

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: Addressing disease emergences induced by *Xanthomonas vasicola* using a molecular epidemiology approach

STSM start and end date: 19/08/2018 to 25/08/2018

Grantee name: Emmanuel WICKER

PURPOSE OF THE STSM

The UMR IPME (GTIPP team) and the University of Pretoria (Teresa Coutinho's group) have started collaborating during the PhD thesis of Valentine NAKATO on *Xanthomonas vasicola* pv. *musacearum* (*Xvm*). This collaboration led to a first SNP-based genotyping of the East-African *Xvm* populations (Nakato et al. 2018c), and to the first MLVA scheme on *Xvm* (Nakato et al. 2018d, in prep).

The purpose of this STSM was to review all collaboration possibilities on the *X. vasicola* species. Indeed, T. Coutinho's team aims addressing the *X. vasicola* pv. *vasculorum* (*Xvv*) host jump recently reported from sugarcane to eucalyptus (Coutinho et al., 2015) using both genomics and a molecular epidemiology approach. Work on *Xvm* thus include V. NAKATO PhD defence and methodological discussions on next pathogen sampling campaigns. Work on *Xvv* will include methodological discussions on sampling designs, phenotyping and genotyping strategy, genomics.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

Teresa COUTINHO is a professor in the Department of Microbiology and Plant Pathology, in the Genomics Research Institute of the University of Pretoria (UP, South Africa). She is also a scientific staff of the Forestry and Agricultural Biotechnology Institute (FABI). In 2017, her team joined the Center of Microbial Ecology and Genomics (CMEG), directed by Dr Don COWAN, in a UP building with well-equipped new labs. Her team research is dedicated to bacterial diseases affecting fruit trees, forest trees. More specifically, research is done on bacterial diseases of eucalyptus, caused by *Pantoea ananatis* (bacterial blight and die-back) and the *Ralstonia solanacearum* species complex (RSSC). On *P. ananatis*, a post-doc (S. SIBANDA) is studying mechanisms of quorum-sensing, while small-RNAs are studied by a PhD student (G. SHIN) and a "Honor" Bsc student. On *R. solanacearum*, G. CARSTENSEN just completed her PhD thesis dealing with genotyping of the RSSC infections, pathogenomics and metagenomics of eucalyptus RSSC-caused wilt (see below).

Research also addresses bacterial diseases of stone fruit trees (plum, apricot, cherry), more specifically on *Pseudomonas syringae* pv. *syringae* (PhD thesis of K. BOPHELA), using comparative genomics.

Moreover, T. COUTINHO is very interested by bacterial adaptation to new crops, and specifically to bacterial host jumps /host shifts from herbaceous species to tree species. The *Xanthomonas vasicola* case is of particular interest on that aspect. In South Africa, the recent sugarcane-maize-eucalyptus host shift is a potentially serious emergence, because both eucalyptus and maize are widely cropped in this country. It can also be regarded as potentially important in Europe, given the importance of maize crops there. In Eastern and Central Africa, the *Xvm* host jump from onset to banana that most likely occurred in Ethiopia

is another case that should be better understood.

This STSM was thus a great opportunity to review all the aspects of future collaboration on *X. vasicola* adaptation, including the skills and expertises of our respective teams, logistic possibilities, etc... It appeared that FABI is well equipped in greenhouses and growth chambers, which opens possibilities for extensive phenotyping assays. However, inoculations with *Xvm* are not possible in South Africa due to quarantine limitations.

During this STSM, we discussed our respective *Xanthomonas* collections and possible **exchange of strains**.

We discussed a project on **genomics of adaptation of *X. vasicola***, which was applied to the Leverhulme Trust.

We discussed on **methods for sampling *X. vasicola* populations** from maize, sugarcane and eucalyptus from neighboring fields, in different regions of South Africa.

As one external examiner of G. CARSTENSEN's PhD thesis, I discussed with her about prospects of publication on her comparative genomics data on *R. solanacearum*. Her defence, previously planned during this week, was postponed at Monday August 27.

I also presented a **1 h-seminar at FABI**, entitled "What makes a plant pathogen cause emerging diseases?", on Thursday August 23. Most of my talk dealt with my past activities of the evolutionary dynamics and molecular epidemiology, using the *R. solanacearum* species complex as a model.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Collection, exchange of strains

T. Coutinho's UP collection already contains *X. vasicola* strains isolated from different herbaceous species (mostly maize, sugarcane, as well as sorghum, from South Africa but also different African countries) and from the eucalyptus outbreak sampled in 2015. Moreover, there are 30 *Xvm* strains sampled in Ethiopia in 2004 that were not genotyped by V. NAKATO. Though these strains were not geolocalized, it is important to genotype them, or even sequence them, to know in which of the 5 newly described sublineages they fall.

