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Transposon-mutagenesis based identification of virulence factors of Paenibacillus larvae

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Paenibacillus larvae, is an etiological agent of American Foulbrood, a deadly disease of the European Honey Bee (Apis mellifera). American Foulbrood is the most important bacterial honey bee disease but relatively little is known of its virulence. In recent years, however, some virulence factors have been identified. In our research, the goal is to identify virulence factors of the pathogen in an unbiased way. This is accomplished by EZ-Tn5 transposon mutagenesis. Using the EZ-Tn5 transposome complex (Epibio), a library of random knock-out transformants was created. The virulence of these transformants was tested using infection assays. Spores were fed to 1st instar honey bee larvae, which were further reared *in vitro*. Of the 158 transformants, only 93 were able to sporulate. Preliminary tests with these 93 transformants rendered 15 with atypical virulence compared with the wild type. These 15 transformants were fully tested using three independent infection groups with 30 larvae each. Statistical test confirmed that 7 transformants had a mortality rate that was significantly lower than the wild type. Identification of the interrupted genes was done using rescue cloning. The genomic DNA was sheered by three restriction enzymes, blunt ended by T4 DNA polymerase and circulized by T4 DNA ligase. Since the transposon carried *R6Kyori* and kanamycin resistance marker, selection of plasmids carrying the transposon could be done by cloning into pir E. coli. In a final step, the regions flanking the transposon were sequenced to identify the knocked out gene.

Biography

Tine Descamps has completed her Master's degree in Biochemistry and Biotechnology in 2013 and is currently pursuing PhD in the Laboratory for Molecular Entomology and Bee Pathology (L-MEB) at Ghent University, Belgium.

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