

# Correlation between Abundance and Isolation of Bacteria in the Gut Microbiota of Stingless Bees of the *Melipona mondury* Species

Stella Braathen de Carvalho<sup>1\*</sup>, Alan Emanuel Silva Cerqueira<sup>1,2</sup>, Amanda Tristão Santini<sup>1</sup>, Evelyn Ruth dos Santos Rodrigues<sup>1</sup>, Ana Clara Duarte Metri<sup>1</sup>, Cynthia Canêdo da Silva<sup>1</sup>

<sup>1</sup> Department of Microbiology, Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil

<sup>2</sup> Department of Integrative Biology, College of Natural Science, University of Texas at Austin, 2415 Speedway, Austin, TX 78712.

\* Corresponding author. E-mail: stella.carvalho@ufv.br

The gut microbiota of bees plays an extensive role in their health and development. Stingless bees harbor a unique gut microbiota that has not yet been well studied and characterized. The purpose of this study was to analyze the gut microbial diversity of *Melipona mondury*, as well as to isolate their associated bacteria. The study was divided into two stages: the analysis of previously published amplicon sequencing data on the gut microbial diversity of *M. mondury*; and the isolation of its associated bacteria from bees collected at the same hives used for the microbial diversity study - located in Mata do Paraíso - Viçosa. For isolation purposes, single bees were dissected, and the gut homogenates were diluted in 96-well plates containing APT or MRS culture medium, followed by incubation for 24 to 72 hours to facilitate the growth of microorganisms with lower abundance. Afterward, cultures were plated in the same culture medium and incubated at 32°C in anaerobic jars to obtain isolated colonies. Subsequently, the colony PCR and Sanger sequencing were performed using the 10F and 1100R primers to assess the 16S rRNA region of the bacterial DNA. The most abundant bacteria observed by the microbial diversity analysis were *Floricoccus* (Streptococcaceae family); *Apilactobacillus*, *Fructobacillus*, and *Lactobacillus* (Lactobacillaceae); *Bifidobacterium* (Bifidobacteriaceae); and *Bombella* and *Commensalibacter* (Acetobacteraceae). Among them, we obtained a *Floricoccus* isolate closely related to ASV11, an abundant sequence in the amplicon dataset. All 10 isolates of the Lactobacillaceae family are closely related to *Convivina intestini*, a bacteria present in the bumblebee microbiome but not close to sequences in the amplicon data. In conclusion, amplicon sequencing data is an important tool to direct the isolation of microorganisms, but isolation strategies can also help obtain microbes not observed in the amplicon data. Future studies will contribute to a better understanding of the role of those microorganisms in bee health and development, as well as to isolate and characterize other bacteria.

**Keywords:** Gut; Microbiology; *Floricoccus*; Lactobacillaceae.

## Correlação entre abundância e isolamento de bactérias da microbiota intestinal da abelha sem ferrão *Melipona mondury*

A microbiota de abelhas sociais exerce um papel importante para a sua saúde e desenvolvimento. O presente estudo teve como objetivo analisar a diversidade microbiana intestinal de abelhas sem ferrão da espécie *Melipona mondury* e isolar suas bactérias associadas. Os microrganismos mais abundantes pertenceram aos gêneros *Floricoccus*, *Fructobacillus*, *Lactobacillus*, entre outros. Foram isolados *Floricoccus* - filogeneticamente próximo as sequências dos dados de diversidade, e *Convivina* - da família Lactobacillaceae. Os dados de diversidade foram importantes para direcionar o isolamento de bactérias de abelhas, e futuros estudos contribuirão para o seu papel na vida desses insetos.

**Palavras-chave:** Intestino; Microbiologia; *Floricoccus*; Lactobacillaceae.

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