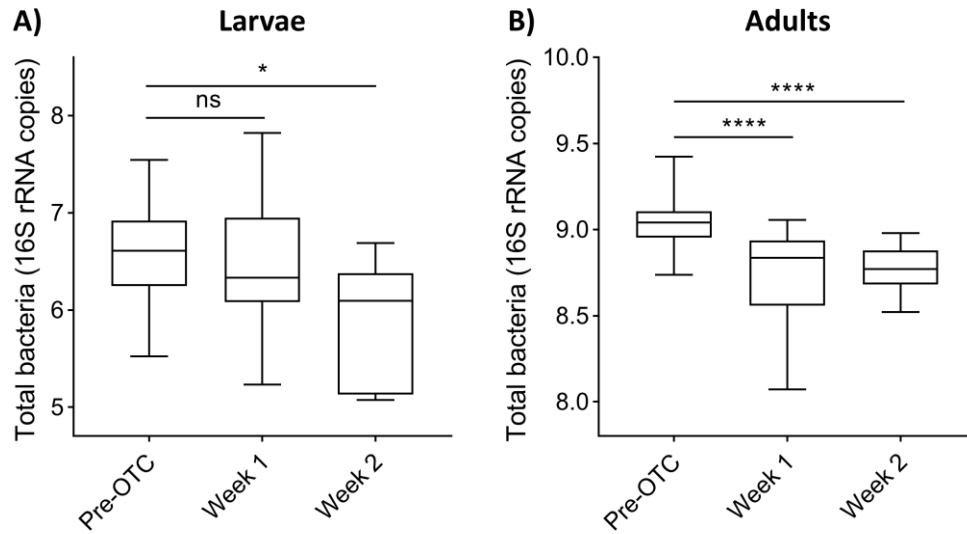
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Supplementary Figure 2. Total bacterial loads in honey bee adults and larvae during antibiotic treatment. Experimental hives were subjected to standard antibiotic treatment (OTC) for two weeks. Total bacterial loads in surface-sterilized (A) adults (dissected abdomens) and (B) larvae (whole bodies) were quantified via qPCR directly prior to hive administration with oxytetracycline (pre-abx) and a one-week intervals thereafter. Data represents the median (line in box), IQR (box), and minimum/maximum (whiskers) of n = 18 individual adult samples and n = 12 pooled larval samples (three larvae per pooled sampled) at each time point (Kruskal-Wallis with Dunn's multiple comparisons). ns = not significant, *P < 0.05, ****P < 0.0001.

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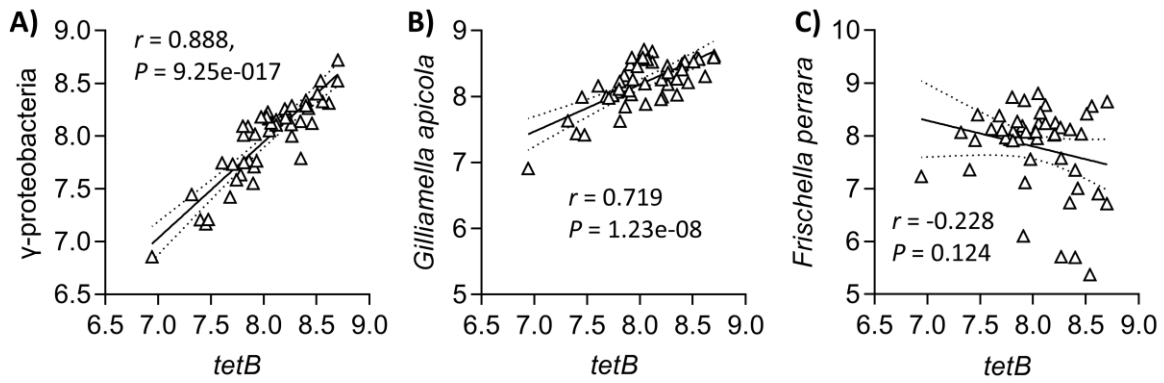
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68 **Supplementary Figure 3. Association between Gammaproteobacteria and *tetB* abundance in adult honey bees.** After the six-week
 69 experimental period of antibiotic treatment followed by probiotic supplementation, intra-individual abundance of (A) total Gammaproteobacteria,
 70 (B) *Gilliamella apicola*, and (C) *Frischella perrara* was compared with *tetB* abundance. Data shown on log₁₀ scale. r = Pearson correlation
 71 coefficient. N=48 adult gut samples. Simple linear regression (solid line) with 95% confidence interval (dotted lines) is shown for each.

