

PH.D. Theses

Mechanisms of *Galleria mellonella* cellular immune response after infection with entomopathogenic fungus *Conidiobolus coronatus*

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More than half of all living species on the Earth are insects. Most of them reproduce very quickly, use diverse sources of food and possess an efficient system of biological defense against pathogens. Thanks to these abilities insects may be found in nearly all environments. Many insects are considered as pests by humans, as they can transmit diseases to humans and livestock, destroy crops and cause economic losses. Therefore, researchers are involved in developing new methods of pest control without the use of chemical insecticides. Nowadays, methods of biological pest control with the use of bioinsecticides are gaining popularity. Biocontrol uses the natural enemies of insects (bacteria, viruses, nematodes, entomopathogenic fungi) to reduce the population of pests.

In this work the emphasis is placed on entomopathogenic fungus, which presents high toxicity and acts specifically on a rather narrow range of hosts. On the market, some fungal bioinsecticides are available, however they contain living organisms (hyphae, spores) which can lead to uncontrolled fungal infections. Hence, an effort is being made to replace the living cells with bioactive ingredients from fungi. In the course of these investigations the effect of different fungal metabolites on insects cells is investigated.

As a model for studying insect immunology and fungal infections the larvae of the wax moth *Galleria mellonella* were chosen. *Conidiobolus coronatus* was chosen as the entomopathogenic fungus, a soil fungus from the collection of Prof. Bałazy (Polish Academy of Sciences, Research Center for Agricultural and Forest Environment, Poznań). All fungus-insect interactions (*C. coronatus* induces 90% mortality of *G. mellonella* larvae) were analyzed using these models.

Insects are not helpless in the face of various pathogens, they build up sophisticated and effective defense systems against numerous microorganisms, including pathogenic fungi. The first barrier which parasites come across is the cover of the body i.e. the exoskeleton (protein, chitin and lipids). Invaders that are able to penetrate successfully into the insect hemocel encounter several innate cellular and humoral reactions that are tightly connected. One of the most important strategies in the insects defense system is the cellular reaction which is based on hemocytes (immune competent cells) circulating in the body cavity. Hemocytes are able to distinguish between self and nonself structures. The cellular immune response comprises distinct mechanisms, phagocytosis, encapsulation, nodule formation, antimicrobial proteins synthesis. Melanization and clotting of foreign structures also occur with the participation of hemocytes. Classification of these

cells is based on morphological characteristics and biological functions but is still not consistent. The research aim of this work was to study insect cellular defense reactions during the confrontation between the insect host and the entomopathogenic fungus.

During this study five types of *G. mellonella* hemocytes have been described and classified: prohemocytes, plazmatocytes, granulocytes, spherulocytes and oenocytes. Additionally, the plasmatocytes were divided into four subclasses with regard to morphology and function. The behavior of hemocytes cultured in vitro was observed for two weeks, after which time the culture began to die. Hence, the best results should be obtained during first 48 hours of cell incubation. Working on these cells also revealed evidence of crosstalk between hemocytes and other components of the immune system, and gave information about their role during fungal infection. To understand the connections between these cells in the study, an attempt was made to separate them into individual classes with the use of a flow cytometer connected to a FACSAria sorter, unfortunately, this method failed as hemocytes are very fragile and sensitive to mechanical manipulation. Therefore another solution needs to be found for cells separation.

Although the molecular basis for antifungal immunity in insects is becoming clearer the mechanisms responsible for killing insect cells are still poorly understood. Changes in the morphology of hemocytes after fungal infection in vivo was observed, the main disorder was reorganization of the cytoskeleton (poor adherence, non specific cell shape and disruption of actin fibers) and increased cell death. The next step was focused on establishing the cause and pathways of premature hemocyte death (apoptosis/necrosis) after contact with the entomopathogenic fungus. Active caspase-3-like proteins were identified in hemocytes from infected larvae, which confirmed the presence of apoptotic cells.

Another goal of this work was to select the compounds most toxic to hemocytes from the fungal metabolites already isolated in our laboratory. In the course of our previous investigations, some insecticidal proteins and low mass metabolites which have deteriorating effects on insect defense system have already been partly classified. These substances were tested on *G. mellonella* hemocytes cultured in vitro. To confirm the action of these substances, they were also tested on the continuous insect cell line Sf9. Of the whole group of fungal metabolites, the most active was selected: a fraction named Ał3. *G. mellonella* plasmatocytes were stimulated to mitotic divisions by Ał3 and culture could survive up to two months. This fraction showed strong toxic effects on Sf9 cells causing fast cell lysis resulted in low cell viability.

The prospects of using the Ał3 fraction in order to create bio-insecticides safe for humans animals and the environment seems to be highly probable and a new generation of insecticide could be introduced on the market in the near future.

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