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PP-098 [Bee Health]

Actinobacteria from Stingless Bees: Isolation, Characterization, and Antimicrobial Potential

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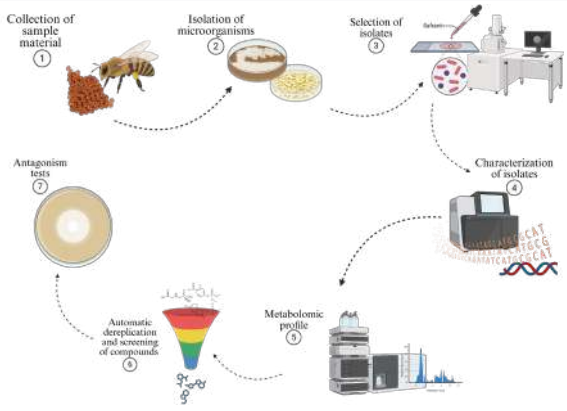
Introduction

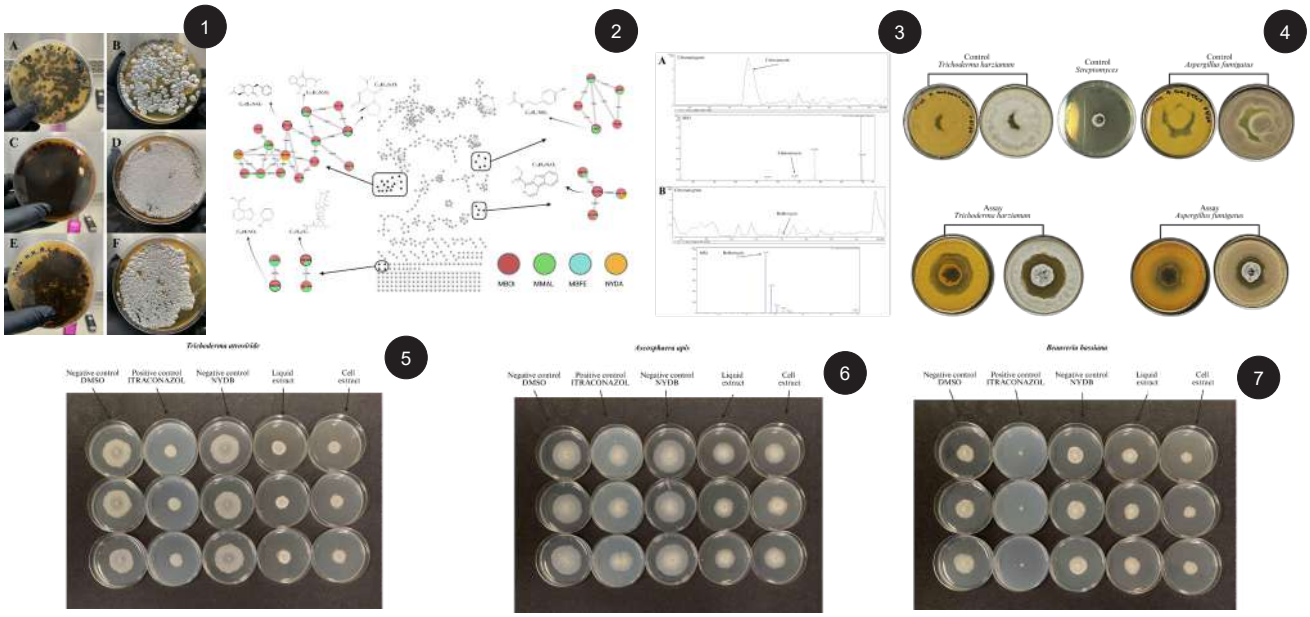
Stingless bees (tribe Meliponini) are eusocial insects commonly found in tropical and subtropical regions, where they play a crucial role in pollinating numerous plant species. Meliponiculture has gained growing interest due to its significance in environmental conservation and the production of medicinal honey. The colonies of these bees harbor a complex and largely unexplored microbiota, including bacteria from the phylum Actinobacteria, known for their capacity to produce bioactive secondary metabolites. Among this group, the genus Streptomyces is recognized as a major source of natural antibiotics. However, the interactions between stingless bees and their microbial communities, especially actinobacteria, remain poorly understood.

