Objectif : Impact of climate (i.e. temperature and relative humidity) on population size and within-plant distribution of *Pseudomonas syringae pv. actinidiae* bacteria as analyzed by absolute qPCR, and on the severity of symptoms induced on kiwi plants (*Actinidia chinensis var. deliciosa*).

Contract type: Master 2 internship

Dates : 1st 2023 semester, precise dates to be defined with the selected candidate
Length: 6 months
Host unit: research unit, INRAE Pathologie végétale
https://www6.paca.inra.fr/pathologie_vegetale/
Team: MISTRAL
Location : 67 Allée des Chênes, CS 60094 - Domaine Saint Maurice, F84143 Montfavet Cedex
Contact : Christelle Lacroix (christelle.lacroix@inrae.fr)

Context

Pseudomonas syringae corresponds to a bacterial species complex, which strains can be epiphytes and/or pathogens on a large angiosperm host range[1-4]. *P. syringae pv. actinidiae* (Psa) strains belong to one of the phylogenetic group within this complex[5,6] and are responsible for bacterial canker disease in kiwi crops[1,4]. These strains are distributed into six different biovars, according to biological and phylogenetic characteristics[7]. Strains from biovar 3 are the most agressive and are recognized as the causal agents of the current and global bacterial canker epidemics in kiwi crops worldwide. Nevertheless, strains from different biovars were responsible of multiple episodes of emergence of this disease over the last few decades.

There are no direct, efficient, and sustainable mean to control this disease. The management of canker disease in kiwi crops rely on prophylactic methods (e.g. desinfection of pruning tools, pruning symptomatic canes, use of copper-based compounds)[1]. These methods are applied to prevent or to limit the dissemination of the disease as soon as symptoms are observed (e.g. foliar necrotic spots, wood cankers, exudates)[1,4,8]. However, these methods are moderately efficient to control the disease, and the severity of symptoms and infection is highly variable according to environmental conditions and remains hardly predictable.

Management of kiwi bacterial canker disease could be improved through surveillance strategies of early infection signs and detection of outbreaks, and through a better understanding of environmental factors that control disease dissemination and severity. These academic and applied lines of research require efficient detection and diagnostic tools that lead both to the qualitative and the quantitative detection of targeted bacteria, in order to estimate population sizes in plant samples according to the factors studied.

Objectives and missions

The objective of this internship is to determine the impact of climatic factors (i.e. temperature and relative humidity) on *P. syringae pv. actinidiae* biovar 3 strain population size, on the severity of symptoms, and on kiwi plant health.

The internship will be composed of two parts which objectives are to i) finish the optimization of a detection and absolute quantification qPCR protocol of Psa biovar 3 strains, and to ii) analyze, using this qPCR protocol, bacteria population size in kiwi plant tissues sampled during previous experiments and stored at -80°C; and to perform data statistical analyses to assess the effects of temperature and relative humidity on bacteria population size, symptoms severity, and plant health.

Specifically, the first objective is to contribute to the optimization of a DNA extraction protocol, to confirm the specificity and to assess the sensitivity of an absolute qPCR protocol.

Absolute detection and quantification was developed on the basis of primers published in the literature[9], the design of a TaqMan-MGB type probe, and of standards corresponding to serial dilutions of genomic DNA extracts of a representative strain of Psa biovar 3. The first tests carried out with pure suspensions of a limited number of bacterial strains of the different biovars demonstrated a good specificity of this protocol (100% true positive and negative) and a detection sensitivity between 10 and $10^{-4}/10^{-5}$ ng of DNA. The first step will be to confirm the specificity of the protocol using a wider range of strains. In addition, several protocols have been tested to extract bacterial DNA from more complex samples (i.e. leaf limbs ground in buffer). The *in fine* selected protocol will maximize the concentration of extracted DNA while limiting the presence of molecules potentially inhibiting the amplification reaction. Finally, the last step will be to evaluate the sensitivity of detection and quantification on the basis of realistic samples (e.g. juice extracted from infected leaf limbs), and to determine how the amount of bacterial DNA estimated in these samples can be translated into an estimate of the size of the bacterial population (i.e. CFU/g fresh material).

The second objective is to determine the effect of different climatic conditions on the population size and *in planta* distribution of Psa Biovar 3 bacteria.

