Title: Live visualization and characterization of *Xanthomonas* virulence activity

Introduction, rationale and preliminary data. Plant pathogenic bacteria in the species *Xanthomonas translucens* cause diseases of cereal crops globally. Our previous research with collaborators Dr. Ralf Koebnik (IRD, France) and Prof. Claude Bragard (UCLouvain, Belgium) showed that *X. translucens* depends on protein secretion for pathogenesis (1). As with other *Xanthomonas* pathogens, *X. translucens* reprograms plant cells with Type III (T3)-secreted transcription activator-like effectors (TALEs) (2, 3). TALEs are injected by *X. translucens* from the bacterial to host cells, the TALEs localize to the nucleus and then bind DNA to activate plant gene expression.

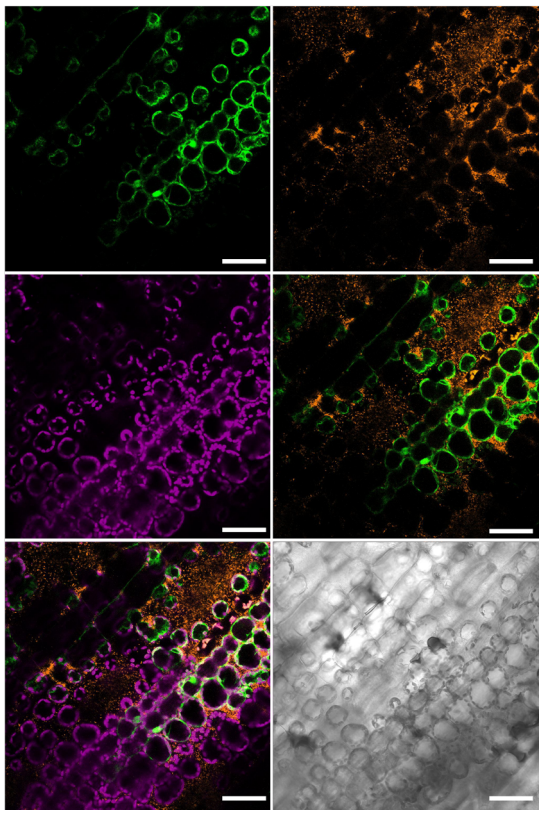

Figure. Effector detector plants: TALE-mediated induction of GFP by *X. translucens*. RFP-expressing *X. translucens* pv. undulosa (orange; left upper panel) targets cells with dTALE_GFP and induces expression of GFP (green) in plant cells (outlined by pink chloroplasts). Bacteria are more abundant adjacent to GFP-expressing plant cells.

Our collaborators (J. Kumlehn, IPK Gatersleben, Germany) created transgenic barley plants whose cells detect TALE injection and activity by fluorescing from GFP (Figure). Briefly, these "effector-detector" transgenic barley have an inducible promoter fused to *gfp*, and this *gfp* is only induced by an artificial TALE (dTALE_GFP) (see figure). Jacobs developed this tool as a Fulbright Scholar at UCLouvain (collaboration with Bragard and Koebnik). This visualization tool allows for easy detection of cells targeted by *X. translucens* T3 effectors (green) by RFP-expressing bacteria (orange) (see Figure). To our knowledge this is the first time both the bacteria and T3 effector activity simultaneously visualized (paper *in prep*). This proposal aims to host a researcher/student interested in understanding the spacial aspects to T3-secreted TALEs.

Host laboratory and desired applicant. The Emerging Infectious Disease Ecology Laboratory (TEIDEL) led by Prof. Jonathan M. Jacobs (The Ohio State University) focuses on the basis of bacterial colonization of plants and translational approaches to improve management of phytobacterial diseases. The team comprises a lab manager, technician, two postdocs, five graduate students and three undergrads. This team is a dynamic, supportive group focused on *Xanthomonas, Xylella* and *Ralstonia* research. The potential COST STSM visiting researcher or student will hopefully have experience in plant bacteriology or related techniques. The visitor will have full access to the Jacobs' laboratory, which is equipped with an Olympus FV3000 laser confocal microscope and a ThermoFisher Attune flow cytometer.