This study aimed to isolate and characterize actinobacteria associated with stingless bees of the species *Melipona bicolor*, *Melipona mondury*, and *Melipona quadrifasciata*, evaluate their potential in microbial interactions, and identify the secondary metabolites they produce.

Materials and Methods



Results and Discussion



Figures 1–7. (1) Isolated actinobacteria. (A and B) Reverse and obverse sides of the plate with an actinobacterium isolated from the cuticle of forager bees of *Melipona bicolor*. (C and D) Reverse and obverse sides of the plate with an actinobacterium isolated from the internal environment of worker bees of *Melipona bicolor*. (E and F) Reverse and obverse sides of the plate with an actinobacterium isolated from larval food of *Melipona mondury*. (2) Molecular network of extracts injected into UHPLC-HRMS in positive ESI mode. Node colors indicate the organism or culture medium from which the compound was annotated. (3) (A) Chromatogram of actinobacterium MBOI and below the MS1 spectrum of the compound Caboxamycin. (B) Chromatogram of actinobacterium MBOI and below the MS1 spectrum of the compound Bafilomycin. Results obtained by UHPLC-HRMS in positive ESI mode. (4) Reverse and obverse sides of the independent co-culture bioassay (14-day test). Control plates of the fungi used on the sides and of the actinobacterium in the center. Below, plates showing inhibition halos against *T. harzianum* and *A. fumigatus* in NYDA culture medium. (5) Obverse side of the mycelial growth inhibition bioassay with *Trichoderma atroviride* CMLD 34 after 3 days of incubation. (6) Obverse side of the mycelial growth inhibition bioassay with *Ascosphaera apis* CMLD 131 after 3 days of incubation. (7) Obverse side of the mycelial growth inhibition bioassay with *Beauveria bassiana* IBCB 66 after 7 days of incubation.

Conclusion

The findings of this study emphasize the potential of actinobacteria associated with stingless bees as important sources of bioactive secondary metabolites with antimicrobial effects. Additionally, the results offer new insights into the role of these bacteria in maintaining colony health in *Melipona bicolor* and *Melipona mondury*, highlighting their significance in protecting bees against pathogenic threats.

Acknowledgements

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Stingless bees (tribe Meliponini) are eusocial insects widely distributed across tropical and subtropical regions, playing a fundamental role in the pollination of various plant species. Meliponiculture has gained increasing attention due to its relevance in environmental conservation and the production of medicinal honey. The colonies of these bees harbor a complex and largely unexplored microbiota, including bacteria from the phylum Actinobacteria, which are well known for their ability to produce bioactive secondary metabolites. Among these, the genus Streptomyces stands out as one of the primary producers of natural antibiotics. However, the interactions between stingless bees and their microbial communities, particularly actinobacteria, remain poorly understood. This study aimed to isolate and characterize actinobacteria associated with stingless bees of the species Melipona bicolor, Melipona mondury, and Melipona quadrifasciata, evaluate their viability in microbial interactions, and identify the secondary metabolites they produce. Using microbiological and chemical approaches, three strains of actinobacteria were successfully isolated: two from M. bicolor, one from the cuticle of forager bees (MBFE) and another from the internal tract of worker bees (MBOI), and one from the larval food of M. mondury (MMAL). No cultivable actinobacteria were obtained from M. quadrifasciata. Morphological characterization and phylogenetic analysis indicated that the isolates belong to the genus Streptomyces, with high similarity to the species Streptomyces halstedii. UHPLC-DAD-QTOF-MS/MS analysis and chemical dereplication strategies revealed that MBOI and MMAL were capable of producing two antimicrobial compounds previously reported in the literature as Streptomyces metabolites: Bafilomycin Cl and Caboxamycin. Coculture assays demonstrated that the MMAL isolate exhibited significant antifungal activity against Trichoderma atroviride, Aspergillus fumigatus, and Trichoderma harzianum. Additionally, organic extracts obtained from the MMAL bacterial culture were evaluated for their inhibitory effects against fungal and entomopathogenic pathogens, including Ascosphaera apis, Beauveria bassiana, and Metarhizium anisopliae, confirming its microbicidal efficacy. The findings of this study reinforce the potential of actinobacteria associated with stingless bees as promising sources of bioactive secondary metabolites with antimicrobial properties. Furthermore, this research provides novel insights into the role of these bacteria in maintaining colony health in Melipona bicolorand Melipona mondury, emphasizing their relevance in protecting bees from pathogenic microorganisms.