TULIP LabEx

Application form to the "Young Scientists for the Future" Call 2016

Project application form

- **Research Unit of the PhD student:** **LIPM, Laboratoire des interactions plantes-microorganismes**
- **Thesis supervisor:** **Nemo Peeters**
- **Co-Supervisor:** **Laurent Noël**
- **Number of post-docs and PhDs currently supervised:** **Nemo Peeters: Alice Morel, PhD (100% 2015-18); Gaofei Jiang, PhD (50% 2012-16); Cyrus Sabbagh, PhD (50% 2016-19). Laurent Noël: Aude Cerutti, PhD (50% 2014-17).**
- **Thesis project title:** **Evolutionary conservation of plant targets of pathogenicity determinants from xylem-colonizing bacteria.**
- **Thesis project description (1.5 page maximum) (Scientific context, Previous results, Project description, References)**.

A common aim of both research groups is to understand the molecular and evolutionary mechanisms driving pathogenicity of the plant pathogenic bacteria *Ralstonia solanacearum* and *Xanthomonas campestris*, two of the most devastating bacterial plant pathogens. Among the known virulence determinants, the type III effector (T3E) proteins are collectively essential for pathogenicity of these bacteria. T3E are injected in plant cells to suppress plant immunity and subvert plant metabolism for the benefit of the invading bacteria. Although these two bacteria enter either plant roots (*R. solanacearum*) or shoots organs (*X. campestris*), both are vascular and systemic pathogens and face similar plant immune responses in the xylem of crops (Tomato for *R. solanacearum* and cabbage for *X. campestris*) or model host plants (*Arabidopsis thaliana*).

Our groups have developed substantial knowledge in the global characterisation of the complete T3E set of these two phytopathogenic bacteria. Strains of the *Ralstonia solanacearum* species complex can contain up to 70 different type III effectors with a pan-genomic set of about 100 different T3Es (1). *Xanthomonas campestris* type III effectome contains 30 T3Es genes (2). Interestingly, both bacteria express a closely related type III secretion system and share 15 T3Es with a likely common origin, such as the TAL effectors (3), and XopP (4), HLK (5). Thus, both bacteria likely target orthologous processes in their respective host plants.

Both our groups have long invested in identifying the plant targets of both *R. solanacearum* and *X. campestris* T3Es. On the one hand tomato targets of *R. solanacearum* T3Es are being identified by yeast-two-hybrid screening of a custom root cDNA library (Nemo Peeters). On the other hand, a set of 28 T3Es from *X. campestris* has been tested for pairwise interaction against the *A. thaliana* ORFeome by yeast-two-hybrid library of *Arabidopsis thaliana* (Laurent Noël). Because genetic screens in plants have also been proven successful in identifying T3E targets (6), *A. thaliana* plants expressing individual *X. campestris* T3E are currently being produced.

In this PhD project we propose to exploit the results of these available T3E interactor screens by prioritising the 15 evolutionary-conserved T3Es between these two pathogens. Because these conserved T3E likely target orthologous processes in their respective host plants, we should be able to greatly reduce the number of false positives in the screens by conducting a thorough and systematic comparison. Our objective is to provide a global analysis of the common targets of these two important vascular pathogens, and evaluate the biological significance to the T3E/target interaction for pathogens fitness and host immunity and physiology.

The research plan will be structured as follows:
-We will first determine whether the putative T3E targets identified in both yeast-two-hybrid screens (see above) also interact with the orthologous T3E. This will be performed by pairwise yeast-two-hybrid by focusing on *A. thaliana*. To this end, cDNA clones of tomato orthologs will be tested. This part will yield a list of T3E targets shared by both pathogens.

-We will in parallel generate transgenic *A. thaliana* expressing the individual *R. solanacearum* T3E conserved between both bacteria as a complement for those expressing *X. campestris* T3E (currently being generated, L. Noël). We will test whether these plants are more susceptible to either *Ralstonia solanacearum* or *Xanthomonas campestris*. Developmental defect potentially caused by specific T3E transgenic expression could serve as a base to develop genetic screens for target identification. These T3E transgenic plants will also be phenotyped by RNAseq to classify T3E into functional groups potentially targeting common pathways. This part will yield a list of T3E important for plant immunity or targeting similar pathways.

-Last, we will test by reverse genetics (T-DNA in *A. thaliana* and CRISPR-Cas9 in Tomato) the biological significance of few selected physical, transcriptomic or genetic interactions for pathogens' pathogenicity and plant immunity and physiology.

Combining the knowledge of plant targets from both approaches for these T3E is a novel and comprehensive approach which will provide a compelling description of their biological role in pathogenicity that have been conserved throughout evolution in these two important bacterial vascular pathogens. Identification of these biologically relevant T3E targets will be valuable for marker-assisted selection of tolerant crops in breeding programs.

References

1. Peeters N, et al. (2013) Repertoire, unified nomenclature and evolution of the Type III effector gene set in the *Ralstonia solanacearum* species complex. *BMC genomics* 14(1):859.
2. Roux B, et al. (2015) Genomics and transcriptomics of *Xanthomonas campestris* species challenge the concept of core type III effectome. *BMC genomics* 16(1):975.
3. de Lange O, et al. (2013) Breaking the DNA-binding code of *Ralstonia solanacearum* TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease. *The New Phytologist* 199(3):773–786.
4. Ishikawa K, et al. (2014) Bacterial effector modulation of host E3 ligase activity suppresses PAMP-triggered immunity in rice. *Nature communications* 5:1–11.
5. Chen L, et al. (2014) Involvement of HLK effectors in *Ralstonia solanacearum* disease development in tomato. *Journal of General Plant Pathology* 80(1):79–84.
6. Wang G, et al. (2015) The Decoy Substrate of a Pathogen Effector and a Pseudokinase Specify Pathogen-Induced Modified-Self Recognition and Immunity in Plants. *Cell host & microbe* 18(3):285–295.

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- **Project summary (200 words) :**

Ralstonia solanacearum and *Xanthomonas campestris* are both xylem-colonizing bacteria which pathogenicity relies on type III effector (T3E) proteins, injected in plants to suppress immunity. This project aims at identifying biologically-relevant interactors of individual T3E common to both pathogens. This project exploits transgenic material currently being developed and screens which are being completed in both host groups. Results from comparative yeast-two hybrid screens, transcriptomic signatures caused by transgenic expression of T3E in Arabidopsis and reverse genetics should uncover novel T3E effector targets or pathways that will be relevant for breeding crops with increased tolerance to these two pathogenic bacteria in particular and possibly to other vascular pathogens.

This phD project will be co-supervised by two experienced researchers from the LIPM working each on the characterisation of these two bacterial plant pathogens. A set of complementary experimental approaches (Yeast-two-hybrid, transcriptomics, molecular genetics) will train the phD candidate in a wide range of approaches which integration should yield a global understanding of T3E-mediated pathogenicity in these two important bacterial pathogens.