

# 6<sup>th</sup> *Xanthomonas* Genomics Conference

July 18 - 21, 2018

&

# 2<sup>nd</sup> Annual EuroXanth Conference

## Program & Abstracts





# **6<sup>th</sup> *Xanthomonas* Genomics Conference (XGC 2018) & 2<sup>nd</sup> Annual EuroXanth Conference**

July 18 – 21, 2018  
Leopoldina in Halle (Saale), Germany

## **Organizer**

**Ulla Bonas**                      Halle (Saale), Germany

## **Scientific Committee**

**Daniela Büttner**                      Halle (Saale), Germany

**Jens Boch**                      Hannover, Germany

**Ralf Koebnik**                      Montpellier, France

**Roland Kölliker**                      Zurich, Switzerland

**Jan E. Leach**                      Fort Collins, USA

**David Studholme**                      Exeter, UK

**Nian Wang**                      Lake Alfred, USA

**Financial support for this conference was generously provided by:**



Funded by the Horizon 2020 Framework Programme  
of the European Union



Deutsche  
Forschungsgemeinschaft



Leopoldina  
Nationale Akademie  
der Wissenschaften



Martin Luther University  
Halle-Wittenberg



# Table of Contents

<b>General Information</b>	<b>2</b>
<b>Program</b>	<b>5</b>
<b>Talks</b>	<b>11</b>
<b>Session I</b>	Genomics and molecular diagnostics <b>13</b>
<b>Session II</b>	Pathogenicity mechanisms and regulation <b>27</b>
<b>Session III</b>	Type III secretion and effector proteins <b>39</b>
<b>Session IV</b>	Other secretion systems <b>57</b>
<b>Session V</b>	Ecology and epidemiology <b>65</b>
<b>Session VI</b>	Plant defense and resistance <b>73</b>
<b>Posters</b>	<b>79</b>
<b>List of Participants</b>	<b>155</b>

# General Information

## Conference Venue



**Leopoldina**  
**Jägerberg 1, 06108 Halle (Saale)**

The registration desk is located in the foyer. The conference will be held in the lecture hall on the first floor.

## Oral Presentations

All oral presentations will be held in the lecture hall on the first floor. Technical equipment including a PC is available. Please give your presentation to the technical staff at the registration desk or in the lecture hall, at least one session before your talk.

### Time schedule:

Keynote lecture	30 minutes + 10 minutes discussion
Invited speakers	25 minutes + 5 minutes discussion
Short talks	15 minutes + 5 minutes discussion

## Poster Presentations

Posters will be displayed in the dining room (ground floor) and the foyer on the 1<sup>st</sup> floor.

Poster session I (even numbers)	Thursday	13.30 – 15.30
Poster session II (odd numbers)	Friday	16.15 – 18.15

Please set up the posters on Wednesday. Poster boards and pins will be provided. Poster numbers are indicated in this booklet and on the poster boards.

## Meals

Refreshments, welcome reception, lunch on Thursday and Friday and the gala dinner are included in the registration fee.



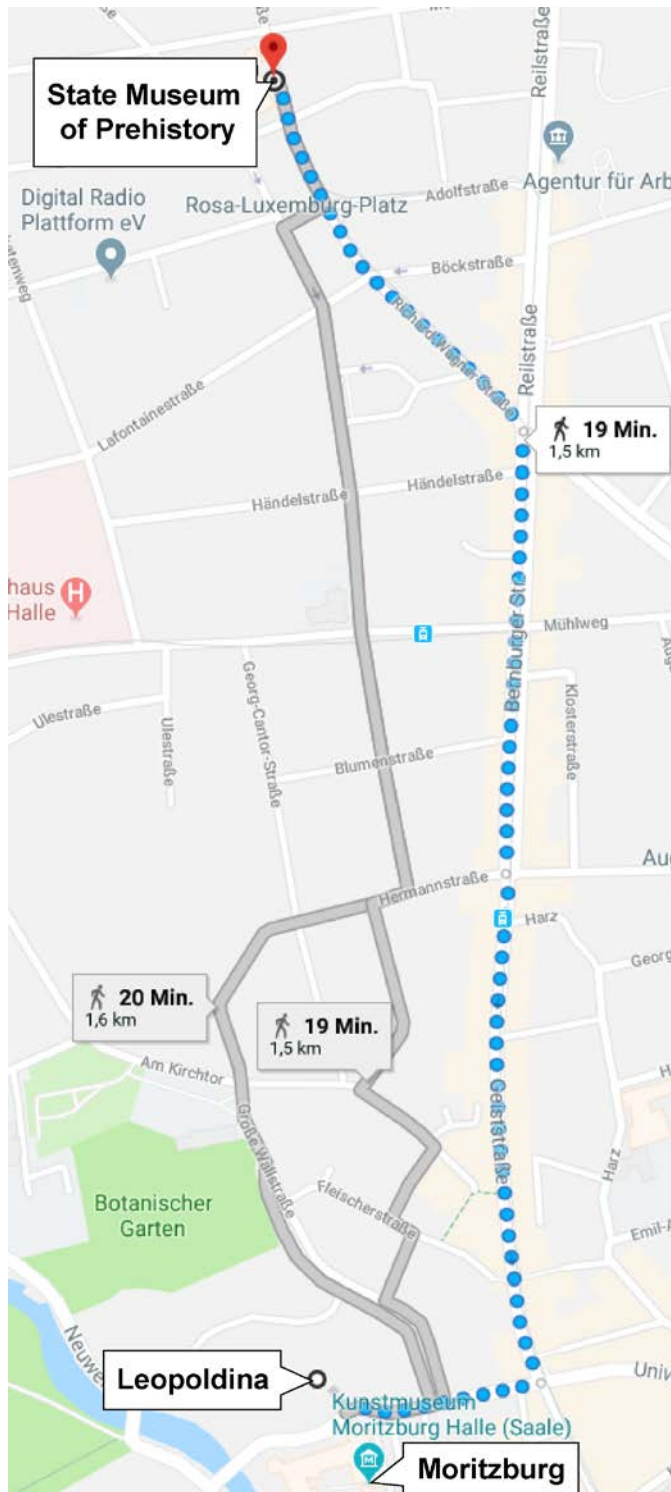
## Events

### Welcome reception

Wednesday, 18.00 h (ground floor and garden of the Leopoldina)

### Social program

Thursday, 16.15 h



Please join the social program according to your sign-up. If you did not register for a social program but want to participate, please contact the staff at the registration desk.

#### *Historic Town Centre Stroll*

(meeting point at the entrance of the Leopoldina at 16.15 h)

#### *Visit of Moritzburg*

(meeting point at the entrance of Moritzburg at 16.15 h; the Moritzburg is located across the street, opposite to the Leopoldina)

