

## Evaluation of molds and yeasts in *Melipona bicolor* honey

Elisana Julek<sup>1,2</sup>, Vitor Luis Fagundes<sup>1,2</sup>, Juliana Chiesse Da Silva Zatta<sup>2</sup>, Suelen Ávila<sup>3</sup>, Desirré Alexia Lourenço Petters-Vandresen<sup>4,5</sup>, Chirlei Glienke<sup>4,5</sup>, Julia Arantes Galvão<sup>1,2</sup>

Post-Graduation Program in Veterinary Sciences - Federal University of Paraná, Curitiba/Paraná, Brazil<sup>1</sup>

Quality Control and Food Safety Laboratory - Federal University of Paraná, Curitiba/Paraná, Brazil<sup>2</sup>

Post-Graduation Program in Food and Nutrition - Federal University of Paraná<sup>3</sup>

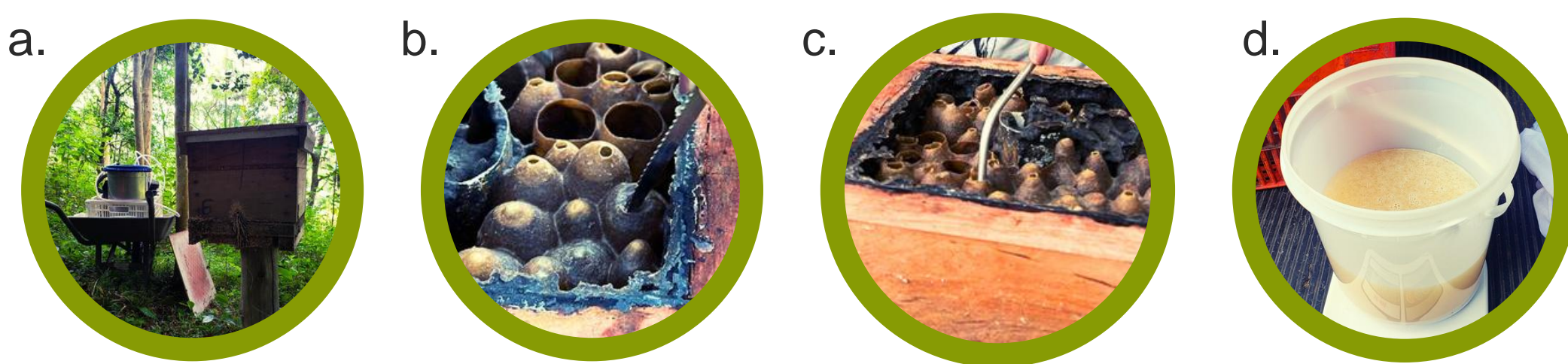
Laboratory of Bioprospecting and Molecular Genetics of Microorganisms (BioGeMM), Department of Genetics, Biological Sciences Sector, Federal University of Paraná<sup>4</sup>  
Post-Graduation Program in Genetics, Department of Genetics, Biological Sciences Sector, Federal University of Paraná<sup>5</sup>

### INTRODUCTION & AIM

The honey of Stingless Bees creates an unfavorable environment for pathogenic microorganisms due to its low pH and high acidity, making it safe for human consumption. However, it is prone to the development of molds, yeasts, and lactic acid bacteria at a pH below 4.5 [1-3]. Therefore, in this study, the objective was to quantify molds and yeasts in stingless bee honey and identify the detected microorganisms.

