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Bacterial supplementation shapes honey bee gut microbiota, metabolism and proteome under controlled and field conditions

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Gut microbiota influence immunity, metabolism, and behavior in honey bees (Apis mellifera). While numerous studies have explored microbiota-driven health benefits, most have been conducted either under controlled laboratory conditions or directly in the field. However, how gut microbiota interventions transition from controlled environments to natural hive conditions remains unclear.

We conducted a cage experiment (controlled conditions) and a field experiment (natural conditions) using four bacterial isolates—Lactobacillus helsingborgensis, Bifidobacterium asteroides, Bombilactobacillus mellis, and Lactobacillus apis—to assess the effects of bacterial supplementation on honey bee gut microbiota and metabolism. In the cage study, newly emerged bees were assigned to four groups: control (C) and bacterial supplementation (B) (strictly controlled microbiome) and control with older bees (COB) and bacterial supplementation with older bees (BOB) (semi-controlled with social interaction). All groups received ad libitum feeding. In the field study, bees were supplemented once by trickling a bacterial mixture (TRT) or a sucrose solution (CONT) directly onto the clustered bees within the hive. Gut samples from 10-day-old bees were analyzed using 16S rRNA sequencing (microbiota), ^{1}H-NMR metabolomics, and LFQ-proteomics (cage only).
Results showed that bacterial supplementation (B, BOB, and TRT) significantly altered gut microbiota composition, increasing Lactobacillus and Bifidobacterium abundance compared to respective controls (C, COB, and CONT). Lactobacillus helsingborgensis was significantly enriched in TRT compared to CONT (q-values < 0.05), demonstrating its resilience under natural conditions. Metabolomic analysis revealed significant shifts in organic acids and neurotransmitter-related metabolites in supplemented groups compared to their respective controls (q-values < 0.05). Proteomic analysis identified upregulation of major royal jelly proteins and defense response pathways in B, while C showed increased expression of ribosomal and other translation-related proteins. Additionally, BOB exhibited increased expression of proteins involved in detoxification and stress responses, while COB showed similar patterns to C (q-values < 0.05).

These findings illustrate how bacterial supplementation influences the gut microbiota, with certain species demonstrating resilience and consistent metabolic patterns across controlled to natural conditions, providing a comprehensive perspective on microbiome modulation.

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