Experiments were previously conducted under controlled conditions (i.e. phytotrons) in which 240 female kiwifruit plants (*Actinidia chinensis var. deliciosa*) were inoculated with (N=120) or without (N=120, negative controls) a suspension of a Psa Biovar 3 strain. Plants were distributed in different phytotrons allowing for varying day/night temperature regimes and relative humidity. Necrosis symptoms at the point of inoculation, and phenotypic traits (e.g. plant height, number of leaves) were measured on all plants 15 and 30 days after inoculation. At the same dates, a portion of each plant was destructively sampled in order to collect pieces of petioles at the level of the inoculation points, and adjacent pieces of tissues (i.e. leaf limbs and stems). These samples were individually ground in phosphate buffer, and stored at -80°C with glycerol. The objective is to analyze these samples according to the absolute quantification protocol developed in the first phase in order to determine the effect of the tested climatic conditions on i) the intra-plant distribution, ii) the population size of the inoculated bacteria and on iii) the severity of symptoms and kiwi plant health.

Main activities

The student will be expected to use his/her skills to:

- Understand the subject, and set up the experimental protocol
- Participate in the coordination of the different steps
- Carry out the different steps of the protocol independently or as a team
- Enter the data and perform statistical analyses
- Gather and format the results
- Keep a laboratory notebook
- Participate in meetings with supervisors, researchers, technicians and engineers of the team to plan and share work
- Communicate orally on his/her work during team meetings
- Write a report

Skills required

The skills searched are :

- Molecular biology (DNA extraction, PCR, qPCR, gel electrophoresis)
- Phytopathology, Ecology
- Microbiology (media manufacturing, strain culturing, suspension preparation)
- Statistical analysis, ideally with R software
- Good knowledge of computer tools (pack office)
- Good level of oral and written communication
- Scientific rigor
- Sense of organization, anticipation and teamwork
- Critical thinking and synthesis
- Adaptability, autonomy
- Ethical and relational skills, diplomacy

Education

Background in molecular biology, ecology and microbiology

Basic knowledge of hygiene and safety rules in molecular biology and microbiology laboratories

Application

Please send all inquiries, as well as your CV and cover letter (max. 2 pages) describing how your education and/or experience match the job profile to the attention of Christelle Lacroix (christelle.lacroix@inrae.fr).

Literature

1 Lamichhane, J. R., Varvaro, L., Parisi, L., Audergon, J. M. & Morris, C. Disease and frost damage of woody plants caused by *Pseudomonas syringae*: seeing the forest for the trees. *Advances in Agronomy* **126**, 235-295, doi:10.1016/B978-0-12-800132-5.00004-3 (2014).

2 Morris, C. E. *et al.* Expanding the Paradigms of Plant Pathogen Life History and Evolution of Parasitic Fitness beyond Agricultural Boundaries. *Plos Pathogens* **5**, e1000693, doi:10.1371/journal.ppat.1000693 (2009).

3 Lamichhane, J. R., Messean, A. & Morris, C. E. Insights into epidemiology and control of diseases of annual plants caused by the *Pseudomonas syringae* species complex. *Journal of General Plant Pathology* **81**, 331-350, doi:10.1007/s10327-015-0605-z (2015).

4 Scortichini, M., Marcelletti, S., Ferrante, P., Petriccione, M. & Firrao, G. *Pseudomonas syringae* pv. *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. *Molecular Plant Pathology* **13**, 631-640, doi:10.1111/j.1364-3703.2012.00788.x (2012).

5 Berge, O. *et al.* A User's Guide to a Data Base of the Diversity of *Pseudomonas syringae* and Its Application to Classifying Strains in This Phylogenetic Complex. *PLoS ONE* **9**, e105547, doi:10.1371/journal.pone.0105547 (2014).

6 Donati, I. *et al.* New insights on the bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*). *Journal of Berry Research* **4**, 53-67 (2014).

7 Vanneste, J. L. The Scientific, Economic, and Social Impacts of the New Zealand Outbreak of Bacterial Canker of Kiwifruit (Pseudomonas syringae pv. actinidiae). *Annual Review of Phytopathology* **55**, 377-399, doi:10.1146/annurev-phyto-080516-035530 (2017).

8 Renzi, M. *et al.* Bacterial Canker on Kiwifruit in Italy: Anatomical Changes in the Wood and in the Primary Infection Sites. *Phytopathology* **102**, 827-840, doi:10.1094/phyto-02-12-0019-r (2012).