Streszczenie w języku angielskim

Knowledge of the mechanisms of host-pathogen interactions helps to understand the molecular role of the individual elements of the insect immune system and pathogen virulence factors. It also contributes to the development of natural, medical, pharmaceutical, and veterinary sciences (Suesdek, 2019; Grether and Okhamoto, 2022). The greater wax moth *Galleria mellonella* is an experimental model used in immunobiology research, including analyses of molecular interactions with pathogens (Cook and McArthur, 2013). In turn, *Pseudomonas entomophila* is a bacterial strain isolated from the alimentary tract of the fruit fly *Drosophila melanogaster*. The sequenced bacterial genome was shown to encode a number of potential virulence factors, e.g. potential insecticidal compounds (Vodovar et al., 2006). *P. entomophila* infections occur through the alimentary tract or damaged cuticle. The investigations conducted so far have focused only on general analyses of the mechanism of infection in *D. melanogaster* (Dieppois et al., 2015). No studies have been conducted to date to fully elucidate the mechanisms of interactions of the newly identified bacterium with the insect host.

The research undertaken as part of the dissertation was aimed at investigating the interactions between *G. mellonella* larvae and *P. entomophila* bacteria. Larval survival and changes in the organism of infected insects were analyzed. A decrease in animal survival was observed with the increase of the infectious dose of the bacteria. Based on the analysis of survival curves, two infectious doses were selected, i.e. 10^3 CFU *P. entomophila* (so–called lower dose of the bacteria) and 10^5 CFU *P. entomophila* (so– called higher dose of the bacteria), and used in further studies. The histological analysis of cross–sections of *G. mellonella* larval intestines revealed that the severity of infection was correlated with the dose of bacteria administered to the insects and increased proportionally to the infection time. Intestinal destruction in the advanced infection stage was observed. The monitoring of selected aspects of humoral immune response showed that antibacterial activity in whole hemolymph appeared after infection of the insects with the lower dose of the bacteria. However, the electrophoretic separation of low-molecular proteins of hemolymph and the analysis of their antimicrobial activity demonstrated that they were present in both research groups and exhibited antibacterial properties. The activation of insect immune response after each dose of the bacteria was confirmed by the induction of the expression of analyzed genes both at the local (guts) and systemic (fat body) levels. There was no uniform trend in the expression of genes encoding

immune peptides with regard to the relationship between the dose of the bacteria and the level of gene expression.

Selected bioactive compounds present in the hemolymph of insects infected with bacteria were analyzed and identified, and the properties of selected low-molecular proteins and peptides were investigated. Among them, a number of proteins and peptides were identified, including completely unknown ones or factors whose role in the immune response of insects has not yet been determined. Particular attention was paid to proteins and peptides whose amount declined after the infection with the higher dose of the bacteria, compared with the group infected with the lower dose. This was consistent with the observations of the absence of antibacterial activity in whole hemolymph of larvae infected with the higher dose of the bacteria. This was the most numerous group of all the analyzed groups. Proline peptide 1, an unknown protein– a product of the LOC1135102370 gene (protein 32), and lysozyme were identified in the hemolymph. They were involved in the immune response of the insects, as indicated by the increase in the expression of genes encoding the analyzed proteins and peptides. In the case of protein 32, a decrease in its amount was observed after infection with the higher dose, compared with the other groups (control and administered the lower dose), although the gene expression exhibited a higher level after the infection with the higher dose of the bacteria. Protein 32 may therefore be targeted by *P. entomophila* virulence factors. Moreover, proline peptide 1, protein 32, and lysozyme were shown to be active against *P. entomophila* in *in vitro* conditions. The hemolymph of the infected insects also contained *Gm cationic protein 8* (GmCP8), i.e. an insect receptor polypeptide acting as an opsonin. The level of expression of the gene encoding the GmCP8 protein and its amount was the same in all groups, regardless of the bacterial infection. The investigations showed that this protein had antimicrobial activity *in vitro* and induced changes in the topography and nanomechanical properties of pathogen cell surfaces. These results indicate the role of the GmCP8 protein in the immune response of *G. mellonella*. The analyzed material was also found to contain two polypeptides representing the group of odorant binding proteins whose role in the immune *G. mellonella* response has not been fully elucidated so far. One of them, i.e. the Ebsp3 protein (*Ejaculatory bulb–specific protein 3–like isoform X1*) has the ability to bind pheromones. The expression of the gene encoding Ebsp3 increased only in the material collected from insects infected with the lower *P. entomophila* dose, which corresponded to the analysis of the Ebsp3 protein amount in hemolymph extracts obtained after the infection with this entomopathogen. The analysis of the *in vitro* antimicrobial properties of the protein revealed its activity against

selected microorganisms at very low concentrations and its cytostatic action. Moreover, it was shown that incubation of the Ebsp3 protein with *P. entomophila* post-culture fluid resulted in degradation of the polypeptide, which may suggest its important role in *G. mellonella* immunity. This protein is probably a "target" of *P. entomophila* virulence factors. In the case of the OBP7 protein, i.e. the other analyzed polypeptide from the group of odorant binding proteins, enhanced gene expression was noted in the infected groups as well as its antimicrobial activity *in vitro*. However, the amount of the OBP7 protein in the hemolymph extracts decreased with the increasing *P. entomophila* infectious dose. These results indicate that the OBP7 protein may also be one of the potential targets of entomopathogen virulence.

The research allowed tracing the *P. entomophila* infection mechanism in *G. mellonella* larvae and confirmed the entomopathogenic properties of the analyzed microorganism. The study facilitated identification of a number of poorly known *G. mellonella* proteins and peptides and elucidation of their role in the immune response and antimicrobial potential.