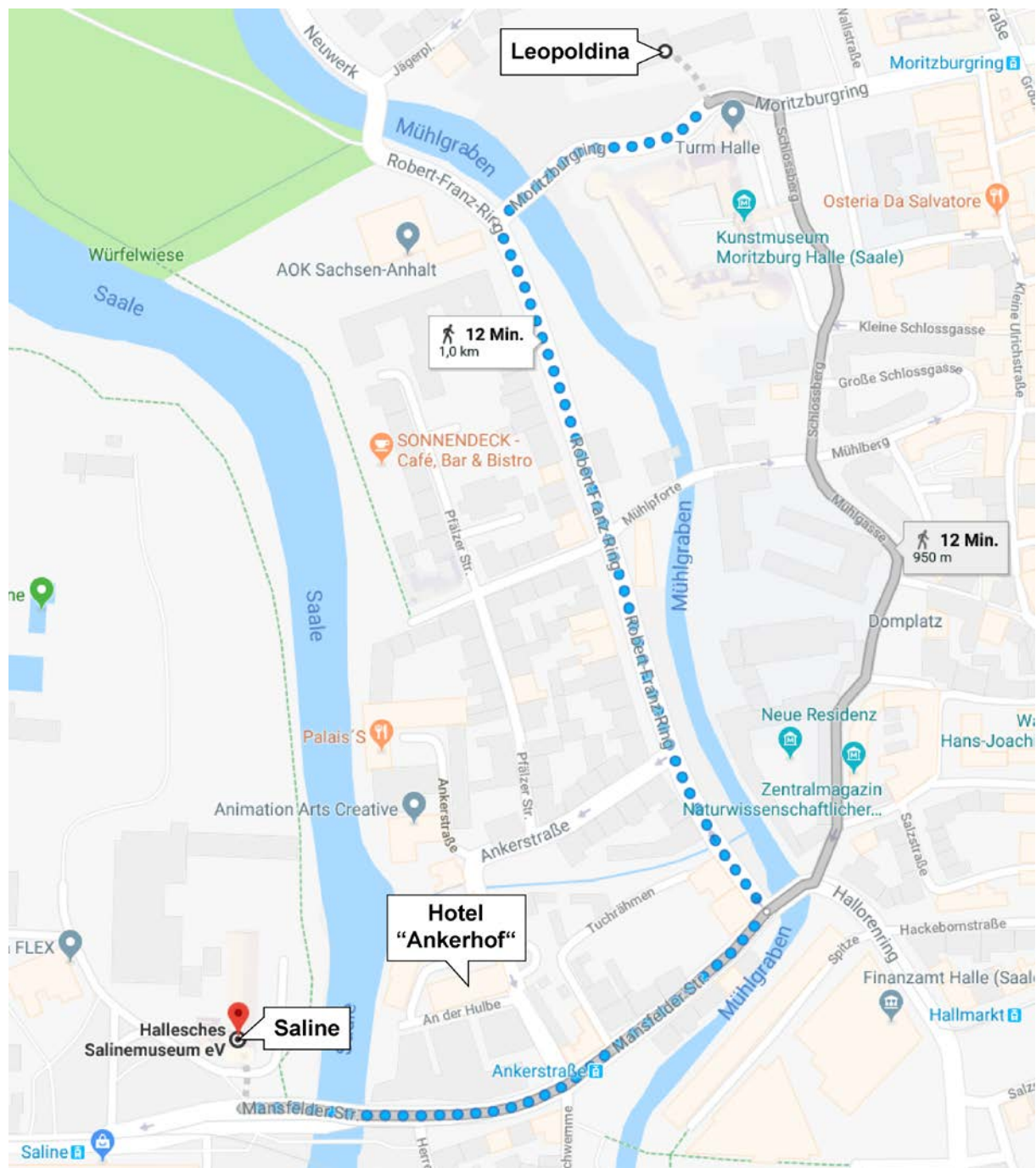
#### *State Museum of Prehistory*

(meeting point in front of the museum at 16.30 h, see map)

You can reach the State Museum of Prehistory by foot [ca. 20 minutes walk] or by tram. If you want to walk, a guide is waiting at the entrance of the Leopoldina at 16.00 h.

## Gala dinner

Friday, 19.00 in the Saline (12 minutes walk, see map)





# Program

## Wednesday, 18.07.2018

**14.00 – 17.30**      **Registration**  
Guided tour of the Leopoldina  
(30 min tours: 14.30/15.15/16.00; reception Leopoldina)

**16.45 – 17.00**      **Welcome address (Ulla Bonas)**

**Session I**      **Genomics and molecular diagnostics**  
Chair: Ulla Bonas

**17.00 – 17.40**      **Keynote lecture:**  
**Jan Leach** (Fort Collins, USA – invited speaker)  
Contributions of *Xanthomonas* genomics to plant disease management

**17.40 – 18.00**      **Lionel Gagnevin** (Montpellier, France)  
Two hundred years of solitude: first genomes of the bacterial pathogen *Xanthomonas* from herbarium specimen

**18.00 – 19.00**      **Reception** (Ground floor and garden of the Leopoldina)

**19.00**      **Meeting of the COST Management Committee** (Lecture Hall)

## Thursday, 19.07.2018

**Session I**      **Genomics and molecular diagnostics**  
continued      Chair: Jeffrey Jones

**08.30 – 09.00**      **Marie-Agnès Jacques** (Beaucouzé, France - invited speaker)  
Evolutionary histories of pathogenicity factors and organisms in the genus *Xanthomonas*

**09.00 – 09.20**      **Ricardo Oliva** (Los Banos, Philippines)  
Exploiting bacterial genomics to develop tools for effective pathogen monitoring in rice

**09.20 – 09.40**      **Marion Fischer-le-Saux** (Beaucouzé, France)  
Divergence and gene flow in *Xanthomonas axonopodis* species complex

**09.40 – 10.00**      **Joana Vicente** (Warwick, UK)  
Diversity of *Xanthomonas campestris* from ornamental *Brassicaceae* reveals a new pathovar pathogenic on wallflower

**10.00 – 10.30**      **Coffee break**

<b>Session I</b> continued	<b>Genomics and molecular diagnostics</b> Chair: Adam Bogdanove
<b>10.30 – 11.00</b>	<b>Laurent Noël</b> (Castanet-Tolosan, France - invited speaker) Extracting biology from <i>Xanthomonas campestris</i> genomic and transcriptomic analyses
<b>Session II</b>	<b>Pathogenicity mechanisms and regulation</b> Chair: Adam Bogdanove
<b>11.00 – 11.20</b>	<b>Paula Martins</b> (São Paulo, Brasil) Extracellular death factor peptides as novel molecules for citrus canker control
<b>11.20 – 11.40</b>	<b>Seiji Tsuge</b> (Kyoto, Japan) A novel regulatory pathway of <i>hrp</i> gene expression in <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> : quantitative regulation of a key <i>hrp</i> regulator HrpX by the regulator controlling xylan/xylose metabolism-related genes
<b>11.40 – 12.00</b>	<b>Nian Wang</b> (Lake Alfred, USA) A phosphorylation switch on Lon protease regulates bacterial type III secretion system in host
<b>12.00 – 13.00</b>	<b>Lunch break</b>
<b>Session II</b> continued	<b>Pathogenicity mechanisms and regulation</b> Chair: Adam Bogdanove
<b>13.00 – 13.30</b>	<b>Renier van der Hoorn</b> (Oxford, UK - invited speaker) Mining the extracellular battlefield of a plant-pathogen interaction
<b>13.30 – 15.30</b>	<b>Poster session I (even numbers)</b>
<b>15.30 – 16.00</b>	<b>Coffee break</b>
<b>16.15</b>	<b>Social program</b> Participation according to registration.  <b>Visit of Moritzburg</b> (meeting point at the entrance of the Moritzburg)  <b>Historic Town Centre Stroll</b> (meeting point at the entrance of the Leopoldina)  <b>State Museum of Prehistory</b> (start at 16.30, meeting point at the museum)

