

# Analysis of the pathogenic interaction between *Paenibacillus larvae* and honeybee larvae

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American foulbrood (AFB) of honeybees is the most virulent bacterial disease of honeybee brood and a notifiable disease in many countries. Its etiological agent is the spore-forming, gram-positive bacterium *Paenibacillus larvae* (*P. larvae*) (3). So far, little is known about the interaction between the bacterium and infected larvae and the molecular nature of virulence factors. Recently, we established the method of fluorescence *in situ* hybridization (FISH) for the analysis of the early steps in the pathogenesis of infections caused by *P. larvae* ERIC I and ERIC II (5). These results allowed us to propose a new model for the pathogenesis of AFB: During the first days after infection, *P. larvae* colonizes the larval midgut living like a commensal from the food ingested by the larvae. Eventually, the honey bee larval gut contains nothing but these bacteria. It is not until then that the bacteria switch to the invasive state, breach the epithelial barrier and invade the haemocoel thereby killing the larvae. Therefore, one of the key steps in the pathogenesis of AFB is the switch of *P. larvae* from the non-invasive state to the invasive state and understanding the regulation of this switch will help us to understand the interaction between *P. larvae* and honeybee larvae.

We are currently analyzing the expression and function of different virulence factors, we recently identified by comparative genomics (2): ADP-ribosylating toxins of the AB-type, non-ribosomally synthesized antibiotics, and enhancin, a virulence factor originally identified in viruses. The sequencing and annotation of the *P. larvae* genome is in its final phase and will be finished soon. This will give us even more information on possible virulence genes and on factors putatively enabling *P. larvae* to successfully colonize the larval midgut and to develop AFB disease in larvae.

The entire genome sequence will also allow the application of nucleic acid microarray techniques for the assessment of transcriptional changes on a whole bacterial genome level. Microarrays contain DNA probes representing all or rationally selected coding sequences in or from a genome. Comparison of mRNA from two conditions by competitive hybridization to these probes is nowadays frequently used to identify differentially expressed genes (1, 4).

In our project we want to identify genetic determinants of *P. larvae* (i) responsible for the switch from the non-invasive to the invasive state (5) and/or (ii) characteristic for either state using microarray analysis. Identified genes (e. g. genes for transcription factors, signalling molecules, proteins involved in metabolism, etc.) will be cloned and expressed for confirming their role in pathogen-host-interaction using functional assays. Future work will be dedicated to further elucidate the interplay of virulence factors in determining disease in honeybee larvae.

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