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Methods of detection and genetic characterization of baculoviruses infecting caterpillars feeding on economically important trees.

Abstract

The *Baculoviridae* family comprises large, rod-shaped viruses with circular, double stranded DNA as a genetic material. They infect larval stages of insects, mainly butterflies (Lepidoptera), sawflies (Hymenoptera) and true flies (Diptera). Baculoviruses are divided into nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) according to their morphology. Major structural occlusion body protein are highly conserved and in NPVs it is called polyhedrin, while in GVs – granulin. The latest classification of *Baculoviridae* family consists of four genera due to genomic sequences and host-dependent evolution: *Alpha-*, *Beta-*, *Delta-* and *Gammabaculovirus*. Alphabaculoviruses are lepidopteran-specific NPVs, while betabaculoviruses are lepidopteran-specific GVs; both are the main interest of my doctoral thesis. Baculovirus genomes ranges in size between 80-180 kbp and encodes 90-180 genes, 38 of which are so called "core genes" – present in every species of *Baculoviride* family.

Baculoviruses have found two main applications - as an efficient protein expression system and - more importantly in the context of my work - as biopesticides for the control of pests invading crops of economic importance. They are safe, specific, selective and do not accumulate in the environment. Although the estimated number of baculoviruses is about 600 species, so far only about 100 of them have been fully sequenced.

The objectives of my doctoral thesis included the development of a method enabling the detection and differentiation of baculovirus species derived from environmental samples and the use of modern techniques for the detection and genetic characterization of baculoviruses isolated from caterpillars of pests of economically important trees.

In my work I have described and analysed full genomic sequences of three alphabaculoviruses – two isolated from gypsy moth caterpillars (*Lymantria dispar* L.) from different regions of Poland – Biebrzański National Park and Rudy, near Racibórz and one that was discovered in caterpillars of pale tussock moth (*Dasychira pudibunda* L.). The LdMNPV- BNP isolate has a length of 157 270 bp, and LdMNPV-RR01 - 159 729 bp, the first encodes - 154 open reading frames, while the second - 166. Phylogenetic analyzes based on amino acid sequences of proteins encoded by 38 core genes and other described genomic features, e.g. the genome structure and the content of homologous repeats or genes encoding atypical proteins such as photolyase in LdMNPV-BNP, or the comparison of nucleotide sequences of other genes (e.g. *polyhedrin*) indicated large differences in both

baculoviruses. LdMNPV-RR01 is very similar to the other nine alphabaculoviruses available in the GenBank database derived from *L. dispar*, while LdMNPV-BNP significantly differs from them. Conducted analyzes may indicate that it is a different species, not just an isolate from the same host.

The third alphabaculovirus DapuNPV is the first genome derived from pale tussock moth, it has the length of 136 761 bp and encodes 161 open reading frames. The analyzes mentioned above performed for this virus, showed that it is very similar to the baculovirus OpMNPV discovered in another geographically distant host - *Orgyia pseudotsugata* (McDunnough). The conclusion is that both of them belong to the same species.

The second part of my doctoral thesis consisted of the development of methods for the detection and differentiation of betabaculoviruses. For this purpose, I selected a representative group of *Betabaculovirus* genus and designed degenerate primers to amplify short fragments of three highly conserved genes – *gran, lef-8* i *lef-9.* The first method is based on the multitemperature single stranded DNA conformational polymorphism (MSSCP) technique, while the second utilizes real-time polymerase chain reaction (real-time PCR). Both methods are fast and inexpensive. They can be used as a screening methods in the control of the stability/genetic variability of betabaculoviruses in the insect populations or for the search of completely new species/isolates.