



Characterization of intrinsic resistance of *Apis mellifera* colonies against the European Foulbrood in Switzerland. Application for breeding protocols

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Key words

Honey bees; European foulbrood; EFB; *Melissococcus plutonius*; *Apis mellifera*; disease resistance; virulence;

Aim of the study

European foulbrood (EFB) is a highly contagious disease of honey bees (*Apis mellifera*) caused by the bacterium *Melissococcus plutonius*. In Switzerland, the number of notified EFB cases has increased dramatically in the last 16 years. However, little is known on the pathogenesis of EFB and until today only the application of costly and laborious control measurements limits the spread of the disease. A better understanding of the disease would be necessary to develop more effective control strategies.

This study was therefore performed to: (i) characterize defense mechanisms in honey bees, with the aim to provide selection criteria for EFB resistance breeding programs; (ii) identify differences between isolates of the pathogen *M. plutonius*; (iii) analyze the host-pathogen-interaction by the means of the immune reaction to find targets for future treatments of EFB.

Material and methods

Queens of honey bee colonies from apiaries with an acute EFB outbreak were collected and kept in host colonies. These queens were distributed in two groups: in queens from colonies with clear disease symptoms (susceptible group); and in queens from colonies without EFB symptoms (resistant group). The larvae of queens of both groups were tested for its survival after artificial infections with *M. plutonius* in the laboratory. Further the offspring of these queens was tested for hygienic behavior by the removal rate of freeze killed brood on the colony level.

A total of 160 Swiss *M. plutonius* isolates was classified via a multilocus sequence typing (MLST) system. Thereof seven were subjected to a more detailed genetic analysis using whole genome sequencing. Seventeen isolates were tested for their virulence in in vitro infection assays and their growth rate in artificial media. The immune response of larva was quantified based on the relative transcript levels of 17 genes by PCR.

Results and significance

Dead brood was on average faster removed in test colonies of the resistant group than of the susceptible group, however, the difference was statistically not significant. Larvae from the resistant group statistically died faster and showed higher mortality rates after artificial infection with *M. plutonius*. A possible explanation for these results is a two-stage mechanism to prevent disease in colonies of the resistant group: Larvae of this group die faster and hygienic workers remove these larvae earlier from the colony.

The MLST system distinguished 13 genetic sub-types of *M. plutonius*, including six endemic to Switzerland. A gene for the production of a toxin, melissotoxin A, was discovered in the whole genome analysis and virulence tests in vitro showed that *M. plutonius* isolates that contain this gene are causing higher mortality rates in infected larvae. Highly virulent *M. plutonius* isolates showed a higher growth rate in artificial media than non- or low virulent *M. plutonius* isolates.

Five out of 17 immune relevant genes were downregulated in honey bee larvae 24h after infection. Genes for the production of antimicrobial effectors abaecin, apisimin and hymenoptaecin were upregulated 72h after infection.

The results presented in this study contribute to a better understanding of the disease, which might help to develop new and less expensive strategies to control EFB in Switzerland and support the idea that breeding more hygienic bee lines is a highly recommended approach to prevent further damage to the Swiss beekeeping industry.

Publications, posters and presentations

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