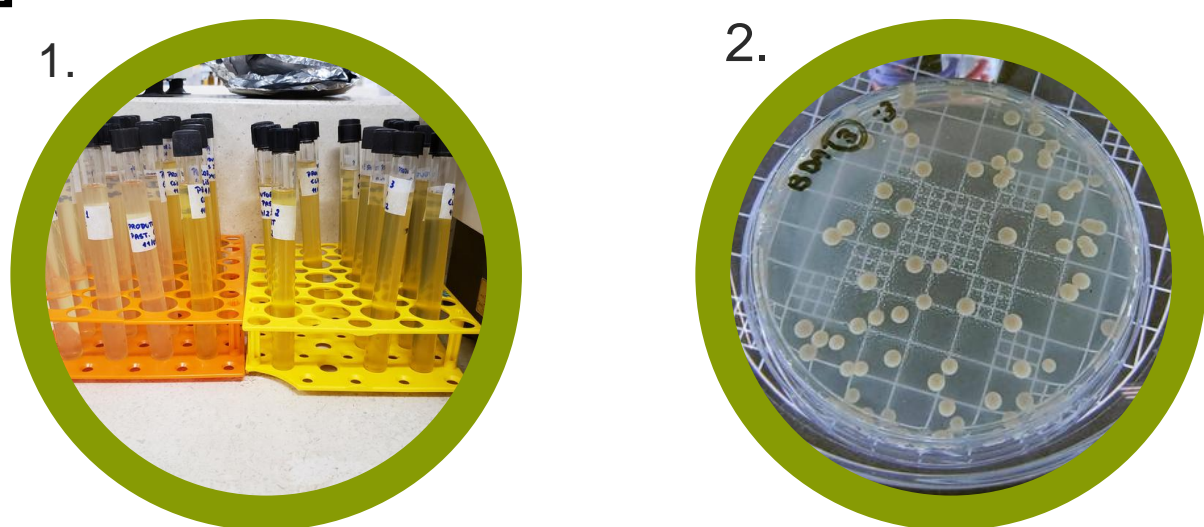
### METHOD

• **Samples:** Raw honey from *Melipona bicolor*.



**Figure 1.** Sample collection: a. Hive location; b. Opening of the pots; c. Honey collection by suction; d. honey after collection

• **Culture medium:** Acidified potato agar to quantify yeasts [4].



**Figure 2.** 1. Aliquots of samples. 2. Microbiological analysis.

• **Molecular identification:** Genomic DNA extraction, ITS region amplification by PCR using V9G and ITS4 primers.

• **PCR cycles:**

- Initial denaturation: 94°C, 5 min;
- 35 cycles: 94°C, 30 sec; Annealing: 48°C, 30 sec; Extension: 72°C, 1 min;
- Final extension: 72°C, 10 min;

• **Sequencing:** BigDye kit, ABI3500 sequencer, V9G and ITS4 primers.

• **Preliminary identification:** NCBI BLAST tool.

### RESULTS & DISCUSSION

- **Growth:** No molds were observed in the samples, with the exclusive growth of yeasts with similar macroscopic characteristics observed in all samples.
- **Counts:** Ranged from 3.28 to 7.30 log CFU.mL<sup>-1</sup> in raw honey.
- **Identification:** The yeast was identified as belonging to the genus *Starmerella*.
- **Food Safety:** Despite the high yeast count, these microorganisms pose no risk to human health.

### CONCLUSION

Although a high quantity of yeasts was observed in the analyzed honey, molecular analysis indicated that they were non-pathogenic microorganisms for humans and associated with stingless bees, highlighting a relevant symbiotic relationship between these insects and the microorganisms present in the honey.

### ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) for their support.

### REFERENCES

1. Ávila, S.; Lazzarotto, M.; Hornung, P.S.; Teixeira, G.L.; Ito, V.C.; Bellettini, M.B.; Beux, M.R.; Beta, T.; Ribani, R.H. Influence of Stingless Bee Genus (*Scaptotrigona* and *Melipona*) on the Mineral Content, Physicochemical and Microbiological Properties of Honey. *J. Food Sci. Technol.* **2019**, *56*, 4742–4748. <https://doi.org/10.1007/s13197-019-03939-8>.
2. Braghini, F.; Biluca, F.C.; Schulz, M.; Gonzaga, L.V.; Costa, A.C.O.; Fett, R. Stingless Bee Honey: A Precious but Unregulated Product-Reality and Expectations. *Food Rev. Int.* **2021**, *38*, 683–712. <https://doi.org/10.1080/87559129.2021.1884875>.
3. Nordin, A.; Sainik, A.V.; Chowdhury, S.R.; Saim, A.B.; Idrus, R.B.H. Physicochemical Properties of Stingless Bee Honey from Around the Globe: A Comprehensive Review. *J. Food Compos. Anal.* **2018**, *73*, 91–102. <https://doi.org/10.1016/j.jfca.2018.06.002>.
4. Downes, F.P.; Ito, K. *Compendium of Methods for the Microbiological Examination of Foods*; 4th ed.; American Public Health Association (APHA): Washington, DC, USA, **2001**; p. 676.