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VERSITA

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Galleria mellonella infected with *Bacillus thuringiensis* involves Hsp90

Communication

Iwona Wojda*, Patryk Kowalski

Department of Immunobiology,
Faculty of Biology and Biotechnology,
Institute of Biology and Biochemistry,
Maria Curie-Sklodowska University,
20-033 Lublin, Poland

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Abstract: Insects are good models for studying the innate immune response. We report that *Galleria mellonella* larvae infected with entomopathogenic bacteria *Bacillus thuringiensis kurstaki* show changes in the level of Hsp90. Our experimental approach was to pre-treat larvae with the Hsp90- binding compound, 17-DMAG, before infection with *B. thuringiensis*. We show that pre-treated animals display a higher level of immune response. This was mainly manifested by enhanced action of their hemolymph directed toward living bacteria as well as lysozyme activity digesting bacterial peptidoglycan. The observed phenomenon was due to the higher activity of antimicrobial peptides which, in contrast to healthy animals, was detected in the hemolymph of the immune-stimulated larvae. Finally, the physiological significance of our observation was highlighted by the fact that *G. mellonella* pre-treated with 17-DMAG showed a prolonged survival rate after infection with *B. thuringiensis* than the control animals. Our report points to a role for Hsp90 in the immune response of *G. mellonella* after infection with *B. thuringiensis* at the optimal growth temperature.

Keywords: *Bacillus thuringiensis • Galleria mellonella • Hsp90 • Insect immune response*

1. Introduction

The insect immune response comprises cellular and humoral events. The former involves hemocytes, which efficiently phagocytose most microorganisms. Larger pathogens such as parasitoid wasp eggs are encapsulated by blood cells and melanin. In contrast, infecting microorganisms activate a humoral immune response. This consists of proteins involved in clotting, melanisation and synthesis of antimicrobial peptides (AMPs). In the well-known insect model *Drosophila melanogaster,* two pathways recognize different classes of pathogens and activate expression of antimicrobial peptide genes. In general, the Toll pathway usually responds to Gram-positive and fungal infections, while the Imd pathway recognizes Gram-negative bacteria [1]. The structural basis for distinguishing between Gram-positive and Gram-negative bacteria is a cell wall constituent, peptidoglycan. The Toll pathway is mainly triggered by lysine-type peptidoglycan, while the Imd pathway is activated upon recognition of diaminopimelic acid-containing peptidoglycan (DAP-type) [2]. In the case of fungi, β-glucan serves as a PAMP (pathogen-associated molecular pattern) [3]. The Toll and Imd pathways both activate transcription factors, Dif and Relish, respectively, which are homologs of mammalian NF-κB. In response insect fat body synthesises and releases into the hemolymph peptides with antimicrobial properties. Insect defence peptides possess distinct activities directed against bacteria and fungi. Many antimicrobial peptides and other immune-related proteins have also been described in the greater wax moth *Galleria mellonella* [4-11]. This insect model is widely used for studies concerning different aspects of host-pathogen interactions [12-18].

Bacillus thuringiensis is a Gram-positive bacteria commonly found in soil and many other sources. Interestingly, its cell wall contains DAP-type peptidoglycan, typical for Gram-negative bacteria. *B. thuringiensis* is well known for its insecticidal

* *E-mail: wojda@hektor.umcs.lublin.pl*

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