# SHORT TERM SCIENTIFIC MISSION (STSM)
# SCIENTIFIC REPORT

**This report is submitted for approval by the STSM applicant to the STSM coordinator.**

**Action number: CA16107**
**STSM title: Complete genome sequence analysis of lytic bacteriophages infecting *Xanthomonas arboricola* pv. *juglandis***
**STSM start and end date: 16/02/2020 to 06/03/2020**
**Grantee name: Dr. Katarina Gašić**

## PURPOSE OF THE STSM

Bacterial blight caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj) is widespread and one of the most important diseases of walnut worldwide. The bacteria attack leaves, nuts, buds, catkins and young twigs. When weather conditions favour spread of the pathogen, losses in walnut production can exceed 80%. The limited efficacy of current disease control strategies including spraying of copper-based bactericides, certainly contributes to the economic importance of this disease. The development of copper or streptomycin resistance by the pathogen and increased public concern about detrimental effects of pesticide residues, initiated efforts in searching for alternatives in control of walnut bacterial blight.

Biological approach in the disease control might be a potential solution and substitute for available bactericides of poor efficacy. Bacteriophages, viruses that infect bacterial cells, have regained attention as natural antimicrobial agents to fight bacterial diseases of plants. During 2019, eleven bacteriophage strains specific to Xaj were isolated from rhizosphere of walnuts trees, aerial tissue of walnut plants and irrigation water in Serbia. The objective of the STSM was sequencing and analysis of the genomes of selected Xaj specific bacteriophages.

## DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

Eleven bacteriophage strains infecting Xaj were previously isolated form different locations and sources in Serbia. Extraction and purification of phages' genomic DNA was done using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Germany), according to manufacturer's instructions. DNA concentration was measured by the Qubit 3.0 fluorometer and Qubit double-stranded DNA (dsDNA) high-sensitivity kit (Thermo Fisher Scientific, Waltham, MA). Genomic sequences of eleven Xaj phages was performed with a MiSeq next-generation sequencing platform (Illumina, Inc., USA). The DNA library was prepared using the Nextera XT kit (Illumina Inc.) for paired-end library preparation and Illumina V2 sequencing kit (Illumina Inc.), according to the guidelines of the manufacturer. The next-generation reads were analysed for quality using the FastQC program (Babraham Bioinformatics, version 0.11.5), with default parameters. Low-quality bases and reads were trimmed and/or removed using the Trim Galore! (Babraham Bioinformatics, version 0.4.4 with paired mode). The quality filtered reads were assembled using the MyPro software package. In the assembly processes, we used the Assembly.py and Integrate.py python scripts for all samples.

Moreover, during STSM we performed some additional tests in order to study phage characteristics. Lytic activity of eleven phages was tested using four Xaj strains. The host bacterium was cultivated in a liquid King's B medium (KB) until the bacterial growth reached the mid-exponential phase. Phages were added at approx. MOI of 1. KB without bacteria was used as negative control, since inoculated KB without phages

used as a positive control. Cultures were incubated at 28°C for 48 h with medium shaking. Bacterial growth was monitored in a Synergy HT multimode reader (BioTek, Winooski, VT). The reader was set to automatically measure the optical density at 600 nm (OD600) of culture samples at 15 min intervals. Experiments were performed in six replicates and reproduced in three independent trials.

In order to study phages' morphology, phage samples were prepared and examined by transmission electron microscopy following a negative staining protocol using. Phage suspension was spotted on a 200 mesh formvar coated copper grid, stained with 3 % (w/v) sodium phosphotungstate. The phages were observed at various magnifications by JEOL JEM-1200EX II transmission electron microscope operating at an acceleration voltage of 64 kV.

**DESCRIPTION OF THE MAIN RESULTS OBTAINED**

Total of eleven phage strains was used for whole genome sequencing procedure. We were able to prepare libraries and sequence on the Illumina Miseq platform all phage DNA samples. The complete genomes of all Xaj phages were assembled. The genome size of the phages ranged between 40 and 60 kb. In a few phage genomes we detected direct terminal repeats, with a self-dot plot using Geneious software. More detailed analysis of the sequenced genomes of phages is in progress.

All phages showed lytic activity to its host Xaj strains. Significant differences were observed between the growth kinetics of untreated control cells and phage-treated cells. However, we detected differences in lytic activity of different phage strains.

By using transmission electron microscope, we classified phage strains in the order *Caudovirales*, family *Podoviridae, Siphoviridae* or *Myoviridae.*

**FUTURE COLLABORATIONS (if applicable)**

Collaboration with the research group of Dr. Tamas Kovacs will be continued through the joint research on Xaj phages. We will continue analysis of Xaj phage genomes by performing phage genomes annotation. Comparative analysis of studied phage strains with others from NCBI GenBank will be performed, as well as phylogenetic characterization based on comparison of different genes. In order to prove that phage strains are safe for use as biocontrol agents, different bioinformatics tools will be used to search for the potential presence of toxin genes and allergens in phage genomes.

I am very grateful to Dr. Tamas Kovacs and to his associates for sharing their knowledge and experience on phage research topic, as well as for their hospitality and successful completion of my STSM. I also thank the COST Action CA16107 EuroXanth for the financial support.