

PHD PROPOSAL FOR THE DOCTORAL SCHOOL « Ecologie, Géosciences, Agronomie, ALimentation »

GENERAL INFORMATION

Thesis title: Assessment of the impact of Effectors of <i>Xanthomonas</i> on plant sugar metabolism and Defenses
Acronym: EXUDE
Disciplinary field 1: Select an element Disciplinary field 2: Select an element
Three keywords: Type3 Effectors, Primary Metabolism, Plant defense pathways
Research unit : UMR 1345 IRHS
Name of the thesis director HDR (Habilitation thesis to supervise research) required: BOUREAU Tristan Email address of the thesis director: tristan.boureau@univ-angers.fr Name of the thesis co-director (if applicable): HDR (Habilitation thesis to supervise research) required: Email address of the thesis co-director (if applicable): Name of the thesis co-supervisor 1 (if applicable): BELIN Etienne Email address of the thesis co-supervisor 1 (if applicable): etienne.belin@univ-angers.fr Name of the thesis co-supervisor 2 (if applicable): Email address of the thesis co-supervisor 2 (if applicable):
Thesis grant (funding origin and amount): MESRI grant (salary) + CLIGDI Project (SFR Quasav 20k€) + Submission to IB22 SPE Department INRAE for further support
Contact(s) (mailing address and E-mail): Tristan Boureau, Team EmerSys, UMR 1345 IRHS, 42 rue Georges Morel, BP 60057, 49071 BEAUCOUZE Cedex
Recruitment process: Recruitment process depends on thesis funding. To select the corresponding recruitment process, please visit the EGAAL website here . This information is needed for proposal publication. <input type="checkbox"/> Doctoral school contest <input type="checkbox"/> —Interview <input type="checkbox"/> Other (indicate):

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All sections must be filled. Once filled, please save the proposal form in pdf format using the following naming: Supervisor Name_Unit_Subject Acronym_EN.pdf

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SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

Socio-economic and scientific context : (10 lines)

Common bean (*Phaseolus vulgaris*) is a legume of major importance for food. Common bacterial blight, caused by *Xanthomonas* strains, is among the most devastating diseases on bean, with potential yield-losses up to 75%. Virulence of *Xanthomonas* strains on bean involves the delivery by the type 3 secretion system of over 30 effectors (T3Es) directly inside the plant cell. Collectively, T3Es suppress plant defenses and divert the host metabolism to the benefit of the bacterium. The precise role of some T3Es is well understood, however the contribution of many T3Es to the virulence of *Xanthomonas* still remains elusive. To the exception of XopD, until now in *Xanthomonas*, only T3Es belonging to the TALE family target the nucleus, where they trigger the expression of sensitivity genes such as sugar transporters. Furthermore in *Xanthomonas*, only one T3Es is known to target chloroplasts (XopAG) and none were shown to target mitochondria.

Assumptions and questions (8 lines)

In common bean, the modulation of the transcription of genes involved in sugar metabolism and salicylic acid and ethylene pathways was linked to differential phenotypes of sensitivity / resistance to *Xanthomonas*. By screening the subcellular localization of most T3E of the *Xanthomonas* strain CFBP 4834 (pathogenic on bean), we identified 5 novel non-TALE T3Es addressed to the nucleus, suggesting that they trigger transcriptional changes of bean. We also identified a T3E triggering the migration of chloroplasts around the nucleus, and another one that targets mitochondria and induces their aggregation. These two T3Es may impact organelle-nucleus communication (retrograde signalling), a process that appears widely involved in plant responses to stresses. The objectives will consist in the comparative analysis of the impact of these T3Es on the sugar metabolism and defenses of bean genotypes with contrasting levels of resistance to *Xanthomonas*.

