P3.36 - DEVELOPMENT OF A FAST METHOD FOR THE EARLY DETECTION OF *PAENIBACILLUS LARVAE* SPORES IN BEEHIVES - A STRATEGY TO CONTROL AMERICAN FOULBROOD

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ABSTRACT

American Foulbrood (AFB) is a highly devastating bacterial disease from honeybees, caused by the spore-forming bacterium *Paenibacillus larvae*. The innocuous resistant spores are carried by honeybees, but when they germinate inside bee larvae, the disease sets in. As antibiotics are not an option, incinerating contaminated hives is mandatory, profoundly impacting the ecological balance and beekeeping economy. Clinical symptoms in hives only become apparent after the infection has progressed and cannot be managed, but if discovered early, preventive, and prophylactic measures can be put in place. Current detection methods provide late responses preventing beekeepers from monitoring the health status of the hive in real time. This work aimed to develop a method for a fast and in situ detection of *P. larvae* spores, even in the pre-clinical stages of the infection. For capturing spores from artificially infected bee samples, we tested an upstream bead-based magnetic separation, employing a spore-binding protein (γD-crystallin). Then, for *P. larvae*-specific detection, a bacteriophage-derived cell wall binding domain (CBD), PlyPl23_CBD, was used after pre-germinating spores. A fluorescent probe (GFP) fused to PlyPl23_CBD enabled the detection of *P. larvae* spores by fluorescence microscopy. A bead-based method for spore-capturing was developed, resulting in a spore recovery rate of approximately 80%. Spore germination assays revealed that, after 210 minutes of germination, spores could be efficiently detected by fluorescence microscopy, using GFP-PlyPl23_CBD. All combined, the sequential steps of the developed methodology enabled spore detection in 5 hours. The outcomes of this study lay the foundation for a quick on-site detection method, capable of early detection of *P. larvae* spores in beehives, that can prevent hives destruction and mitigate a potential Ecological imbalance.