

The association of insects with bacteria (symbionts) is very important for the evolution and/or feeding habits of insects. Insects-associated bacteria may, for example, participate in: a) the plant–insect interaction, b) xenobiotics detoxification or in c) the development of insect host insecticide resistance. Insect may also use microbiota–related enzymes to overcome enzyme inhibitors produced by plants in response to insect feeding. Relationship between the insect and its microbiota remains poorly known for many species, including cereal leaf beetle (CLB, *Oulema melanopus*, Coleoptera, Chrysomelidae) - a serious pest of cereals. Both adults and larvae damage grain crops by feeding on leaves. The application of different chemical groups of insecticides is the main method of controlling CLB population. But the chemical protection today and in the future may not be as efficient as formerly. Intensive and continuous application of the same organic pesticides can lead to the evolution of insecticide resistance. Thus, there is need of alternative, more environmentally friendly, methods for the control of insect pests.

The **aims of project are:**

- Checking the degree of CLB resistance to insecticides.
- Determination of microbial symbionts associated with CLB responsible for the evolution of insect resistance to insecticides (insecticide-degrading bacteria).
- Analysis of changes in bacterial community upon insecticide treatment of CLB larvae.
- Checking if CLB – associated bacteria are involved in the adaptation of their insect host to the protease inhibitors (PIs) of plants.

The involvement of CLB – associated bacteria in the adaptation to insecticide or protease inhibitors exposure is also unknown. Therefore the following tasks are planned to understand the role of CLB symbionts in detoxification of harmful substances to their insect host.

a) Analysis of the impact of insecticide on the CLB larvae (with natural gut microflora or reduced bacteria content). Larvae will be treated with: a) appropriate active substance of insecticide, b) only with antibiotics, c) first with antibiotics followed by active substance of insecticide, d) water (control). Insect mortality will be recorded.

b) Identification of the best growing bacteria after insecticide treatment will be made. In addition, the active substance of insecticide degradation by the selected best growing bacteria will be assessed by using GC- and LC-MS/MS analysis (depending on active substance of insecticide).

c) Evaluation of the effect of insecticide treatment on the bacterial community associated with CLB. CLB larvae will be treated with different doses of the tested active substance of insecticide (water treatment-control). DNA extracted from guts of each treatment will be subjected for amplicon library construction followed by NGS to identify CLB-associated bacteria after treatment with active substance of insecticide.

d) Checking whether CLB use bacterial-related enzymes to overcome the harmful effects of protease inhibitors. Insects will be treated with antibiotics (water treatment - control), next with selected protease inhibitors. The profile of protease activity will be analyzed by using: FITC-casein assay and *in-gel* protease assay.

e) Phylogenetic and statistical analysis of obtained data.

Explaining the participation of the insect microbiota in the degradation of xenobiotics, including the synthetic organic compounds raises important issues. Obtained results will provide the basis for implementation of new strategies to control the insect pest, including CLB by manipulating their gut microbiota in the future. They can also have an impact on reducing the amount of insecticides used, environmental pollution and harmful effects on non-target insects. Proposed studies may have a significant impact on such disciplines as agriculture, entomology, plant and environmental protection.