***Objectives*.** Our research showed T3 secretion is essential for *X. translucens* vascular pathogenesis (1), but it is unknown what cells are targeted by T3-secreted TALE effectors across the leaf landscape. This experiment proposal will cover two objectives. We will 1) determine barley cells targeted by

X. translucens over time and 2) define the population of *X. translucens* expressing T3 secretion components during leaf infection.

Objective 1. Determine barley cells targeted by X. translucens over time. The visiting researcher will gain experience directly with *X. translucens* inoculation on barley. For all experiments we will use RFP-expressing, non-vascular barley and wheat pathogen *X. translucens* pv. undulosa. We will examine *X. translucens* infection from a naturalistic spray inoculation. Upon infiltration we can observe GFP expression at 6 hours post inoculation. We will observe and quantify cells targeted over time upon spray inoculation over a 7-day period. We expect to see stomata guard cells as initial targets as this is the entry point and an expansion through the mesophyll. We will also use RFP-expressing *X. translucens* to follow the bacteria with the effector detection and GFP plant cell expression. This research will directly connect to publishable research on the spatial dynamics of leaf infection and T3 secretion.

Objective 2. Define the population of X. translucens expressing T3 secretion during leaf infection. X. translucens does not uniformly colonize the leaf space. The T3 secretion system in *X. translucens* is directly regulated by HrpX, and the goal of this aim will be to monitor expression of HrpX-inducible P_{hpaT}. HpaT is required for pathogenesis and T3 effector injection as it is a putative component of the translocon. With microscopy, we will observe RFP-expressing *X. translucens* with an inducible P_{hpaT}::*gfp*. Therefore cells expressing GFP will be considered active for the T3 secretion. Bacterial cells are easily distinguishable from plant cells expressing GFP, and we will locate and quantify cells expressing GFP as an output for T3 secretion. We will finally use flow cytometry to specifically quantify over the whole population cells expressing the T3 secretion system. The visitor will strengthen his/her microscopy abilities and also expand their laboratory skills by validating his/her work with flow cytometry.

Contribution to EuroXanth COST and significance. Bacterial infection is dynamic, and this project provides a visual representation to our molecular understanding of *Xanthomonas* pathogenesis. Therefore, this research directly supports the working group 2 objective: "To identify key bacterial factors in the microbe-eukaryote interaction at different steps of the infection/dissemination cycle by coordinating research on the pathogen's biology". This research will strengthen connections with an International Partner Country laboratory and hopefully provide training to a European researcher interested in leading techniques in visualization and gene expression.

Planned period of time: 3 months (September to November 2020)

Cost estimates for STSM ($3400 = 3100 EUR):

1) Housing ($500/mo): $1500
2) Travel (flight and taxi): $1000
3) Subsistence ($300/mo): $900

*The Jacobs laboratory will cover experiment fees and additional costs for the visit.

References

1. C. Pesce, *et al.*, Comparative genomics identifies a novel conserved protein, HpaT, in proteobacterial type III secretion systems that do not possess the putative translocon protein HrpF. *Front. Microbiol.* **8**, 1177 (2017).
2. N. Falahi Charkhabi, *et al.*, Complete genome sequencing and targeted mutagenesis reveal virulence contributions of Tal2 and Tal4b of *Xanthomonas translucens* pv. undulosa ICMP11055 in bacterial leaf streak of wheat. *Front. Microbiol.* **8**, 1488 (2017).
3. Z. Peng, *et al.*, *Xanthomonas translucens* commandeers the host rate-limiting step in ABA biosynthesis for disease susceptibility . *Proc. Natl. Acad. Sci. U.S.A.* **116**, 20938-20946 (2019).