## Friday, 20.07.2018

<b>Session II</b> continued	<b>Pathogenicity mechanisms and regulation</b> Chair: Jana Streubel
<b>08.30 – 08.50</b>	<b>Cornelius Schmidtke</b> (Halle/Saale, Germany) Complexity of type III effector gene expression in <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>
<b>08.50 – 09.10</b>	<b>Chenyang He</b> (Beijing, China) Interplay between two <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> response regulators TriP and PdeR in modulating virulence and exopolysaccharide production
<b>Session III</b>	<b>Type III secretion and effector proteins</b> Chair: Jana Streubel
<b>09.10 – 09.30</b>	<b>Daniela Büttner</b> (Halle/Saale, Germany) Functional characterization of a cytoplasmic ring component of the type III secretion system from <i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>
<b>09.30 – 09.50</b>	<b>Suayib Üstün</b> (Uppsala, Sweden) How <i>Xanthomonas</i> hijacks the interplay between proteolytic degradation pathways
<b>09.50 – 10.20</b>	<b>Coffee break</b>
<b>Session III</b> continued	<b>Type III secretion and effector proteins</b> Chair: Ralf Koebnik
<b>10.20 – 10.50</b>	<b>Guido Sessa</b> (Tel Aviv, Israel - invited speaker) Interference of <i>Xanthomonas</i> type III secreted effectors with plant immunity
<b>10.50 – 11.10</b>	<b>Jessica Erickson</b> (Halle/Saale, Germany) The <i>Xanthomonas</i> effector XopL affects stromules in <i>Nicotiana benthamiana</i>
<b>11.10 – 11.30</b>	<b>Stefanie Mücke</b> (Hannover, Germany) Functional convergence of TALEs addressing susceptibility hubs
<b>11.30 – 11.50</b>	<b>José Gadea</b> (Valencia, Spain) Characterisation of TAL-effector-mediated resistance to citrus canker using a new variant of <i>Xanthomonas citri</i>
<b>11.50 – 12.50</b>	<b>Lunch break</b>

<b>Session III</b> continued	<b>Type III secretion and effector proteins</b> Chair: Boris Szurek
---------------------------------	--

- |                      |   |
|----------------------|---|
| <b>12.50 – 13.20</b> | <b>Bing Yang</b> (Iowa, USA - invited speaker)<br>Bacterial TAL effector and host target gene interactions in bacterial blight of rice                |
| <b>13.20 – 13.40</b> | <b>Mathilde Hutin</b> (Ithaca, USA)<br>OsERF#123, a new susceptibility gene for bacterial blight of rice  |
| <b>13.40 – 14.10</b> | <b>Ramesh Sonti</b> (New Delhi, India - invited speaker)<br>Inducers and suppressors of host innate immunity in rice- <i>Xanthomonas</i> interactions |
| <b>14.10 – 14.40</b> | <b>Coffee break</b>   |

<b>Session IV</b>	<b>Other secretion systems</b> Chair: Matthieu Arlat
-------------------	---

- |                      |   |
|----------------------|---|
| <b>14.40 – 15.10</b> | <b>Chuck Farah</b> (São Paulo, Brazil - invited speaker)<br>Bacterial wars: Functional and structural studies on the bacteria-killing type IV-secretion system from <i>Xanthomonadaceae</i> |
| <b>15.10 – 15.30</b> | <b>Jonathan Jacobs</b> (Columbus, USA)<br>A single cell wall degrading enzyme acts as a molecular switch for vascular and non-vascular <i>Xanthomonas</i> pathogenesis                      |
| <b>15.30 – 15.50</b> | <b>Anna Joe</b> (Davis, USA)<br>ABC transporter RaxB is required for processing and secretion of sulfated RaxX to yield a biologically active form that resembles a plant hormone peptide   |
| <b>15.50 – 16.10</b> | <b>Ofir Bahar</b> (Rishon LeZion, Israel)<br>Bacterial outer membrane vesicles modulate the plant immune response and induce resistance to infection  |
| <b>16.15 – 18.15</b> | <b>Poster session II (odd numbers)</b>  |
| <b>19.00</b>         | <b>Gala dinner</b> (in the Saline, 10 minutes walk)   |

## Saturday, 21.07.2018

Session V	
Ecology and epidemiology	
Chair: Jan Leach	
09.00 – 09.30	<b>Steven Lindow</b> (Berkeley, USA - invited speaker) The many cell density-dependent behaviors of <i>Xylella fastidiosa</i> : achieving disease control via pathogen confusion
09.30 – 09.50	<b>Claude Bragard</b> (Louvain-la-Neuve, Belgium) What is the risk of <i>Xylella fastidiosa</i> for EU? Risk assessment and pest categorization
09.50 – 10.10	<b>Massimiliano Morelli</b> (Bari, Italy) Assessment of the effects of antimicrobial and quorum-sensing regulating substances on biofilm formation and cell growth of <i>Xylella fastidiosa</i> “De Donno” strain
10.10 – 10.30	<b>Valentine Nakato</b> (Kampala, Uganda) Deciphering the emergence of <i>Xanthomonas vasicola</i> pv. <i>musacearum</i> in Eastern and Central Africa using MLVA
10.30 – 11.00	<b>Coffee break</b>
Session VI	
Plant defense and resistance	
Chair: Jens Boch	
11.00 – 11.30	<b>Leena Tripathi</b> (Nairobi, Kenya - invited speaker) Development of host plant resistance to <i>Xanthomonas campestris</i> pv. <i>musacearum</i>
11.30 – 11.50	<b>Neha Potnis</b> (Auburn, USA) Harnessing the genome plasticity of bacterial spot species complex to guide a search for novel disease resistance in pepper
11.50 – 12.10	<b>Roland Kölliker</b> (Zurich, Switzerland) Characterisation of resistance genes and virulence factors in the <i>Lolium multiflorum</i> – <i>Xanthomonas translucens</i> pv. <i>graminis</i> pathosystem
12.10	<b>Closing remarks</b>





# TALKS



# Session I

## Genomics and molecular diagnostics

### Contributions of *Xanthomonas* genomics to plant disease management

**Jan E. Leach**

Colorado State University, Bioagricultural Sciences, Plant Sciences, Fort Collins, CO USA  
80537

#### **Abstract**

Translating tools and advances to manage diseases of important crops is a major driver of *Xanthomonas* genomics research programs. Genomics approaches have contributed to a deeper understanding of *Xanthomonas* biology and evolution as well as how this important group of pathogens cause disease or activate host defense responses. Genome-derived information has led to the development critical diagnostic tools as well as tools for genome editing. The scientific advances have been significant. Yet, diseases caused by *Xanthomonas* remain important problems for crop production. Using predominantly the rice pathogens in the *Xanthomonas oryzae* group as examples, I will explore emerging environmental and technological challenges that continue to thwart disease control, and that will influence future research directions.





## Two hundred years of solitude: first genomes of the bacterial pathogen *Xanthomonas* from herbarium specimen

Adrien Rieux<sup>1</sup>, Philippe Roumagnac<sup>2</sup>, Boris Szurek<sup>3</sup>, Myriam Gaudéul<sup>4</sup>, Nathalie Becker<sup>1,4</sup>, **Lionel Gagnevin**<sup>5</sup>

<sup>1</sup> CIRAD, UMR PVBMT, Saint-Pierre, La Réunion, France

<sup>2</sup> CIRAD, UMR BGPI, Montpellier, France

<sup>3</sup> IRD, UMR IMPE, Montpellier, France

<sup>4</sup> MNHN, UMR ISYEB, Paris, France

<sup>5</sup> CIRAD, UMR IMPE, Montpellier, France

**Keywords:** paleogenomics, ancient DNA, degraded DNA, evolution, pathogen emergence

### Abstract

In order to better control current diseases of plants and prevent future epidemics, it is crucial to develop an improved understanding of the factors underlying pathogen emergence, adaptation and spread. Recent methodological developments in molecular epidemiology now allow tackling such question through fine reconstruction of disease dynamics in space and time. To date, essentially all studies on plant pathogens have focused on “contemporary” individuals sampled over a fairly limited period of time (around 30 years at a maximum) but recent developments in DNA sequencing technology now make possible reconstructing historical genomes dating back to previous centuries. We intended to apply such development to the case of historical crop pathogenic bacterial genomes retrieved from herbarium collection material. In this talk I will expose our first results and discuss future research directions.



