Work Plan for Short-Term Scientific Mission

COST Action EuroXanth (CA 16107)

STSM title: Towards a new MLSA typing scheme of clade-1 xanthomonads and identification of a type III effector causing a non-host hypersensitive response

Prospective host: Dr. Ralf Koebnik
Host institution: Institut de Recherche pour le Développement (IRD), Laboratory for Plant-Microbe-Environment Interactions (IPME), Research Team on Genomics and Transcriptomics of the Interactions between Plants and Prokaryotes (GTIPP), 911 avenue Agropolis, 34394 Montpellier – France.
Collaboration partners: Prof. Claude Bragard, UCLouvain (Belgium) and Prof. Jonathan M. Jacobs, The Ohio State University (USA)
Planned dates and length of stay:
3 months (60 working days), probably September 21 – December 20, 2020.

Description of the proposed work plan of the STSM

1. Aim and motivation

Accurate molecular typing of bacterial pathogens is important to identify and classify them, to monitor epidemics and population dynamics, to take regulatory measures and to implement management procedures (Bull et al. 2015; Louws et al. 1999). Multilocus sequence analysis (MLSA) is the current method of choice that resolves phylogenetic relationships efficiently at genus, species and infraspecies levels at a reasonable cost (Glaeser and Kämpfer 2015). Two general schemes have been developed for MLSA of xanthomonads (Almeida et al. 2010; Young et al. 2008), and one of them has been further refined for typing of *Xanthomonas arboricola* strains (Cesbron et al. 2014).

Phylogenetic analyses of the genus *Xanthomonas* consistently revealed that it comprises two major clades. Notably, all above-mentioned MLSA schemes have been developed for clade-2 xanthomonads (e.g. *X. arboricola*, *Xanthomonas axonopodis*, *Xanthomonas campestris*, *Xanthomonas oryzae*, *Xanthomonas vasicola*). However, these schemes lack robustness for clade-1 xanthomonads, which is not surprising since they are sometimes considered to belong to another genus than clade-2 xanthomonads (Young et al. 2008).

We have initiated the development of a modified MLSA scheme using optimized PCR primers for clade-1 species. In this project, we wish to apply this new scheme to a representative set of strains of all described clade-1 species. In addition, we have observed that some strains of *X. translucens* cause a hypersensitive response (HR) on some non-host plants. Preliminary data suggest that HR induction depends on a functional type III secretion system. Based on comparative genomics, we wish to identify and confirm the responsible type III effector(s). Considering the lack of effective resistance genes against *X. translucens*, studies on non-host resistance have great potential to establish robust genetic control of the diseases.

2. Contribution to the scientific objectives of the EuroXanth COST Action

This project will focus on xanthomonads of clade-1, which comprises some species of significant interest, such as the sugarcane pathogens *Xanthomonas albilineans* and *Xanthomonas sacchari*, *Xanthomonas hyacinthi*, and pathovars of *Xanthomonas translucens* affecting small-grain cereals (bacterial leaf streak) and/or forage grasses (bacterial wilt) (Birch 2001; Cohen et al. 2020; Sapkota et al. 2020). Notably,

X. translucens pv. *translucens* is classified as an A1 quarantine organisms by the European Plant Protect Organisation (EPPO).

In this project we will collaborate with Prof. Claude Bragard, who is a Member of the Management Committee (MC) of the EuroXanth COST Action, and with Prof. Jonathan M. Jacobs, who acts as a MC Observer of the EuroXanth COST Action. Therefore, this collaboration between the host institute, the UCLouvain (Belgium), The Ohio State University (USA), and the STSM fellow from a fourth country will have a structuring effect on the research landscape in the participating COST countries. By providing a robust molecular typing scheme and candidate type III effectors that trigger non-host HR, we consider this project as highly relevant for the EuroXanth COST Action.

3. Strategy and techniques

The work plan includes:

- Genome mining for partial sequences of five housekeeping genes from clade-1 strains.
- PCR amplification and DNA sequencing of corresponding DNA fragments from at least 24 additional clade-1 strains for which genomic information is not available.
- Phylogenetic analyses of the obtained data.
- Plant inoculations and monitoring of hypersensitive responses.
- Comparative genomics comparing HR-positive and HR-negative strains.
- Molecular cloning of candidate avirulence genes, followed by plant inoculation assays.
- Drafting of a scientific manuscript.

4. References

1. Almeida NF, Yan S, Cai R, Clarke CR, Morris CE, Schaad NW, Schuenzel EL, Lacy GH, Sun X, Jones JB, Castillo JA, Bull CT, Leman S, Guttman DS, Setubal JC, Vinatzer BA (2010). PAMDB, a multilocus sequence typing and analysis database and website for plant-associated microbes. Phytopathology 100: 208-215.
2. Birch RG (2001). *Xanthomonas albilineans* and the antipathogenesis approach to disease control. Mol. Plant Pathol. 2: 1-11.
3. Bull CT, Koike ST (2015). Practical benefits of knowing the enemy: modern molecular tools for diagnosing the etiology of bacterial diseases and understanding the taxonomy and diversity of plant-pathogenic bacteria. Annu. Rev. Phytopathol. 53: 157-180.
4. Cesbron S, Pothier J, Gironde S, Jacques MA, Manceau C (2014). Development of multilocus variable-number tandem repeat analysis (MLVA) for *Xanthomonas arboricola* pathovars. J. Microbiol. Methods 100: 84-90.
5. Cohen SP, Luna EK, Lang JM, Ziegle J, Chang C, Leach JE, Le-Saux MF, Portier P, Koebnik R, Jacobs JM (2020). High-quality genome resource of *Xanthomonas hyacinthi* generated via long-read sequencing. Plant Dis., in press. doi: 10.1094/PDIS-11-19-2393-A.
6. Glaeser SP, Kämpfer P (2015). Multilocus sequence analysis (MLSA) in prokaryotic taxonomy. Syst. Appl. Microbiol. 38: 237-245
7. Louws F, Rademaker J, de Bruijn F (1999). The three Ds of PCR-based genomic analysis of phytobacteria: diversity, detection, and disease diagnosis. Annu. Rev. Phytopathol. 37: 81-125.
8. Sapkota S, Mergoum M, Liu Z (2020). The *translucens* group of *Xanthomonas translucens*: Complicated and important pathogens causing bacterial leaf streak on cereals. Mol. Plant Pathol. 21: 291-302.
9. Young JM, Park DC, Shearman HM, Fargier E (2008). A multilocus sequence analysis of the genus *Xanthomonas*. Syst. Appl. Microbiol. 31: 366-377.