I also used my expertise in *Ralstonia* bacterial wilt to advise T. Coutinho's team in the choice of representative strains. Because Teresa is looking for South-African RSSC strains isolated from potato, I shared with her a part of the *Ralstonia* RUN collection file (22 SA isolates, among which 8 are from potato).

Genomics of adaptation of *X. vasicola*

This subject is being addressed though the co-supervising (IPME/UP) of the MSc student Nomakula ZIM. We precised the framework of her MSc project, which started last June and will spend two years. To date, they are currently 8 *Xvv* genomes isolated from eucalyptus and 8 genomes isolated from maize, and already have sequenced *Xvv* genomes from sugarcane. Since her study combines comparative genomics and host range phenotyping, we discussed the two aspects. For host range phenotyping, it was chosen to inoculate at least 2 strains of each group (eucalyptus, maize, sugarcane) on the hosts maize, eucalyptus, sorghum, sugarcane, banana. We also agreed that phenotyping should combine both (i) symptom scoring (on a typical 4 weeks period) to access to latency, incidence and severity, and (ii) bacterial load at the end of experiment, to assess the ability to establish latent infections.

For genomics, I shared the list of *X. vasicola* genomes currently available on NCBI, and advised her to analyse her reads on the new *X. vasicola* reference SAM119 (combination of PacBio and Illumina, released by the University of Exeter). Annotation strategies were discussed (I advised to use Eugene-PP). After this first comparative genomics step, I suggested to address the genome dynamics, using ClonalFrameML at a first glance.

Additional samplings on maize and eucalyptus

The objective is to isolate about 20-25 strains per site, with each site, ideally with eucalyptus and maize being cropped in close proximity.

Teresa proposed to plan samplings and isolations in 2019, during the austral summer when symptoms are most obvious. Regions sampled will include eucalyptus-maize areas in the Gauteng state, and also in the Western Cape state.

The **PhD defence of V. NAKATO** was performed on Friday August 24, in FABI. From this thesis to date, one article was published (Nakato et al. 2018a), one article was accepted in *Plant Pathology* (Nakato et al. 2018b), one is submitted (Nakato et al. 2018c) and another one is in preparation (Nakato et al. 2018d). V. NAKATO was graduated, and obtained a FABI award for her review in *Molecular Plant Pathology*.

FUTURE COLLABORATIONS (if applicable)

To reinforce the collaboration, we applied for a Leverhulme Trust grant focused on the genomic aspects of host shift in *X. vasicola*. The response is expected soon.

We also applied for a bilateral French-South African program (PHC PROTEA) that would fund travels and per diem for short missions, including exchange of professors and students.

The work on *Xvm* will continue in a IITA-IPME-UP collaboration, with additional samplings planned in Burundi and Rwanda in late 2018-2019.

The co-supervising of Noma ZIM project on *X. vasicola* comparative genomics, can probably lead to a PhD project starting in late 2019 if MSc results are promising.

Collaboration will also include *R. solanacearum*, at least on the analysis of genomic data. One prospect is a co-publication of the results of Gabrielle CARSTENSEN's PhD thesis.

References

Nakato V, Mahuku G, Coutinho T (2018a). *Xanthomonas campestris* pv. *musacearum*: a major constraint to banana, plantain and enset production in central and east Africa over the past decade. *Molecular Plant Pathology* 19, 525–36.

Nakato GV, Christelová P, Were E, Nyine M, Coutinho TA, Doležel J, Uwimana B, Swennen R, Mahuku G (2018b). Sources of resistance to *Xanthomonas campestris* pv. *musacearum*, the causal agent of banana *Xanthomonas* wilt. *Plant Pathology*, **in press**.

Nakato GV, Studholme D, Blomme G, Grant M, Coutinho T, Were E, Wicker E, Mahuku G (2018c). SNP-based genotyping and whole-genome sequencing reveal previously unknown genetic diversity in *Xanthomonas campestris* pv. *musacearum*, causal agent of banana *Xanthomonas* wilt, in its presumed Ethiopian origin. *Plant Pathology*, **submitted**.

Nakato GV, Fuentes Rojas JL, Verniere C, Blondin L, Mahuku G, Coutinho T, Wicker E (2018d). Development of a Multi Locus Variable Number of Tandem Repeat Analysis Scheme (MLVA-19) for epidemiological surveillance of *Xanthomonas campestris* pv. *musacearum*, the pathogen causing bacterial wilt on banana and enset. *Applied and Environmental Microbiology*, **in preparation**.