## Evolutionary histories of pathogenicity factors and organisms in the genus *Xanthomonas*

Déborah Merda<sup>1</sup>, Martial Briand<sup>1</sup>, Eran Bosis<sup>2</sup>, Thibaut Leroy<sup>3</sup>, Mahendra Mariadassou<sup>4</sup>, Adrien Rieux<sup>5</sup>, Marion Fischer-Le Saux<sup>1</sup>, and **Marie-Agnès Jacques**<sup>1</sup>

<sup>1</sup> IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, 49071, Beaucouzé, France

<sup>2</sup> Department of Biotechnology Engineering, ORT Braude College, Karmiel 2161002, Israel

<sup>3</sup> UMR 1202 BIOGECO, INRA, F-33610 Cestas, France

<sup>4</sup> MalAGE, INRA, Université Paris, Saclay, 78350 Jouy en Josas, France

<sup>5</sup> CIRAD, UMR PVBMT, 97410 St Pierre de la Réunion, France

### Abstract

Deciphering the evolutionary history of virulence factors and bacterial populations is required to understand disease emergence. *Xanthomonas* are plant-associated bacteria being pathogens or commensals. The main *Xanthomonas* virulence factors are the type three secretion system (T3SS) and its effectors (T3E). Our aim is to unveil the evolutionary events responsible for pathovar emergence. From comparisons of genome sequences representing the diversity of this genus, we inferred three ancestral acquisitions of the T3SS gene cluster during *Xanthomonas* evolution followed by subsequent losses in some commensal strains and re-acquisition in some species. As most commensal strains are found in *Xanthomonas arboricola*, we focused on this species. First, a reduced core type 3 effectome was inferred in the putative ancestor of this species. From this one, stepwise accumulation of numerous T3E genes was detected in successful pathogens responsible for epidemics. This T3E gene accumulation would have led to the emergence of an epidemic clone from a recombinogenic background population. Divergence time of the three main pathovars in *X. arboricola* fit with anthropomorphic activities and recent epidemics. Finally, commensal and pathogenic strains continue to evolve together, as detected by a persistent gene flux between these two groups. Altogether these analyses demonstrate that from a common ancestor that had a limited set of virulence factors, three evolutionary routes were followed involving gradual acquisition of T3Es, maintenance of the T3E ancestral set, or loss of these ancestral virulence factors. Gene flux from commensal strains to pathovars may favor *de novo* acquisition of adaptive traits, including virulence factors.



## Exploiting bacterial genomics to develop tools for effective pathogen monitoring in rice

Ian Quibod<sup>1</sup>, Genelou Grande<sup>1</sup>, Eula Oreiro<sup>1</sup>, EiEi Aung<sup>1</sup>, Denice Palmos<sup>2</sup>, Frances Borja<sup>1</sup>, Veronica Roman-Reyna<sup>1</sup>, Thea Coronejo<sup>1</sup>, Cintia Saloma<sup>2</sup>, Ramil Mauleon<sup>1</sup>, Casiana Vera Cruz<sup>1</sup>, **Ricardo Oliva**<sup>1</sup>

<sup>1</sup> International Rice Research Institute, Los Banos, Philippines

<sup>2</sup> Philippines Genome Center, University of the Philippines, Manila, Philippines

**Keywords:** race, pathogenomics, bacterial blight, rice, *Xanthomonas oryzae* pv. *oryzae*

### Abstract

The recurrent emergence of highly aggressive clones of plant pathogens in agricultural ecosystems represents an important threat to food security. Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is the most important bacterial disease of rice. At the population level, *Xoo* is usually composed of a number of phenotypic groups (races), which show *R*-gene-specific interactions.

Estimating the race composition of *Xoo* in the rice paddy is critical to design sustainable management strategies. In this work, we used bacterial genomics to develop DNA markers for effective surveillance of *Xoo* races in the Philippines archipelago. We first identified the evolutionary forces that shape contemporary groups during the last 40 years of *Xoo* outbreaks. Through comparative genomics on 100 highly informative strains, we identified six *Xoo* populations, which diverge before the colonization of the islands. The patterns of positive selection, recombination, and diversification of effector genes suggest that each population experienced a distinct adaptation process that led to the creation of modern races. Using this information, we developed single nucleotide polymorphism (SNP) markers that allow us to detect the pathogen groups in the rice leaf. We validated the robustness of SNP markers for three cropping seasons using a trapping system based on near-isogenic lines carrying different *R* genes and demonstrated that real-time surveillance of *Xoo* is possible. In this scenario, we are proposing an interactive platform, called Pathotracker, which integrates early-season race diagnostics, modeling of weather patterns, and disease resistance profiles to accurately predict which variety should be used in the following season. The platform will allow us to manage bacterial blight epidemics in real-time and to define breeding priorities for the region.





## Divergence and gene flow in *Xanthomonas axonopodis* species complex

Karine Durand, Martial Briand, Marie-Agnès Jacques, Christophe Lemaire, **Marion Fischer-Le Saux**

IRHS, INRA, Université d'Angers, Agrocampus-Ouest, SFR 4207 QuaSaV, 49071, Beaucouzé, France

**Keywords:** population genomics, homologous recombination, demographic inference, speciation

### Abstract

Deciphering the mechanisms responsible for the divergence of bacteria is crucial to estimate the evolutionary dynamics of pathogens. The *Xanthomonas axonopodis* species complex includes bacteria responsible for plant diseases with important socio-economic impacts on many crops. Using 73 genomes representative of the diversity of this complex, we address the questions of the nature of the evolutionary forces and the demographic history that have shaped the genetic structure and the species boundaries, within this species complex. Based on phylogenomics and population genomics, we detected five genetic groups corresponding to species delineations recently reported with the exception of *X. citri*, which was now split into two distinct genetic groups. Genetic exchanges by homologous recombination between groups were extremely low compared to their level within groups. When detected, gene flow between groups was positively correlated to ecological niche sharing and not correlated to phylogenetic proximity. For instance, gene flow was observed between strains from phylogenetically distant groups sharing the same host (bean and *Araceae*, respectively). Demographic history inferences supported a scenario of divergence with very low gene flow between the two *X. citri* groups. In contrast, scenarios of divergence followed by recent secondary contact were inferred between *X. euvesicatoria* and the two *X. citri* groups, respectively. These secondary contacts responsible for a much higher gene flow between phylogenetically distant groups of *Xanthomonas* than the one detected between recently diverged groups, put the question of the role of agricultural intensification and globalization in promoting pathogen emergence through acquisition of new traits.



## Diversity of *Xanthomonas campestris* from ornamental *Brassicaceae* reveals a new pathovar pathogenic on wallflower

**Joana G. Vicente**<sup>1</sup>, David E. Stead<sup>2</sup>, Steve Roberts<sup>3</sup>, Vânia H. Passo<sup>1</sup>, Eric B. Holub<sup>1</sup>, David J. Studholme<sup>4</sup>

<sup>1</sup> School of Life Sciences, University of Warwick, Wellesbourne Campus, Warwick CV35 9EF, UK

<sup>2</sup> Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK

<sup>3</sup> Plant Health Solutions, 20 Beauchamp Road, Warwick CV34 5NU, UK

<sup>4</sup> Biosciences, University of Exeter, Exeter EX4 4QD, UK

**Keywords:** wilt, candytuft, stock, pathogenicity, sequencing

### Abstract

The bacterial pathogen *Xanthomonas campestris* can cause damaging diseases on popular ornamental plants belonging to the *Brassicaceae* family, including: wallflower (*Erysimum* spp.), candytuft (*Iberis* spp.) and garden stock (*Matthiola incana*). Previous isolates from wallflowers were identified as *X. campestris*, *X. campestris* pv. *campestris* (*Xcc*) or *X. campestris* pv. *incanae* (*Xci*), whilst an isolate from candytuft was the type-strain of *X. campestris* pv. *armoraciae* and later included within *Xcc*. Isolates from garden stocks are included in *Xci*.

