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**Modulacja humoralnej odpowiedzi immunologicznej barciaka większego (*Galleria mellonella*) w przebiegu zakażenia bakteriami *Bacillus thuringiensis***

Modulation of humoral immune response of the greater wax moth (*Galleria mellonella*) during infection with bacteria *Bacillus thuringiensis*

**Summary**

Insects immune system relies only on innate defence mechanisms. This feature has often been turned into an asset, for example, in an investigation on innate immunity mechanisms which were conducted on various insect representatives. One branch of invertebrate immunology focuses on the correlations that occur between the level of the immune system activity and the environmental conditions affecting the infected organism. Among factors influencing the insect immunity, a mechanical injury, a nutrient availability, an ambient temperature, as well as a past or ongoing infections, should be mentioned. Insect studies show that environmental temperature and prior infection with a non-lethal dose of the pathogen can affect the development of immune reactions (see for example: Adamo, 1998; Blanford and Thomas, 1999; Konkel and Tilly, 2000; Arthurs and Thomas, 2001; Elliot *et al*., 2002; Thomas and Blanford, 2003; Ouedraogo *et al*., 2003; Bundey *et al*., 2003; Sadd and Schmid-Hempel, 2006; Pham *et al*., 2007; Linder *et al*., 2008; Roth *et al*., 2009; Rodrigues *et al*., 2010; Catalán *et al*., 2012; Anderson *et al*., 2013; Wu *et al*., 2015; Wu *et al*., 2016). But can these factors (which affect the insect's organism before it becomes infected) alter the level of immune response to the subsequent invasion of the pathogen?

In this thesis I studied the immune response of the greater wax moth *Galleria mellonella* to *Bacillus thuringiensis* infection after previous exposure of these insects to either: the elevated temperature or to the contact with the pathogen. The thesis focused on the humoral aspects of the immune mechanisms. The level of immune system activation was estimated on the basis of immunological parameters such as antimicrobial activity detected in hemolymph and expression of genes encoding immune-related proteins and peptides in fat body of infected wax worms. The level of antibacterial activity was determined by the ability of the hemolymph to inhibit the growth of Gram-negative (*Escherichia coli* D31) and Gram-positive (*Bacillus circulans*) bacteria. Microbial growth inhibition zones were tested not only in full cell-free hemolymph (using diffusion method), but also around some peptides present in protein profiles after hemolymph electrophoresis (using bioautography). Additionally the ability to *Micrococcus* *lysodeikticus* peptidoglycan degradation (so-called lysozyme-type activity) and phenoloxidase activity were checked in full cell-free hemolymph. In general, these parameters revealed the extent of stimulation of *G. mellonella* larvae immune system in response to the given immunogen. The level of defense activity checked in the hemolymph of greater wax moth larvae was analyzed in relation to the expression of genes encoding proteins or peptides having an antimicrobial properties, or proteins recognizing and opsonizing the pathogen. Among them cecropin, gallerimycin, galiomycin, anionic peptide II, insect metalloproteinase inhibitor (IMPI), hemolin, and apolipophorin III (apoLp-III) are worth mentioning. Expression of these polypeptides was estimated on the basis of the amount of mRNA transcripts (detected by the reverse transcription and Real-Time qPCR) in the fat body of treated insect. Furthermore the one- and two-dimensional protein profiles and the profiles of low-molecular polypeptides (which were obtained using SDS-PAGE and IEF SDS-PAGE or Tris-tricine electrophoresis respectively) were compared. After one-dimensional electrophoresis the amount of apoLp-III was also analyzed - in this case specific antibodies that recognized this protein were used (in so-called immunodetection).

The obtained data allow to conclude, that both temperature fluctuations and previous immunological experience can significantly modulate the resistance of *G. mellonella* larvae to subsequent infection with lethal dose of *B. thuringiensis*. However, depending on the used immunomodulator (elevated temperature or prior contact with the pathogen) parameters of the humoral response were altered in a various way. Exposure of the greater wax moth larvae to the elevated temperature directly before pathogen application, resulted in increased antibacterial activity in full cell-free hemolymph - in comparison to the level of this activity observed in infected larvae reared constantly at optimal temperature. This evidence correlated with the enhanced expression of genes encoding defense peptides (cecropin, gallerimycin, galiomycin) in the fat body of insects subjected to temperature stress just prior to infection (in comparison to the expression of these genes in the fat body of infected larvae kept at optimal conditions. The exposure of wax worms to heat stress directly before injection of *B. thuringiensis* did not affect the expression of the IMPI gene and the apoLp-III gene in the fat body of the studied insects. On the other hand, previous contact of *G. mellonella* larvae immune system with pathogen cells increased the level of antibacterial activity during reinfection with *B. thuringiensis*. That effect was not due to the increased expression of genes encoding the given immune peptides (cecropin, gallerimycin, galiomycin). The analysis of phenoloxidase activity in *G. mellonella* larvae hemolymph showed no significant differences between the insects injected with *B. thuringiensis* for the first time and the insects infected again. Moreover, it has been shown that the modulation of some aspects of the *G. mellonella* larvae humoral response, as a result of their previous contact with the pathogen, was dose- and virulence-dependent. The phenomenon of enhanced resistance of the greater wax moth larvae to subsequent infection with *B. thuringiensis* was associated with the so-called immune priming. Priming of invertebrate immunity is related to the development of a kind of "immunological memory" in re-infected insects.

Presented data seemed to be a certain contribution to the knowledge of the insects immunity alteration by the environmental conditions and factors. This thesis indicates that insects immune response depends on the various environmental factors acting on the particular immune system mechanisms.

**Key words:** *G. mellonella*, *B. thuringiensis*, modulation of immune response, temperature stress, immune priming

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