Assigning the proteome complement of honey from Africanized Apis mellifera: paving the way to set up an analytical platform for sanitary control of quality

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Honey is a complex biological product created by bees from plant nectar. The primary roles of honey are to nourish the colony, support larval development, and provide immune defense. Despite its low protein content (0.2%-1.6%), honey\'s proteins significantly alter its flavor, control pH, and prevent infections. Various microorganisms interacting with the colony (bacteria and fungi), as well virus, and parasitic arthropods, leave protein traces in honey, which are crucial for sanitary quality control.

The proteins were extracted using the method of "Saturated Phenol", digested with trypsin and analyzed by mass spectrometry using a LC-20AD Prominence, coupled to an amaZon SL Ion Trap mass spectrometer (Bruker). The data were acquired using Bruker Compass Data Analysis 4.3 software, which were processed using MASCOT 2.3.02 server engine search, and protein searches were performed using 10 distinct databases obtained from NCBI. Quantitative proteome analyses were carried out using the strategy of spectral counting method and emPAI strategy to provide a relative quantification of proteins in a mixture. The prediction of signal sequences was performed using the algorithm SignalP 6.0.

The study of Africanized *A. mellifera* honey has unveiled significant insights into its proteomic content, showcasing the contributions of different organisms to its diverse sub-proteomes: i) **Plant Proteins**: Proteomic analysis identified plant proteins involved in defense and antimicrobial activities, particularly peroxidases. The findings highlighted the range of plant sources bees use for nectar collection, including families like Fabaceae and Myrtaceae; ii) **Bacterial Proteins**: A complex sub-proteome of bacterial proteins from diverse species was discovered, underscoring the microbiota\'s role in honey quality and bee health. Key bacterial species were identified for their involvement in pathogen defense and microbiota balance within the hive environment; iii) **Fungal Proteins**: The analysis revealed a diverse range of fungal proteins, including both pathogenic and non-pathogenic species; iv) **Viral Proteins**: Proteomic analysis detected viral proteins from 47 different virus species. This aspect of proteomic analysis is crucial for understanding viral transmission dynamics and emphasizes the importance of maintaining bee health for sustainable pollination-dependent ecosystems; v) **Parasitic Arthropods**: Proteomic techniques detect proteins from parasitic arthropods like mites, beetles, and flies in honey.

In conclusion, proteomic analysis of Africanized *A. mellifera* honey has proven instrumental in uncovering hidden microbial interactions, assessing colony health dynamics, and ensuring honey quality and safety. This approach not only advances our understanding of bee microbiomes but also informs strategies for sustainable beekeeping and ecosystem conservation. Further integration of proteomic techniques with comprehensive field studies will continue to enhance our understanding of the complex dynamics between bees, their pathogens, and the environment, thereby promoting the health and resilience of bee populations worldwide.

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