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Honeybees harbor a core microbiota in their digestive tract, including species of lactobacilli and bifidobacteria, which play a crucial role in maintaining host health and defending against harmful pathogens. One key function of these bacteria is their ability to inhibit the growth of bacterial pathogens. This study assessed the inhibitory properties of various isolates from the honeybee gut against five strains of honeybee pathogens, providing a foundation for selecting strains that may enhance the bees' natural defense.

In this study, 111 bacterial strains (62 lactobacilli and 49 bifidobacteria) were isolated from the digestive tracts of honeybees collected from eight different sites in the Czech Republic. These strains were identified using 16S rRNA gene sequencing and subsequently tested for their ability to inhibit four clinical isolates of *Paenibacillus* larvae, one clinical isolate of *Melissococcus plutonius*, and one of the opportunistic pathogen *Serratia marcescens* by the plate agar diffusion method.

The results showed that 29 strains (15 bifidobacteria and 14 lactobacilli) were able to effectively inhibit at least two clinical isolates of *P. larvae*, with inhibition zones ≥ 21 mm and representing approximately 26% of all strains tested. Additionally, the inhibition of *M. plutonius* and *S. marcescens* was observed in three and 20 strains, respectively. The most significant inhibitory effects were found in species *Bifidobacterium asteroides*, *B. cholestis*, *B. polysaccharolyticum*, *Bifidobacterium* sp., *Lactobacillus apis*, *L. helsingborgensis*, *L. kullabergensis*, and *L. melliventris*.

These findings emphasize that the probiotic potential of lactobacilli and bifidobacteria varies by strain. Some strains exhibited stronger inhibitory effects against multiple clinical *P. larvae* isolates, underscoring their promise as potential probiotics for honeybee health. Due to the variation in inhibitory activity among strains, selecting those with proven broad-spectrum antimicrobial effects against relevant honeybee pathogens is crucial. This selection forms the basis for using specific probiotic strains as a strategy to mitigate bacterial diseases in honeybee populations, ultimately supporting colony health and resilience.