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: Skills exchange-towards learning the standardized methods for *Xylella fastidiosa* and plant endophytic bacteria isolation and detection
STSM start and end date: 01/10/2020 to 18/12/2020
Grantee name: Ana Cláudia Pereira da Silva

PURPOSE OF THE STSM

Xylella fastidiosa is the causal agent of several diseases in crops of great socio-economical value, such as olive, almond, and oak trees worldwide, and in particular in Mediterranean countries such as Italy, Spain and Portugal. In 2019, the presence of *Xylella* was officially confirmed in Portugal. Despite the great threat of *Xylella* to the Portuguese agricultural sector, research in this area is almost inexistent and should be promoted. The home institution, InnovPlantProtect CoLab (InPP), is a newly established collaborative laboratory in Portugal that aims to develop strategies for the control of different plant pathogens, including *X. fastidiosa*. To develop this project the InPP aims to create a laboratory and greenhouse facilities with the biosafety conditions required to work with quarentine organisms. The host laboratory of Dr. Blanca Landa at the Institute for Sustainable Agriculture, Spain, is one of the reference laboratories in Europe for the study of olive microbiome and of *X. fastidiosa* pathosystem, and accounts with knowledge and facilities for their study. This internship aims to provide me hands-on training on the standardized methods for *X. fastidiosa* isolation and detection, as well as to introduce me to technologies, methods and tools that can be implemented at the home laboratory.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

During the STSM, different tasks were performed involving the isolation and detection of *Xylella fastidiosa* as well as identification of different subspecies. The initial part was aimed at getting acquinted in working in a biosafety level 2 environment, including understanding all the requirements of a biosafety level 2+ laboratory (structure, material needed, equipment, etc) and to learn the different standard operating procedures to work there, including the procedures on how to enter and to leave the laboratory as well as how the entry and exit of plant material is operated and logged.
For the isolation of *X. fastidiosa* from plant material, a protocol that had been optimized at the host institution was carried out. Standard protocols for bacteria growth and DNA extraction from *X. fastidiosa* growing in culture plates were also performed. In fact, DNA that I extracted from different *X. fastidiosa* strains were sent to the home instititution (InPP, Elvas) so that we can isolate and clone specific genes of interest to the project that is going to initiate soon.
For *Xylella fastidiosa* detection, I was shown different protocols for plant and insect extraction as well as protocols that are used at the host laboratory and recommended by EPPO to detect the presence/absence of the bacteria in different plant material (Harper et al., 2010; Ouyang et al. 2013).

Considering identification: different protocols that are used for the identification of different *X. fastidiosa* sequence types (ST) were also performed, based on both conventional MultiLocus Sequence Typing (MLST; Yuan et al., 2010) and Nested MLST (Cesbron et al., 2020).
Infection of woody hosts with *X. fastidiosa*, as proposed in the work plan, was not performed due to the fact these experiments were not being carried out at the moment. Although, knowledge on how to extract *X. fastidiosa* and plant DNA from olive leaves, for posterior detection of the presence or absence of the bacteria in that tissue, was acquired.
Another aim was to acquire experience in extracting and handling endophytic bacteria. For that, xylem sap was extracted from olive branches using a Scholander pressure chamber and aliquots of the xylem extracts were cultured in specific media and daily observed for any bacterial growth.
During the time at Dr. Blanca´s laboratory I have compilled and written a series of protocols based on the experiments that I was performing there and that were sent to my home institution so that the work of acquiring reagents and any specific equipment for our *Xylella* project could be initiated.

References:

Cesbron et al., 2020, *Agronomy* 10: 1099; https://doi.org/10.3390/agronomy10081099.
Harper et al., 2010, *Phytopathology* 100: 1282-8, doi: 10.1094/PHYTO-06-10-0168.
Ouyang et al., 2013, *PLoS One* 8: e81647, doi: 10.1371/journal.pone.0081647.
Yuan et al., 2010, *Phytopathology* 100: 601-11, doi: 10.1094/PHYTO-100-6-0601.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The main purpose of this STSM was to get acquainted with working with the quarantine bacteria *Xylella fastidiosa* in a biosafety laboratory level 2 environment and to gain knowledge on the different methodologies routenily used at the host laboratory for *X. fastidiosa* isolation, detection and identification. For that, I had the opportunity to learn and perform different protocols for this purpose that can be later be used at my home institution. Getting experience in working in a biosafety level 2 environment was particularly relevant, as we aimed to construct this type of facility there.
As the main results, we have performed DNA extraction from *X. fastidiosa* from three different strains (De Donno, Temecula and CFBP 8416) growing in culture plates. An A_{260}/A_{280} ratio of 1.71-1.88 was obtained, indicative of the good DNA quality. The DNA was sent to the home institution and will be particularly valuable for starting one of the main projects considering the study of an effective biopesticide against *X. fastidiosa*.
A protocol for the isolation of *X. fastidiosa* from plant material was carried out using plant material (leaves and branches from *Santolina chamaecyparissus* and *Cistus albidus*) coming from the Spanish islands. After approximately 10 days, colonies that might correspond to *X. fastidiosa* were observed, although different steps of isolation are still being executed in order to isolate single colonies to confirm if they belong to that species.
Different protocols were also learned and used for the identification of different *X. fastidiosa* sequence types (ST) based on both conventional MultiLocus Sequence Typing (MLST; Yuan et al., 2010) and Nested MLST (Cesbron et al., 2020). Using the former protocol, we were able to identify, in six different samples, two different STs, ST 80 and ST 81, corresponding to *X. fastidiosa* subsp. *pauca* and *X. fastidiosa* subsp. *multiplex*, respectively.
To acquire experience in isolating plant endophytic bacteria, xylem sap extraction from olive branches using a Scholander pressure chamber was performed accordingly to Anguita-Maeso et al. (2020). Aliquots of the xylem extracts were cultured in culture medium R2A. After which, a daily observation of the plates was carried out in order to observe bacterial growth. Due to time restrictions, the isolation of the bacteria present in the plates was not achieved, although the protocols needed to complete the following tasks were observed as well as the bioinformatics tools used for the analysis and identification were explained.

References:

Anguita-Maeso et al. 2020, *Front Plant Sci* 10: 1708, doi: 10.3389/fpls.2019.01708.
Cesbron et al., 2020, *Agronomy* 10: 1099; https://doi.org/10.3390/agronomy10081099.
Yuan et al. 2010, *Phytopathology* 100: 601-11, doi: 10.1094/PHYTO-100-6-0601.

FUTURE COLLABORATIONS (if applicable)

With the knowledge gained during this STSM, we will be able to set up at the home institution our own laboratory and greenhouse facilities with the biosafety conditions required to work with *X. fastidiosa*. Moreover, this exchange has also provided myself, the home and the host laboratory a wider scientific network that might prove fundamental in future collaborations and funding applications due to the common scientific interests between the two institutions as well as their geographical proximity.