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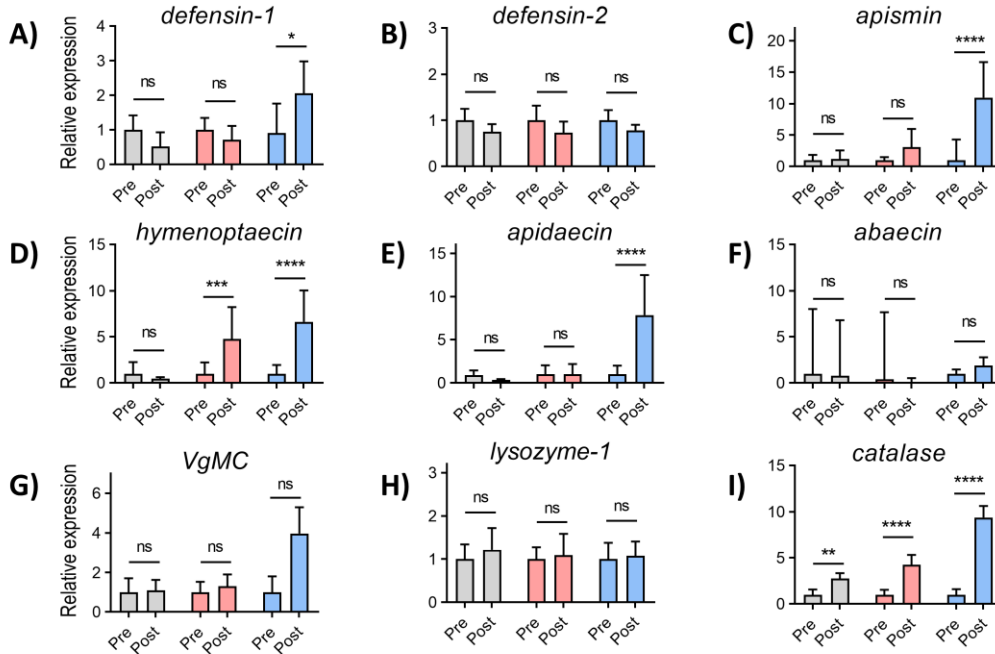
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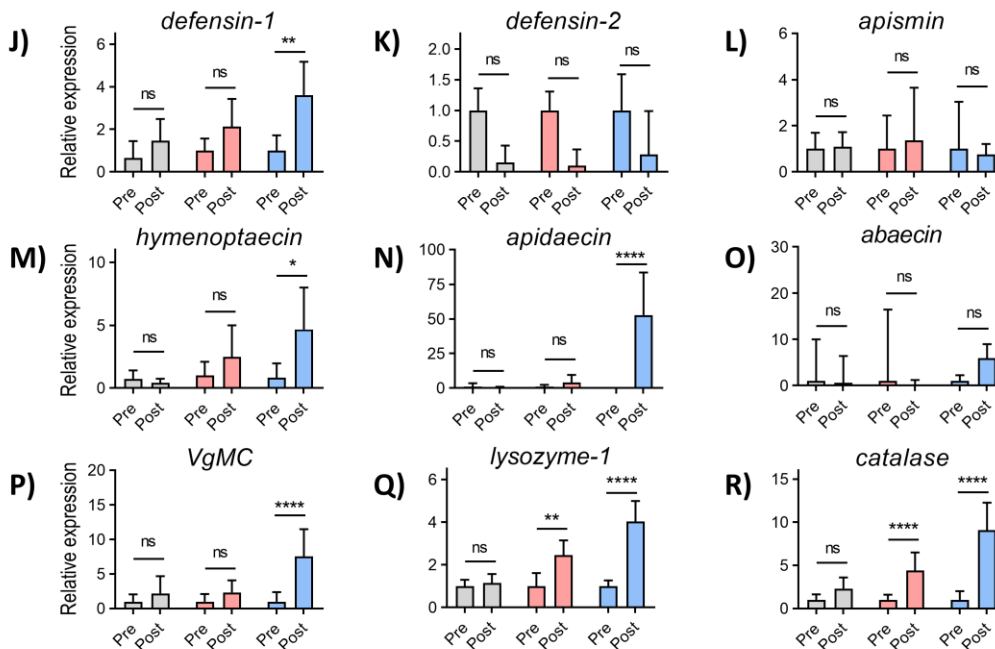
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Heads