In the current study, isolates were obtained from wallflowers with vascular wilt symptoms, growing in commercial plant nurseries in England, and from culture collections. Pathogenicity testing on a range of ornamental *Brassicaceae*, brassicas, radish and tomato revealed that there are two pathotypes of wallflower isolates, distinct from isolates obtained from candytuft, garden stocks and brassicas and should therefore belong to different pathovars. In addition, one isolate from wallflower produced leaf spot symptoms and was identified as *X. campestris* pv. *raphani* (*Xcr*), a pathovar not previously identified in wallflower.

On the basis of *gyrB* DNA sequences, isolates specifically pathogenic to wallflowers comprised a distinct group separated from groups corresponding to *Xcc*, *Xcr* and *Xci*. Whole-genome sequencing of three isolates from wallflower confirmed their distinctness from previously sequenced *X. campestris*. We propose that the isolates from wallflowers that are mainly pathogenic only on wallflowers, should be included in a new pathovar.

Strategies for control of bacterial diseases of ornamental *Brassicaceae*, including selection and/or breeding of resistant cultivars, should take into account the different pathogens that cause disease.





## Extracting biology from *Xanthomonas campestris* genomic and transcriptomic analyses

**Laurent D. Noël**<sup>1</sup>, Matthieu Arlat<sup>1</sup>, Richard Berthomé<sup>1</sup>, Alice Boulanger<sup>1</sup>, Sebastien Carrere<sup>1</sup>, Aude Cerutti<sup>1</sup>, Nicolas Denancé<sup>1</sup>, Ivanna Fuentes<sup>1</sup>, Emmanuel Gonzalez Fuente<sup>1</sup>, Carine Gris<sup>1</sup>, Endrick Guy<sup>1</sup>, Françoise Jardinaud<sup>1</sup>, Alain Jauneau<sup>1</sup>, Emmanuelle Lauber<sup>1</sup>, Julien Luneau<sup>1</sup>, Jean-Marc Routaboul<sup>1</sup>, Brice Roux<sup>1</sup>, Maël Baudin<sup>2,8</sup>, Tristan Boureau<sup>3</sup>, Martial Briand<sup>3</sup>, Adam Bogdanove<sup>5</sup>, Adam Deutschbauer<sup>6</sup>, Erin Doyle<sup>4</sup>, Lionel Gagnevin<sup>7</sup>, Ahmed Hajri<sup>3</sup>, Marie-Agnès Jacques<sup>3</sup>, Ralf Koebnik<sup>7</sup>, Jennifer D. Lewis<sup>2,8</sup>, Stéphane Poussier<sup>3</sup> and Boris Szurek<sup>7</sup>

<sup>1</sup> LIPM, Université de Toulouse, INRA, CNRS, UPS, Castanet-Tolosan, France

<sup>2</sup> USDA ARS Plant Gene Expression Center, Albany, CA, USA

<sup>3</sup> IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, Beaucouzé, France

<sup>4</sup> Biology Department, Doane University, Crete, Nebraska, USA

<sup>5</sup> Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA

<sup>6</sup> Lawrence Berkeley National Laboratory, Berkeley, CA, USA

<sup>7</sup> IRD, Cirad, Univ. Montpellier, IPME, Montpellier, France

<sup>8</sup> Department of Plant & Microbial Biology, University of California Berkeley, Albany, CA, USA

**Keywords:** virulence, avirulence, type III effector proteins, comparative genomics, transcriptomics, hydathode, TALE, black rot disease, *Xanthomonas campestris*, cauliflower, TnSeq

### Abstract

*Xanthomonas campestris* species can cause vascular or non-vascular diseases on *Brassicaceae* crops and served since the early 80s as a model to study the genetic bases of bacterial pathogenicity on plants. To date, sequences of hundreds of *Xanthomonas* genomes have been determined and yielded important information about within-genus, -species and -pathovar genomic diversity. Yet, extracting biologically relevant knowledge from these datasets still remains challenging. I will present some promising approaches applied to *Xanthomonas campestris* to illustrate how comparative genomics, functional genomics, next-generation forward genetic screens and transcriptomic analyses can be used to elucidate some of the mechanisms underlying bacterial adaptation to plant environments and bacterial infection strategies.

These projects benefit from the FNX (French Network on *Xanthomonas*) network and from the EuroXanth COST action.



## Session II

### Pathogenicity mechanisms and regulation

#### Extracellular death factor peptides as novel molecules for citrus canker control

**Paula Martins**, Maria Júlia Festa Franzini, Simone Cristina Picchi, Alessandra Alves de Souza

Instituto Agronômico de Campinas – Centro de Citricultura Sylvio Moreira, 13020-902, Brasil

#### Abstract

Extracellular death factors (EDF) are peptides originated from intracellular metabolism which are able to go freely in and out of neighbouring cells. These molecules, although different in origin, are supposed to act on the toxin counterpart of toxin-antitoxin systems (TA), enhancing its activity and eventually leading to cell death. TAs in phytopathogens are still poorly understood, and their biological role, as well as their possible involvement in disease development are unknown. *Xanthomonas* is the unique phytopathogen to which genome-wide assessment has been done, showing differential TA occurrence in both number and types among this genus. In this work we show for the first time that five known EDFs as well as one new EDF, specific for *Xanthomonas citri*, are able to impair disease progression during *in vitro* experiments using Rangpur Lime plantlets. They were inoculated with bacterial suspensions of *X. citri* to which peptides were separately added, and evaluated after 7, 14, 21 and 28 days based on a severity scale that goes from 1 to 5, being 1 plants with less symptoms and 5 plants with more canker lesions. We observed that at day 28, control plantlets reached severity values of 4, but EDF-supplemented plantlets showed slower disease progression, never surpassing severity level 3. Among the 6 EDF peptides tested, *X. citri*-specific EDF presented the lowest severity level at day 28, reaching only 1.4 in the scale. Therefore, EDF peptides could be seen as an innovative alternative, opening a new field for disease control for many plant diseases.



