SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: 16107 EuroXanth
STSM title: Validation of a new LAMP assay for the identification and detection of *Xanthomonas campestris* pv. *campestris* in brassica plants and seeds using the ICGENE system**.**

STSM start and end date: 20/11/2017 to 08/12/2017
Grantee name: Patrizia Bella

PURPOSE OF THE STSM

Xanthomonas campestris infecting *Brassicaceae* plants was divided in six pathovars: *campestris*, *aberrans, armoraciae, barbareae, incanae* and *raphani*. Recently, the results of pathogenicity tests and MLSA assay suggested the presence of only three pathovars inducing different diseases: black rot of crucifer crops caused by *X. campestris* pv. *campestris* (*Xcc*), leaf spot disease caused by *X. campestris* pv. *raphani* (*Xcr*) on both *Brassicaceae* and *Solanaceae* and bacterial blight induced by *X. campestris* pv. *incanae* (*Xci*) on ornamental plants. Black rot caused by *Xcc* is one of the most severe seed-borne diseases of *Brassicaceae* involving economically important species of crop vegetables, ornamentals and weeds. This disease has a worldwide distribution and typical symptoms are V-shaped yellow to dark-brown lesions starting from the margin of the lower leaves and blackening of the veins. Based on the response on eight differential Brassica lines, nine physiological races were identified within *Xcc.* Conventional PCR and real-time PCR protocols targeting different genes (*hrpF* gene, *hrcC* gene, and an unknown conserved sequence within the *Xcc* genome) were developed to identify the bacterium and for its detection in plants and seeds.
In recent years, loop-mediated isothermal amplification (LAMP) has emerged as a popular tool for pathogen detection in different phytopathological systems. It is a rapid isothermal method to amplify DNA by using a set of four to six primers and a polymerase with a strand displacement activity. This technique allows a greater level of specificity compared with conventional PCR; it is less sensitive to inhibitors and is characterized by easy handling and high reliability. Furthermore, it can be performed in a heat block or water bath, thereby removing the need for specialized equipment. In addition, positive amplification can be observed by colorimetric or fluorescent dyes removing the need to run gels.

The aim of this STSM was to validate a kit and a protocol for the identification and the detection of *Xcc* in plant samples and in seeds by using the ICGENE system that was developed by an Italian company, Enbiotech s.r.l. (Palermo, Italy; http://enbiotech.eu/en) based on LAMP technology. This company has signed a Memorandum of Understanding with the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy) and has provided the device (ICGENE mini) and all ready-to-use reagents to perform the LAMP assay. The STSM was carried out at the School of Life Sciences, Wellesbourne Campus, University of Warwick at the laboratory of Professor E. Holub and J. Vicente where a collection of *X. campestris* strains was available to validate the LAMP assay.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

LAMP assay was performed with the ICGENE mini instrument and the kit developed for *Xcc* provided by the Enbiotech. The kit is composed of ready-to-use reagents for a simplified and rapid DNA extraction, target gene amplification, real time detection of the fluorescence emitted from the sample and automatic interpretation of the final results. All samples analysed during the STSM (bacterial strains, leaf and seed samples) were subjected to the same protocol according to the company instruction.

The activities to validate the kit were scheduled in three phases: a) defining the analytical specificity of the assay using target and non-target samples; b) defining the analytical sensitivity of the assay; c) detection of *Xcc* in naturally infected leaves and seed lots.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The LAMP assay by using the Kit and the instrument provided positive results *Xcc* strains belonging to the nine races isolated from different countries.
One infected seed among 10 000 healthy seeds was detected using LAMP assay and no amplification were obtained from non-inoculated Kale seeds in about 30 min. *X. campestris* was detected in all leaf samples both from leaf extracts and leaf segments.
The LAMP assay with the ICGENE system has several advantages: a) easy to perform also by non-specialized researcher; b) no expensive and time-consuming DNA extraction is necessary; c) very fast method since the results are ready in approximately 40 min, including the simplified DNA extraction; d) less sensitive to plant inhibitors since the target strains can be detected directly from seed extracts and leaf fragments; e) light portable instrument for the on-site diagnosis.

FUTURE COLLABORATIONS (if applicable)

This STSM has strengthened the existing collaboration between the applicant and Dr J. Vicente and has allowed the establishment of a new collaboration between two institutions, University of Warwick and the University of Palermo.

Furthermore, the results obtained during the STSM will be submitted for publication to an international peer reviewed journal.

On the basis of the research activity of the host institution on the genome sequencing of several strains of different *X. campestris* pathovars a future collaboration between the two Institutions and the Enbiotech could involve the identification of specific gene targets to design new primers to differentiate the most important *X. campestris* pathovars.