Use of metabarcoding for bacterial identification in light and dark pollen from Mandaguari (Scaptotrigona postica), a native bee from the Brazilian Cerrado

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Scaptotrigona postica (Mandaguari-black) is a stingless bee (SB) species native to the Brazilian Cerrado, which produces and stores large quantities of fermented pollen (called sambura or bee bread), a protein food that feeds the hive. In addition to proteins, bee bread contains essential amino acids, bioactive compounds, minerals, fatty acids, fiber, microorganisms and bee salivary secretions. Honey and pollen are stored in cerumen pots, a mixture of wax and propolis, that also contain bioactive compounds and microorganisms, which are transferred to the pollen during the storage period and contribute to the fermentation process. These microorganisms, besides establishing a symbiotic relationship with bees and their inputs, assist in the fermentation process of pollen, influencing its maturation, protection against pathogens and development of brood cells. The development of high-throughput techniques has favored the elucidation of microbial communities present in different ecosystems, including food. In this study, bacterial ecology was analyzed using the metabarcoding technique (metataxonomy) by sequencing the 16S rRNA gene of light and dark pollen from Mandaguari. After extracting pollen DNA, molecular identification of microorganisms was performed by preparing 16S ribosomal gene amplicon libraries. The library was sequenced using Illumina® Nextseq™. The DADA2 method was used for bioinformatics, and errors and chimeric sequences were removed. ASV (amplicon sequence variant) were compared with the SILVA v.132 database. Alpha diversity was assessed using estimates from the following indices: Chao1, Simpson, Faith's PD, Evenness, and Shannon. In light pollen, the most frequent phyla identified were Firmicutes and Proteobacteria (Alphaproteobacteria), while in dark pollen, the phyla Firmicutes, Fusobacteriota, Proteobacteria (Alphaproteobacteria and Gammaproteobacteria) were identified. In light pollen, an abundance of the Lactobacillaceae family (> 99%) was observed, with a predominance (> 90%) of the *Lactobacillus* genus. In dark pollen, although the Lactobacillaceae family was also predominant (> 95%), there was greater bacterial diversity (alpha diversity), with a reduction in the abundance of the *Lactobacillus* genus (75%) and an increase in genera little (or not) observed in light pollen, such as *Acetobacter, Bombella, Phyllobacterium, Acinetobacter, Fructobacillus, Bacillus, Erwinia, Leuconostoc*. Freshly collected pollen is lighter and less acidic. The causes of pollen darkening include oxidation of phenolic compounds and lipid oxidation. Additionally, during storage, the lactic acid bacteria (LAB) undergo lactic fermentation, with progressive darkening and a decrease in pH due to the production of lactic acid. Microorganisms more attuned to acidic environments, such as different genera of LAB, e.g. *Lactobacillus, Leuconostoc* and *Fructobacillus*, participate in biochemical processes that modify the nutritional quality and improve the digestion and absorption of pollen by bees, in addition to stimulating the bees\' immune system. Moreover, LAB contributes to the protection of hives from pests and pathogens. The metabarcoding technique revealed differences in the microbial composition of light and dark pollen, with a greater diversity of bacterial genera in the dark pollen of S. postica. However, in both foods there was a predominance of LAB. LAB are very interesting microorganisms for biotechnological applications and the LAB isolated from SB pollen may be promising as probiotics, both for human and bee health.

References

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