**A novel regulatory pathway of *hrp* gene expression in *Xanthomonas oryzae* pv. *oryzae*: quantitative regulation of the key *hrp* regulator HrpX by the regulator controlling xylan/xylose metabolism-related genes**

Yumi Ikawa<sup>1</sup>, Sayaka Ohnishi<sup>1</sup>, Akiko Shoji<sup>1</sup>, Ayako Furutani<sup>2</sup> and **Seiji Tsuge**<sup>1</sup>

<sup>1</sup> Graduate School of Agriculture, Kyoto Prefectural University, Kyoto 606-8522, Japan

<sup>2</sup> Gene Research Center, Ibaraki University, Inashiki 300-0393, Japan

**Keywords:** *hrp*, LacI-type transcriptional regulator, xylose metabolism, *Xanthomonas oryzae* pv. *oryzae*

**Abstract**

*hrp* genes encoding components of the type III secretion system are indispensable for virulence of *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight of rice. Expression of *hrp* genes is induced only in plants or certain nutrient-poor media, so called *hrp*-inducing media, and two key *hrp* regulators, HrpG and HrpG-regulated HrpX that regulates other *hrp* genes, play crucial roles in *hrp* gene expression along with other known and unknown factors. We found that *hrp* gene expression of *X. oryzae* pv. *oryzae* is specifically induced when xylose is added in the *hrp*-inducing medium, and that the second key *hrp* regulator HrpX abundantly accumulated only in the presence of xylose, leading to high *hrp* gene expression although *hrpX* expression is independent on sugar source. Furthermore, in a mutant lacking the LacI-type transcriptional regulator XylR, HrpX accumulation and *hrp* gene expression were highly induced even in the medium without xylose. XylR also negatively regulated one of two xylose isomerase genes (*xylA2*, not *xylA1*) that encode essential enzymes in xylose metabolism by converting xylose to xylulose, by binding to the motif sequence in the upstream region of *xylA2*. In the wild type, addition of xylose specifically induced the expression of *xylA2*, but in the XylR deletion mutant, the expression was observed even without xylose. The results suggest that, in the presence of xylose, inactivation of XylR leads to greater xylan/xylose utilization and, simultaneously, to higher accumulation of HrpX, which leads to higher *hrp* gene expression in *X. oryzae* pv. *oryzae*.



## A phosphorylation switch on Lon protease regulates bacterial type III secretion system in host

Xiaofeng Zhou<sup>1</sup>, Doron Teper<sup>1</sup>, Maxuel O. Andrade<sup>1</sup>, Tong Zhang<sup>2</sup>, Sixue Chen<sup>2</sup>, Wen-Yuan Song<sup>3</sup>, **Nian Wang<sup>1</sup>**

<sup>1</sup> Citrus Research and Education Center, Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA

<sup>2</sup> Department of Biology and the University of Florida Genetics Institute, University of Florida, Gainesville, FL 32611, USA

<sup>3</sup> Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA

**Keywords:** Type III secretion system, *Xanthomonas*, Lon, phosphorylation

### Abstract

Most pathogenic bacteria deliver virulence factors into host cytosol through type III secretion systems (T3SS) to perturb host immune responses. The expression of T3SS is often repressed in rich medium, but is specifically induced in the host environment. The molecular mechanisms underlying host-specific induction of T3SS expression is not completely understood. Here we demonstrate in *Xanthomonas citri*, that host-induced phosphorylation of the ATP-dependent protease Lon stabilizes HrpG, the master regulator of T3SS, conferring bacterial virulence. Ser/Thr/Tyr phosphoproteome analysis revealed that phosphorylation of Lon at serine 654 occurs in the citrus host. In rich medium, Lon represses T3SS by degradation of HrpG via recognition of its N terminus. Genetic and biochemical data indicate that phosphorylation at serine 654 deactivates Lon proteolytic activity and attenuates HrpG proteolysis. Substitution of Lon serine 654 to alanine resulted in repression of T3SS gene expression in the citrus host through robust degradation of HrpG, and reduces bacterial virulence. Our work provides a novel molecular mechanism underlying host-dependent activation of bacterial T3SS.





## Mining the extracellular battlefield of a plant-pathogen interaction

Pierre Buscaill<sup>1</sup>, Balakumaran Chandrasekar<sup>1</sup>, Daniela Sueldo<sup>1</sup>, Nattapong Sanguankiatichai<sup>1</sup>, Farnusch Kaschani<sup>2</sup>, Kyoko Morimoto<sup>1</sup>, Emma Thomas<sup>1</sup>, Markus Kaiser<sup>2</sup>, Gail Preston<sup>1</sup>, Yuki Ichinose<sup>3</sup> and Renier van der Hoorn<sup>1</sup>

<sup>1</sup> Department of Plant Sciences, University of Oxford, Oxford, UK

<sup>2</sup> ZMB Chemical Biology, Faculty of Biology, University of Duisburg-Essen, Essen, Germany

<sup>3</sup> The Graduate School of Environmental and Life Science, Okayama University, Japan

**Keywords:** apoplast, hydrolase, *Nicotiana benthamiana*, *Pseudomonas syringae*

### Abstract

The extracellular space (apoplast) in leaves is an important battlefield of plant-pathogen interactions, also for bacteria. To discover novel host manipulation mechanisms in the apoplast, we study changes in the extracellular enzymes upon infection using activity-based proteomics, using the interaction between *Nicotiana benthamiana* and *Pseudomonas syringae* as a model system. With proteomics and chemical probes that react with the active sites of enzymes, we are able to monitor activities of >140 apoplastic enzymes. The activity of the majority of these enzymes changes during infection. Amongst the 25 protein activities that are post-translationally repressed, we detected a beta-galactosidase that is suppressed by a metabolite secreted by *P. syringae*. Genetic depletion of this enzyme makes the host plant more susceptible for *P. syringae* infection. Further research into this enzyme revealed that this enzyme plays an important role in defense, explaining why the enzyme is inhibited during infection. Our work stresses the role of apoplastic events in plant-bacteria interactions and has increased our interest on investigating the role of the other 24 enzymes that are suppressed during infection.



## Complexity of type III effector gene expression in *Xanthomonas campestris* pv. *vesicatoria*

Jan Niklas Kemna<sup>1</sup>, Alice Wedler<sup>1</sup>, Karen Barthel<sup>1</sup>, Jan Grau<sup>3</sup>, Evelyn Löschner<sup>1</sup>, Stefanie Mücke<sup>1,2</sup>, Ulla Bonas<sup>1</sup>, **Cornelius Schmidtke**<sup>1</sup>

<sup>1</sup> Institute for Biology, Dept. of Genetics, Martin Luther University Halle-Wittenberg, Halle, Germany

<sup>2</sup> Present address: Leibniz University Hannover, Institute of Plant Genetics, Hannover, Germany

<sup>3</sup> Institute of Computer Science, Martin Luther University Halle-Wittenberg, Halle, Germany

### Abstract

Transcriptome analyses of *Xanthomonas campestris* pv. *vesicatoria* (Xcv) strain 85-10, the causal agent of bacterial spot disease on pepper and tomato, led to the identification of noncoding RNAs as novel virulence factors, e.g. the small RNA sX13. Recent RNA-seq analyses also revealed loci with previously unnoticed coding potential. More than 200 5'-untranslated regions of mRNAs contain small ORFs, with often less than 50 codons, which were ignored during genome annotation. One example is the complex transcriptional and posttranscriptional regulation of an Xcv type III effector locus characterized by an mRNA with a long 5'-untranslated region, alternative promoters and small ORFs. Furthermore, we will present a novel antisense RNA-based 'silencing' approach to address potential virulence functions of candidate genes prior to or as alternative to time-consuming deletion mutagenesis experiments.