The main steps of the thesis and scientific procedure (10-12 lines)

The impact of 3 T3Es (XopT : addressed to nucleus, XopV : targeting chloroplasts, XopI : targeting mitochondria) will be characterized on two bean genotypes with contrasting levels of resistance to *Xanthomonas*. Constructs available in the group for the transient expression on bean will be used to characterize the impact of these T3Es on transcriptional activity. Transcriptomics data will be supplemented with the determination of activities of key sugar metabolism and oxidative burst enzymes. The 3 ET3 candidates will be cloned on expression vectors for transformation into the effectorless *Xanthomonas* complemented with a type 3 secretion system described in Meline et al. (2019). The transformed *Xanthomonas* strains will be inoculated on bean plantlets. The impact of such an inoculation on the development of plantlets will be phenotyped. No defense pathway mutants (ethylene, salicylic acid) are available in our group. Therefore in order to assess the defense pathways targeted by each T3E, the impact of the inoculated strains will be phenotyped on a collection of mutants of *A. thaliana* by carrying a morphometric analysis of the development of rosettes.

Methodological and technical approaches considered (4-6 lines)

The analysis of the transcriptional response to the expression of the T3Es will be carried by RNA-Seq. The activities of key enzymes of the sugar metabolism (invertases, sucrose synthases, hexose phosphorylation, glycolytic enzymes) and associated to the oxidative burst (SOD, aPOX, APX, CAT, GR, MDHR, DHAR, GST) will be quantified in 96-wells microtiter plates using the method described by Jammer (2015), in collaboration with the group of T. Roitsch (a stay in his lab under consideration). Phenotyping of inoculated bean plantlets and collections of *A. thaliana* mutants will be performed by imaging (RGB and chlorophyll fluorescence) on the high-throughput phenotyping installations that were recently built on the platform PHENOTIC.

Scientific and technical skills required by the candidate

Skills required by the candidate are classical techniques in microbiology (culture, transformation) and molecular biology (cloning, DNA and RNA purification).

During the PhD the candidate will train in bioinformatics for the analysis of RNA-Seq results, in biochemistry techniques (total protein extracts and characterization of enzymatic activities), as well as image analysis to analyze phenotyping results.

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THESIS SUPERVISION¹

Unit name: UMR 1345 IRHS	Team name: EmerSys
Unit director name: Jean Pierre RENOUE	Team director name: Marie-Agnès JACQUES
Mailing address of the unit director: jean-pierre.renou@inrae.fr	Mailing address of the team director: marie-agnes.jacques@inrae.fr
Thesis director Surname, first name: BOUREAU Tristan Position: Associate Professor Obtained date of the HDR (Habilitation thesis to supervise research): 02-10-2015 Employer: Université d'Angers Doctoral school affiliation: EGAAL Rate of thesis supervision in the present project (%): 70% Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 0 Number of current thesis supervisions/co-supervisions: 0	
Thesis co-director Surname, first name: Position: Obtained date of the HDR (Habilitation thesis to supervise research): Employer: Doctoral school affiliation: Rate of thesis supervision in the present project (%): Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): Number of current thesis supervisions/co-supervisions:	
Thesis co-supervisor 1 (if applicable) Surname, first name: BELIN Etienne	

¹ In EGAAL Doctoral School, if only one scientist in thesis supervision = 100% of supervision rate; if 2 people involved in thesis supervision = from 50% to 70% of supervision rate for the director; if 3 people involved in thesis supervision = 40% / 30% / 30% of supervision rate distribution among supervisors.

Position: Associate Professor

Habilitation thesis to supervise research yes no If yes, date diploma received:

Employer: Université d'Angers

Doctoral school affiliation: MathSTIC

Rate of thesis supervision in the present project (%): 30

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 30

Number of current thesis supervisions/co-supervisions: 1

Thesis co-supervisor 2 (if applicable)

Surname, first name:

Position:

Habilitation thesis to supervise research yes no If yes, date diploma received:

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Private partner (if CIFRE funding, private funding,...)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

International partner (if Cotutelle thesis)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Professional status of previous PhD students supervised by both director and co-supervisors (from 5 years)

Please provide the following information for each PhD students supervised

Surname, first name: HAJRI Ahmed

Date of PhD beginning and PhD defence: jan 2007- dec 2009

Thesis supervision: MANCEAU Charles

Professional status and location: Researcher, ANSES Maison Alfort

Contract profile (post-doc, fixed-term, permanent): fixed-term

List of publications from the thesis work: 8

Surname, first name: ROUSSEAU Céline

Date of PhD beginning and PhD defence: nov2010- mars 2014

Thesis supervision: MANCEAU Charles

Professional status and location: Ingeneer, ALCUIN, ANGERS.