Guts



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91 **Supplementary Figure 4. Immune- and antioxidant-related gene expression in honey bee adults following in-hive LX3 supplementation.**
 92 Hives received standard treatment with oxytetracycline hydrochloride for two weeks and then were longitudinally monitored for an additional
 93 four-week supplementation period in which hives received either no further treatment (NTC), pollen patty with vehicle supplementation (VEH),
 94 or pollen patty with LX3 supplementation (LX3). Expression of immune or antioxidant genes in the heads (A-I) and guts (J-R) of adult honey
 95 bees collected pre- and post-supplementation period were quantified via RT-qPCR. Mean \pm standard deviation (two-way ANOVA with Sidak's
 96 multiple comparisons) of $n=18$ adult heads and $n=18$ adult guts per treatment group with technical duplicate repeats are shown. * $P<0.05$,
 97 ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$, ns = not significant.

98 **Supplementary Tables**

99 **Supplementary Table 1.** Primers used for qPCR-based quantification of bacteria.

Target	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
<i>B-actin</i>	TTGTATGCCAACACTGTCTTT	TGGCGGATGATCTTAATTT
<i>Rp5S</i>	AATTATTTGGTCGCTGGAATTG	TAACGTCCAGCAGAATGTGGTA
Total bacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
Alphaproteobacteria	CIAGTGTAGAGGTGAAATTC	CCCCGTCAATTCCTTTGAGTT
Betaproteobacteria	CTTAGAGATAGGAGAGTG	TAATGATGGCAACTAATGACAA
Gammaproteobacteria	TCGTCAGCTCGTGTGTGA	CGTAAGGGCCATGATG
Bifidobacterium	TACGGCCGCAAGGCTA	TCRTCCCCACCTCCTCCG
Bacteroidetes	CRAACAGGATTAGATACCCT	GGTAAGGTTCTCGCGTAT
Firmicutes	TGAAACTYAAAGGAATTGACG	ACCATGCACCACCTGTC
<i>Paenibacillus larvae</i>	CGGAGACGCCAGGTTAG	TTCTTCCTTGCCAACAGAGC
<i>Lactobacillus plantarum</i>	ATTCATAGTCTAGTTGGAGGT	CCTGAACTGAGAGAATTTGA
<i>Lactobacillus rhamnosus</i>	TGCTTGCATCTTGATTTAATTTTG	GGTTCTTGGATYTATGCGGTATTAG
<i>Lactobacillus kunkeei</i>	GAGAAGCATTACTAAGCCAAC	CATATTGACCTTACCACCAGAT

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109 **Supplementary Table 2.** Primers used for RT-qPCR determination of immune- and antioxidant-
 110 related gene expression.

Target	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
<i>Alpha-tubulin</i>	GCACGTGAAGATCTAGCAGCTC	GCACCTTCTCCTTCACCTTCAG
<i>Rp5S</i>	AATTATTTGGTCGCTGGAATTG	TAACGTCCAGCAGAATGTGGTA
<i>Microsomal glutathione-S-transferase</i>	TTGCTCTGTAAGGTTGTTTTGC	TGTCTGGTTAACTACAAATCCTTCTG
<i>Glucuronyltransferase</i>	CACGGATACATCCTGCAGTCATC	GAGAATGACGAGATACAGAACTGTCAC
<i>Defensin-1</i>	TGCGCTGCTAACTGTCTCAG	AATGGCACTTAACCGAAACG
<i>Defensin-2</i>	GCAACTACCGCCTTTACGTC	GGGTAACGTGCGACGTTTTTA
<i>Hymenoptacein</i>	CTCTTCTGTGCCGTTGCATA	CGTCTCCTGTCATTCCATT
<i>Apisimin</i>	TGAGCAAAATCGTTGCTGTC	AACGACATCCACGTTTCGATT
<i>VgMC</i>	AGTTCCGACCGACGACGA	TTCCCTCCCACGGAGTCC
<i>Apidaecin</i>	TAGTCGCGGTATTTGGGAAT	TTTACGTGCTTCATATTCTTCA
<i>Abaecin</i>	CAGCATTCGCATACGTACCA	GACCAGGAAACGTTGGAAAC
<i>Catalase</i>	GTCTTGGCCCAAACAATCTG	CATTCTCTAGGCCACCAAAA
<i>Lysozyme</i>	ACACGGTTGGTCACTGGTCC	GTCCCACGCTTTGAATCCCT

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