CHARACTERIZATION OF *XANTHOMONAS* SPP. ISOLATES OBTAINED FROM FABACEAE PLANTS

Dovilė Čepukoit[1*], Karolis Sivickis[1], Monika Kałużna[2], Daiva Burokienė[1]

[1]Nature Research Centre, Laboratory of Plant Pathology, Vilnius, Lithuania
[2]Research Institute of Horticulture, Department of Phytopathology, Laboratory of Bacteriology, Skierniewice, Poland
dovile.cepukoit@gamtc.lt

Xanthomonas genus bacteria are plant pathogens that can cause a variety of diseases, including necrosis, vascular or parenchymatous diseases on leaves, stems or fruits of many plants. Various species of these pathogenic bacteria are extensively studied due to their ability to cause economic, industrial and ecological losses. Recently, the classification, genetic diversity, and phylogenetic relationships are being studied more extensively. The aim of this study was to identify and to characterize the species of *Xanthomonas* genus using molecular methods and to estimate which species of these pathogenic bacteria may cause serious diseases on Fabaceae plants in Lithuania.

During the 2017-2018 plant material from different species of Fabaceae was collected. In this study more than 280 bacterial isolates were obtained. The yellowish bacterial colonies were tested for Gram stain and Gram-negative isolates were selected for further molecular studies. For genetic characterization DNA from bacterial isolates was extracted using CTAB method [1] and protocol of Aljanabi and Martinez [2]. *Xanthomonas*-like isolates were analyzed by PCR using genus specific primers. Phenotypic characterization of *Xanthomonas* isolates was confirmed using pathogenicity and hypersensitive reaction tests on potato, tomato and tabaco plants. Genetic diversity and phylogenetic analysis with four molecular methods was performed: three enzymes for PCR melting Profile (PCR MP) were used; repetitive PCR (rep-PCR) with primers for BOX, ERIC and REP were performed, dendrograms were constructed using FREETREE software; four housekeeping genes for multilocus sequence analysis (MLSA) of *Xanthomonas* spp. bacteria were used, sequences were analyzed using the SeqMan software package LASERGENE (DNASTAR, Madison, USA), following the results phylogenetic trees were constructed with the MEGA 7 software package; for detection of type three effector (T3E) genes, PCRs with 15 primers were performed. Furthermore, we completely investigated only a small part of the bacterial collection and the other part is still under investigation.

---

[1] Wilson, K., 2001: Preparation of genomic DNA from bacteria. Current protocols in molecular biology, 56(1): 2-4.
[2] Aljanabi S. M., Martinez I., 1997: Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic acids research, 25(22): 4692–3.