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: Multilocus sequence typing (MLST) for characterization and subspecies identification *Xylella fastidiosa* strains
STSM start and end date: 11/03/2018 to 16/03/2018
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PURPOSE OF THE STSM

Xylella fastidiosa, originally confined to the Americas, is a quarantine plant pathogenic bacterium now emerging in Europe and Asia. It is a genetically and phenotypically diverse species subdivided into several subspecies. There are also different genotypes within each subspecies. In reported findings in Europe different subspecies of *Xylella fastidiosa* were found. To a certain extent different subspecies are limited to different host plants, therefore when *Xylella fastidiosa* is detect in the country or in new region it is very important to know which subspecies and genotype is causing the disease because it is based on this information that the plant health authorities decide on the extent of the measure, i.e. which host plants will be included in the measures.

Within the species *fastidiosa*, multi-locus sequence typing (MLST) is often used as the method of choice to study within-species diversity. The purpose of the STSM was to learn how to perform MLST analysis on the pure culture of different *Xylella fastidiosa* strains and on the different plant extracts infected with *Xylella fastidiosa,* how to analyze and interprete the obtained data using different software programs, and how to compare data to publicly available databases.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

One of the most important thing in detection and identification of *Xylella fastidosa* is how the sample extracts from plant material are prepared. The extracts are prepared differently for isolation on the media and for molecular detection with qPCR and for MLST analysis. During this STSM both approaches were shown and practiced on 25 naturally infected plant samples from different host plants. Extracts prepared for isolation on the media of *Xylella fastidiosa* were plated on BCYE medium. Extracts for molecular analysis were prepared for further molecular analysis.

After this work it was shown how PCRs for MLST are prepared in the INRA research group, which modified the method published in EPPO PM 7/24 (2) protocol for *Xylella fastidiosa* detection for increased sensitivity and reliability of the method. For pure cultures, the published method is good enough, but the main problem is that *Xylella fastidiosa* is a slowly growing bacterium and often isolation of a pure culture from the plant extract fails. There are attempts to do MLST analysis also on the isolated DNA from plant extracts. For some combinations of host

plants and subspecies this works well, but for some extracts, the method is not working. Therefore, the INRA research group developed new external primers to perform nested PCR with already published primers that were used as inner primers. Some of the inner primers were also modified to obtain more reliable results.
Then the work were mainly done on the analysis of the sequence data obtained by the INRA research group from different *Xylella fastidiosa* subspecies with the different freely available softwares to show correct interpretation of obtained data.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Because of the time scale of this STSM overall results of naturally infected host plants were not possible to be obtained, but will be provided from INRA researchers to me to see if the isolation of *Xylella fastidiosa* from plant material was successful.

Therefore, the analysis of MLST data was performed on previous data that INRA researchers obtained in combination with available data in the PubMLST database (https://pubmlst.org/). The raw sequences of different housekeeping genes were first assembled to obtain consensus sequences and then trimmed to the length of sequences available in public databases. Different tools, such as MEGA software, were presented to perform these steps. Then the use of PubMLST database (https://pubmlst.org/) and available tools on this website were performed to identify the sequence type of the analyzed strains. Additional analyses were performed using different freely available online tools, such as eBURST (http://eburst.mlst.net/) and PHILOViZ (https://online.phyloviz.net/index), where algorithms predict the descent from the predicted founding genotype to the other genotypes in the group.

Overall, the STSM included all steps of MLST analysis that is needed to implement the method in the laboratory. Most of the necessarily needed standard operating procedures are already prepared.

FUTURE COLLABORATIONS (if applicable)

The laboratories will exchange the data obtained during this STMS and will be in further contacts for additional collaborations.