Abstract

*Paenibacillus larvae* is a Gram-positive sporulating bacterium that causes American foulbrood (AFB). It is one of the most dangerous bacterial pathogens of the honeybee (*Apis mellifera*). *P. larvae* spores are highly infectious to bee larvae and resist physicochemical influences. *P. larvae* is subtyped using repPCR with ERIC primers (Enterobacterial Repetitive Integrance Consensus) into five genotypes (ERIC I-V), which possess different colony morphology, metabolism and especially virulence. There is a significant genetic variability among isolates of *P. larvae*, which may contribute to differences in virulence.

*P. larvae* isolates used in this work were obtained from clinical cases of American foulbrood as well as from debris collected from bee hives with no American foulbrood symptoms from all over the Czech Republic in cooperation with the Beekeeping Research Institute, s.r.o., Dol. The isolates were obtained from larvae and hive debris. Both virulent and avirulent strains were sequenced using the SMRT (single molecule real time) method on the Sequel platform (PacBio). This method is suitable for Whole Genome Sequencing (WGS), because it allows sequencing of long reads with high accuracy, eliminating the effect of a large number of repetitive sequences during the genome assembly. Furthermore, sequencing was also performed on the MiSeq platform (Illumina). Sequences were obtained from 16 strains, which were classified into several groups (clusters). Gene analysis was performed with respect to the occurrence between isolates of ERIC I and ERIC II genotypes. Genes that may have a potential effect on virulence were searched. Strains of *P. larvae* DSM25430 (ERIC II) and DSM7030 (ERIC I) were used for comparison.

Consequently, an insertion sequence IS256 specific for *P. larvae* was also identified. That element occurs in the genome with a large number of copies. Therefore, it can be potentially used for a PCR screening to identify *P. larvae* from hive debris. Two sets of primers were designed and tested in this work on samples of culture of *P. larvae* of four ERIC genotypes.

**Key words:** *Paenibacillus larvae*, virulence, American foulbrood, Whole Genom Sequencing