## Interplay between two *Xanthomonas oryzae* pv. *oryzae* response regulators TriP and PdeR in modulating virulence and exopolysaccharide production

Haiyun Li<sup>1</sup>, Fang Tian<sup>1</sup>, Dingrong Xue<sup>1</sup>, Xiaochen Yuan<sup>2</sup>, Fenghuan Yang<sup>1</sup>, Huamin Chen<sup>1</sup>, Ching-Hong Yang<sup>2</sup>, **Chenyang He**<sup>1</sup>

<sup>1</sup> Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

<sup>2</sup> Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA

**Keywords:** two-component regulatory system, phosphodiesterase, virulence, epistatic regulation, *Xanthomonas oryzae* pv. *oryzae*

### Abstract

PdeR, a response regulator of the two-component system with the cognate histidine kinase PdeK, has been shown to be an active phosphodiesterase (PDE) for intracellular c-di-GMP turnover and positively regulate the virulence of *Xanthomonas oryzae* pv. *oryzae*, the causal pathogen of bacterial blight of rice. To further reveal the key components involved in the PdeR-mediated c-di-GMP regulation of virulence, 16 PdeR-interacting proteins were identified in the yeast two-hybrid (Y2H) assays. Among them, PXO\_04421 (named as TriP, a putative transcriptional regulator interacting with PdeR) was verified via the Y2H and glutathione-S-transferase pull-down assays, and its regulatory functions in bacterial virulence and exopolysaccharide (EPS) production were accessed by using biochemical and genetic analysis. The REC domain of TriP specifically interacted with the EAL domain of PdeR. TriP promoted the PDE activity of PdeR to degrade c-di-GMP in presence of PdeK. In-frame deletion in *triP* resulted in abolished polar localization of PdeR in the cells.  $\Delta triP$  showed significantly reduced virulence on susceptible rice leaves and impaired EPS production compared with wildtype, whereas the double mutant  $\Delta triP \Delta pdeR$ , like  $\Delta pdeR$ , caused longer lesion lengths and produced more EPS than  $\Delta triP$ . Cross-complementation showed *in trans* expression of *pdeR* in  $\Delta triP$  restored its EPS production to near wildtype level, but not *vice versa*. Our results suggest that TriP is a novel key regulator that is epistatic to PdeR in positively regulating virulence in *X. oryzae* pv. *oryzae*.



## Session III

### Type III secretion and effector proteins

#### Functional characterization of a cytoplasmic ring component of the type III secretion system from *Xanthomonas campestris* pv. *vesicatoria*

Jens Hausner<sup>1</sup>, Sylvestre Marillonnet<sup>2</sup>, Christian Otten<sup>3</sup> and **Daniela Büttner**<sup>3</sup>

<sup>1</sup> Present address: Icon Genetics GmbH, Halle (Saale), Germany

<sup>2</sup> Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany

<sup>3</sup> Martin Luther University Halle-Wittenberg, Institute of Biology, Department of Genetics, Halle (Saale), Germany

**Keywords:** type III secretion, cytoplasmic ring, modular cloning

#### Abstract

*Xanthomonas campestris* pv. *vesicatoria* is the causal agent of bacterial spot disease on tomato and pepper plants. Pathogenicity of *X. campestris* pv. *vesicatoria* depends on a type III secretion (T3S) system, which translocates effector proteins into plant cells. The T3S system is encoded by a chromosomal 23-kb *hrp* (hypersensitive response and pathogenicity) gene cluster, which is organized in eight transcriptional units and contains 25 genes. Additional accessory genes are located in the flanking region of the *hrp* gene cluster. Nine components of the T3S system are conserved in plant- and animal-pathogenic bacteria, suggesting that the core architecture of the secretion apparatus is similar in different bacterial species. Mutant and biochemical approaches revealed that the conserved cytoplasmic HrcQ protein from *X. campestris* pv. *vesicatoria* forms complexes, which might serve as docking sites for secreted proteins and associate with the cytoplasmic ATPase complex and components of the export apparatus in the inner membrane. To facilitate future genetic manipulations of T3S genes in *X. campestris* pv. *vesicatoria*, we generated a modular T3S gene cluster using the Golden Gate-based MoClo system, which will be used for functional and localization studies with putative ring components of the T3S system.





## How *Xanthomonas* hijacks the interplay between proteolytic degradation pathways

**Suayib Üstün**<sup>1</sup>, Daniela Spinti<sup>2</sup>, Frederik Börnke<sup>2,3</sup>, Daniel Hofius<sup>1</sup>

<sup>1</sup> Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden

<sup>2</sup> Leibniz-Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany

<sup>3</sup> Institute for Biochemistry and Biology, University of Potsdam, Germany

**Keywords:** effectors, autophagy, proteasome

### Abstract

Autophagy and the ubiquitin-proteasome system (UPS) are the major pathways for protein degradation in eukaryotes. They orchestrate many cellular processes during development and in response to environmental stimuli such as microbial infections. In plants, the contribution of the UPS to immune responses and its targeting and exploitation by pathogens are well documented, but the role of autophagy remains elusive. Recent advances in the plant-microbe interaction field revealed pro- and anti-bacterial roles of autophagy. For instance, *Pseudomonas syringae* pv. *tomato* activates autophagy for proteasome degradation and enhanced virulence. Here, we show that the T3E XopL of *Xanthomonas campestris* pv. *vesicatoria* (Xcv), possibly modulates autophagy responses by interacting with an autophagy component. *In planta* interaction studies revealed that both proteins interact in vesicle-like mobile structures in the host cytoplasm. Given the fact that XopL constitutes a novel class of E3 ligases, we propose that XopL might interfere with autophagy via ubiquitination and proteasome-dependent degradation of its interaction partner. Preliminary results of the possible effect of XopL on its target indicate that XopL degrades this autophagy component in a proteasome-dependent manner. Using a dual-luciferase based quantitative autophagy assay we identified that Xcv blocks autophagic degradation via T3E XopL. Thus, we conclude that T3E XopL exploits the UPS to degrade an autophagy component leading to altered autophagy responses.



**Interference of *Xanthomonas* type III secreted effectors with plant immunity**

Bharat Bhusan Majhi, Georgy Popov, Guy Sobol, Doron Teper, Anil Madhusoodana Girija, Shivakumar Sreeramulu and **Guido Sessa**

School of Food Security and Plant Sciences, Tel Aviv University, 69978 Tel Aviv, Israel.  
eMail: guidos@tauex.tau.ac.il

**Abstract**

Gram-negative plant-pathogenic bacteria utilize the type III secretion system to deliver effector proteins into host cells, interfere with host cellular functions, and promote disease. A main function of type III effectors is to disarm plant defense responses by manipulating signaling components of plant immunity. *Xanthomonas euvesicatoria* (Xe) strains, which cause bacterial spot disease in tomato and pepper plants, deliver a pool of about 35 type III effectors into plant cells. We utilized a combination of functional screens, biochemical and genetic approaches to identify Xe type III effectors that manipulate plant immunity and uncover their biochemical properties, mode of action and plant targets. By a screen carried out in *Arabidopsis* protoplasts, we defined the pool of Xe effectors that interfere with gene expression and defense responses associated with pattern-triggered immunity (PTI). Further analysis of a subgroup of PTI-suppressing effectors revealed their interaction and possible manipulation of a novel family of receptor-like cytoplasmic kinases involved in plant immunity. We also demonstrated that certain Xe effectors interfere with effector-triggered immunity (ETI). For example, XopQ inhibits ETI by interacting with a tomato scaffolding protein of the 14-3-3 protein family that is required for plant immunity. Conversely, XopAU, which encodes an active protein kinase, activates plant defense responses by interacting with and activating the immunity-associated MAP kinase kinase MKK2. Significance and models depicting molecular strategies used by Xe effectors to manipulate plant immunity will be discussed.



