

Gut microbial diversity of *M. quadrifasciata anthidioides* and isolation of their associated bacteria

Ana Clara Duarte Metri^{1*}, Alan Emanuel Silva Cerqueira^{1,2}, Amanda Tristão Santini¹, Evelyn Ruth dos Santos Rodrigues¹, Stella Braathen de Carvalho¹, Cynthia Canêdo da Silva¹

¹ Department of Microbiology, Federal University of Viçosa (UFV), Av. Peter Henry Rolfs, s/nº, Viçosa, MG, Brazil, 36570-900.

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

*Ana Clara Duarte Metri. E-mail: ana.metri@ufv.br

The microbial community inhabiting the bee's gut exhibits a diversity and abundance of microorganisms that engage in beneficial symbiotic relationships for the maintenance of immune and physiological activities. Stingless bees (*Meliponini* tribe) are abundant in tropical and subtropical regions and possess an atrophied stinger. The objective of this study was to analyze previously generated amplicon sequencing data on the gut microbial diversity of *Melipona quadrifasciata anthidioides* (MQA), commonly known as mandaçaia, and direct the isolation of some of the observed symbiotic and environmental bacteria, for future studies on their roles for the conservation of this bee species. Data analysis showed that Bifidobacteriaceae, Lactobacillaceae, and Acetobacteraceae were the most abundant families. At the genus level, the dominant ones were *Apilactobacillus*, *Lactobacillus*, *Bifidobacterium*, *Weissella*, *Rosenbergiella*, and *Bombella*. Based on that, we collected bees from 3 MQA colonies, dissected, diluted in 96-well plates with MRS or APT culture medium - to favor the growth of homofermentative and heterofermentative slow-growing bacteria, and incubated. The cultures from wells with visible growth were plated in a solid medium and incubated in anaerobic jars. Isolated colonies were subcultured to obtain pure cultures, which were then preserved in 20% glycerol. The DNA was extracted, and the 16S rRNA region was submitted to PCR and Sanger sequencing. A total of 13 bacteria were identified, including 7 *Fructobacillus* - from the Lactobacillaceae family and 6 *Lactococcus* - Streptococcaceae family. Phylogenetic analyses evaluated the similarity between our isolates and sequences from microbial diversity analysis. Results indicated that Lactobacillaceae isolates were not closely related to the most abundant Lactobacillaceae amplicon sequence variant (ASV) in MQA bees; however, the ASV39 is also related to the *Fructobacillus* genus. The most abundant Streptococcaceae ASVs are related to the *Floricoccus* genus, which we could not isolate in this work. In conclusion, our isolates virtually differed from those observed by metataxonomic studies, but we were able to isolate different bacterial genera, especially those from the Lactobacillaceae family, highly abundant in the gut of MQA bees. Therefore, culture and non-culture-based approaches can complement each other in microbial diversity studies. Further studies are necessary to sequence the whole genome of the isolates to understand better the role of those microorganisms in these stingless bee's health.

Keywords: Stingless bee; Lactobacillaceae; Symbionts; Streptococcaceae.

Diversidade microbiana intestinal de *M. quadrifasciata anthidioides*

A comunidade microbiana intestinal das abelhas exerce relações simbióticas benéficas para a manutenção de atividades imunológicas e fisiológicas. O objetivo deste trabalho foi analisar dados sobre a microbiota intestinal de *Melipona quadrifasciata anthidioides* para direcionar o isolamento de bactérias associadas a essas abelhas. Foram obtidos isolados bacterianos filogeneticamente diferentes das ASVs (variantes de sequenciamento amplicon) observadas nos dados de sequenciamento mas pertencentes a famílias altamente abundantes, como Lactobacillaceae. Abordagens dependentes e independentes de cultivos podem ser complementares em estudos de diversidade microbiana. Estudos futuros contribuirão para o entendimento da importância desses microrganismos para as abelhas sem ferrão.

Palavras-chave: Abelha sem ferrão; Lactobacillaceae; Simbiontes; Streptococcaceae.

Acknowledgement: Research funded by FAPEMIG, CNPq and CAPES.