Contract profile (post-doc, fixed-term, permanent): permanent

List of publications from the thesis work: 3

Surname, first name: CHAMBON Arthur

Date of PhD beginning and PhD defence: nov 2014- fev 2017

Thesis supervision: SAUBION Frédéric

Professional status and location: -

Contract profile (post-doc, fixed-term, permanent): -

List of publications from the thesis work: 3

Surname, first name: MELINE Valérian

Date of PhD beginning and PhD defence: nov2015- feb 2019

Thesis supervision: BOUREAU Tristan

Professional status and location: Post Doc, PURDUE University USA

Contract profile (post-doc, fixed-term, permanent): Post Doc

List of publications from the thesis work: 2

Five main recent publications of the supervisors on thesis subject:

1. Méline V, Brin C, Lebreton G, Ledroit L, Sochard D, Hunault G, **Boureau T, Belin E.** « A Computation Method Based on the Combination of Chlorophyll Fluorescence Parameters to Improve the Discrimination of Visually Similar Phenotypes Induced by Bacterial Virulence Factors. ». *Frontiers in Plant Science* . 2020 Vol 26;11:213. doi: 10.3389/fpls.2020.00213. eCollection 2020.
2. Méline V., Delage W., Brin C., Li-Marchetti C., Sochard D., Arlat M., Rousseau C., Darrasse A., Briand M., Lebreton G., Portier P., Fischer-Le Saux M., Durand K., Jacques M. - A., **Belin E., Boureau T.** « Role of the acquisition of a Type 3 Secretion System in the emergence of novel pathogenic strains of *Xanthomonas*». *Molecular Plant Pathology* . 2019. Vol. 20 n°1 p. 33-50.
3. Denancé N., Szurek B., Doyle E. L., Lauber E., Fontaine-Bodin L., Carrère S., Guy E., Hajri A., Cerutti A., **Boureau T.**, Poussier S., Arlat M., Bogdanove A. J., Noël L. D., **2018.** « Two ancestral genes shaped the *Xanthomonas campestris* TAL effector gene repertoire ». *New Phytologist*. 2018. Vol. 219 n°1 p. 391-407
4. Jacques M-A, Arlat M, Boulanger A, **Boureau T**, et al., **2016.** Ecology, Physiology, and Genomics to Understand Host Specificity in *Xanthomonas*. *Annual Review of Phytopathology*
5. Merda D, Bonneau S, Guimbaud J-F, Durand K, Brin C, Darrasse A, **Boureau T**, Lemaire C, Jacques M-A and Fischer-Le Saux M. **2016** The role of nonpathogenic bacterial strains in shaping emergences of new epidemic clones in agroecosystems. *Environmental Microbiology Reports* .
6. Rousseau C, Hunault G, Gaillard S, Bourbeillon J, Montiel G, Simier P, Campion C, Jacques M-A, **Belin E, Boureau T., 2015.** Phenoplant: a web resource for the exploration of large chlorophyll fluorescence image datasets. *Plant Methods*.
7. Cesbron S, Briand M, Essakhi S, Gironde S, Boureau T, Manceau C, Fischer-Le Saux M and Jacques M-A, 2015. Comparative genomics of pathogenic and nonpathogenic strains of *Xanthomonas arboricola* unveil molecular and evolutionary events linked to pathoadaptation. *Frontiers in Plant Science* . 2015. Dec 22;6:1126. doi: 10.3389/fpls.2015.01126. eCollection 2015
8. Degrave A, Siamer S, **Boureau T**, Barny M-A, **2015.** The AvrE superfamily: Ancestral type III effectors involved in suppression of PAMP-triggered immunity. *Molecular Plant Pathology*.

THESIS FUNDING

Origin(s) of the thesis funding: Grant MESRI. Lab fees : CLIGDI Project (SFR Quasav 20k€). Grant Application to be written and submitted to Département SPE INRAE for further running costs. Mobility grant / co-supervision to apply for.

Gross monthly salary: 1957€

Thesis funding state : Select an element Non acquired

Funding beginning date/Funding ending date: octobre 2021 –septembre 2024

Date: 12-03-2021

Name, signature of unit director:

Renou Jean Pierre



Name, signature of team director:

Marie-Agnès Jacques



Name, signature of thesis project director:

Tristan Bourreau

