1. SUMMARY

Invertebrates are widely distributed animals. They can be found in almost any kind of habitat. Their successful survival strategies are based on short life span combined with numerous offspring and, more importantly, all invertebrate species have developed a variety of defense mechanisms efficiently recognizing and responding to non-self substances.

The defense mechanisms of earthworms were studied over the past four decades. It became apparent that the earthworms, as well as other invertebrates, lack specific immunoglobulins, lymphocytes or other features of the adaptive immune system described in vertebrates, but possess innate defense components.

In this thesis, we focused on the detail description of some defense molecules involved in innate immunity of earthworms.

1. **Lysozyme** is an enzyme with strong antibacterial activity described in many organisms. We characterized the lysozyme of *Eisenia andrei* (formerly *E. fetida andrei*) earthworm both structurally and functionally. Molecular characterization of lysozyme provides a new tool for monitoring of innate immunity in earthworms.
2. A cytolytic effect of the coelomic fluid of *E. fetida* was observed in experiments with TNF-sensitive tumor L929 cell line. Subsequent isolation of lytic proteins led to the identification of 42-kDa protein, which was named **coelomic cytolytic factor** – CCF. CCF was shown to be present also in coelomic fluid of another earthworm species, *Lumbricus terrestris*. Therefore we elucidated the presence of CCF-like molecules also in other earthworms from *Lumbricidae* family, characterized their primary sequence and compared the biological properties of these molecules.
3. CCF was described to share functional analogies based on the similar saccharide recognition specificity with mammalian cytokine, tumor necrosis factor (TNF). Both TNF and CCF were shown to induce an increase of membrane conductance in some mammalian cells resulting in membrane depolarization. We investigated the interaction of CCF with peritoneal macrophages and subsequent activation of these cells.
4. The role of calreticulin in the defense mechanisms was previously described in both vertebrates and invertebrates. We proved the presence of **calreticulin** in the coelomic fluid of *E. fetida* earthworms, characterized the primary sequence and determined the expression in different organs.
5. We clarified the relationship between two hemolytic molecules – **lysenin and fetidin** and determined the level of their expression in coelomocytes of individual *E. fetida* earthworms.
6. The prophenoloxidase cascade represents one of the most important defense mechanism in many invertebrates. We proved the presence of **phenoloxidase and its putative inhibitor** in the coelomic fluid of *E. fetida* earthworms, but the level of phenoloxidase activity is lower as compared to other invertebrate species. Moreover, the activation of prophenoloxidase cascade is slower.