## The *Xanthomonas* effector XopL affects stromules in *Nicotiana benthamiana*

**Jessica L. Erickson**<sup>1,2</sup>, Norman Adlung<sup>1,3</sup>, Christina Lampe<sup>1,2</sup>, Ulla Bonas<sup>1</sup>, Martin H. Schattat<sup>2</sup>

<sup>1</sup> Dept. of Genetics, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle, Germany

<sup>2</sup> Dept. of Plant Physiology, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle, Germany

<sup>3</sup> Present address: Dept. of Bioproducts and Biosystems, Biochemistry, Aalto University, Helsinki, Finland

**Keywords:** stromule dynamics, microtubules, XopL, E3 ubiquitin ligase, *N. benthamiana*

### Abstract

Stroma-filled tubules (stromules) emanate from the surface of all plastid types, in species throughout the plant kingdom. Stromule morphology is flexible, and tubules may extend, branch, and retract within minutes or even seconds. Stromules are frequently observed when plants experience stress. Recently, stromules have been suggested to act as a route for pro-defense signal transfer between plastids and the nucleus during effector-triggered immunity (ETI) culminating in the hypersensitive response (HR). However, evaluating the relevance of stromules to cell/plant viability during pathogen attack or under abiotic stress is difficult since there is currently no known mutation that prevents stromule formation. As an alternative to mutational and gene silencing approaches we probed for stromule-relevant processes using type III-secreted effector proteins (T3Es) from *Xanthomonas campestris* pv. *vesicatoria*. During infection, T3Es are translocated directly into the plant cell where they specifically target host cellular components to provide benefit to the pathogen. Transient expression of 21 effectors in the lower epidermis of *N. benthamiana* leaves revealed that XopL, an E3 ubiquitin ligase, almost completely abolished stromules.



## Functional convergence of TALEs addressing susceptibility hubs

**Stefanie Mücke**<sup>1</sup>, Maik Reschke<sup>1</sup>, Sebastian Becker<sup>1</sup>, Claudia A. Schwietzer<sup>1</sup>, Annett Erkes<sup>2</sup>, Jan Grau<sup>2</sup> and Jens Boch<sup>1</sup>

<sup>1</sup> Leibniz University Hannover, Institute of Plant Genetics, Hannover, Germany

<sup>2</sup> Martin Luther University Halle-Wittenberg, Institute of Computer Science, Halle (Saale), Germany

**Keywords:** *Xanthomonas oryzae* pv. *oryzae*, TALE, rice, susceptibility hub, convergence

### Abstract

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*) cause bacterial leaf blight and leaf streak, respectively, both devastating diseases of rice. Major virulence factors of *Xoo* and *Xoc* are transcription activator-like effectors (TALEs), which operate as transcription factors in eukaryotic cells. TALE proteins bind DNA via 34-amino acid-repeats with each repeat containing an RVD (repeat variable diresidue), which recognizes one base in the target DNA. and use large numbers of TALEs to manipulate their host cells.

We sequenced two *Xoo* strains from the Philippines and India to investigate differences and similarities between *TALE* gene repertoires of *Xoo* strains from independent geographic locations. TALEs were assigned into TALE classes based on similarity of their DNA target sequence. TALE classes were further categorized depending on their abundance in different Asian *Xoo* strains and a common core set of TALEs was identified.

Potential target genes of each TALE were identified using a combinatorial approach of *in silico*-prediction and TALE-dependent gene induction in infected rice tissue via RNAseq. To analyze the effect of individual TALEs, members of all 23 TALE classes present in the two sequenced *Xoo* strains were reconstructed and introduced into an *Xoo* strain without any natural TALEs. Strains expressing individual TALEs were used to analyze gene induction of potential target genes in rice and in GUS-reporter studies.

We identified new potential susceptibility hubs that are addressed by the different rice pathogens, *Xoo* and *Xoc*. Our data further suggest, that xanthomonads have common infection strategies across different host plants.





## Characterisation of TAL-effector-mediated resistance to citrus canker using a new variant of *Xanthomonas citri*

Roxana A. Roeschlin<sup>1,2</sup>, Maria Celeste Molina<sup>1,2</sup>, Facundo Uviedo<sup>1,2</sup>, Lucila García<sup>1</sup>, María A. Favaro<sup>1</sup>, María A. Chiesa<sup>1</sup>, Javier Forment<sup>2</sup>, María R. Marano<sup>1</sup>, **José Gadea**<sup>2</sup>

<sup>1</sup> Instituto de Biología Molecular y Celular de Rosario (IBR)- Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET). Área Virología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Ocampo y Esmeralda, S2000FHN Rosario, Argentina.

<sup>2</sup> Instituto de Biología Molecular y Celular de Plantas (IBMCP). Universidad Politécnica de Valencia-C.S.I.C, Ingeniero Fausto Elio, s/n. 46022 Valencia, España.

### Abstract

*Xanthomonas citri* subespecie *citri* (*X. citri*) is the causal agent of Asiatic citrus canker. We have identified a natural variant, *X. citri* AT, that triggers a host-specific defense response in *Citrus limon* and *C. sinensis*. *X. citri* AT triggers in resistant plants a hypersensitive response (HR-like) associated with the interference on biofilm development and arrest of bacterial growth, involving an extensive transcriptional re-programming. Furthermore, this defense response protects plants from disease upon subsequent challenges by pathogenic *Xanthomonas*.

The *X. citri* AT bacterial gene causing the deployment of HR-like responses in the plant appears to be a short (7.5 repeats) variant of the pathogenic pthA4 TAL-effector. The mode of action of this short TAL is mediating transcriptional activation of plant genes, as mutated version of the TAL effector in nuclear localization hampers the triggering of the HR-like response and the SV40 nuclear localization signal restores the HR response. DNA-binding assays using double-stranded microarrays indicate that this short TAL-effector is able to bind DNA. Initial experiments in the Identification of targets demonstrate that natural short TAL-effectors can bind DNA in a code-dependent manner and activate transcription. The identification of the gene responsible of the HR response is underway. This knowledge will help to rationally exploit the plant immune system as a biotechnological approach to manage citrus canker, and reveals biological functionality for short TAL-effectors.



## **Bacterial TAL effector and host target gene interactions in bacterial blight of rice**

**Bing Yang**

Iowa State University, Ames, IA 50011, USA

**Keywords:** TAL effector; *Xanthomonas oryzae*; rice; genome editing

### **Abstract**

Bacterial blight of rice, caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo) in rice, is also well-known as a model for studying host/microbe interaction and represents one of the most well-studied crop diseases. TALEs (transcription activator-like effectors), as a group of type III effector proteins and once translocated into the host cells from pathogens, recognize and activate host genes to condition disease susceptibility and also trigger host resistance responses dependent on the nature of target genes in plants. TALEs and their target genes have become the foci of the molecular battles between Xoo and rice. The continuing battles have led to incredibly diverse virulence mechanisms in pathogen and counteracting defense mechanisms in host. Extensive efforts have been made to understand the TALE biology, identify host target genes, and elucidate their interaction and resulting physiological relevance to rice blight and other crop diseases in general. My presentation aims to summarize how much we have learned about TALEs and their role in bacterial blight of rice, as well as associated susceptibility and resistance genes in the host. The presentation also intends to provide a prospect of engineering genetic resistance by applying precise genome editing of TALE-associated target genes in rice.



**OsERF#123, a new susceptibility gene for bacterial blight of rice**

**Mathilde Hutin**<sup>1</sup>, Tuan Tu Tran<sup>2</sup>, Joseph J Belanto<sup>3</sup>, Li Wang<sup>1</sup>, Alvaro L Perez-Quintero<sup>2</sup>, Matthew R Willmann<sup>1</sup>, Daniel F Voytas<sup>3</sup>, Boris Szurek<sup>2</sup>, Adam J Bogdanove<sup>1</sup>

<sup>1</sup> School of Integrative Plant Science, Cornell University, Ithaca, New York, USA

<sup>2</sup> UMR IPME, IRD-CIRAD-Université Montpellier, Montpellier, France

<sup>3</sup> Department of Genetics, Cell Biology & Development and Center for Genome Engineering, 321 Church Street SE, University of Minnesota, Minneapolis, USA

**Abstract**

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease of rice, a crop that feeds more than half of the world's population. *Xoo* virulence critically depends on the transcription activator-like effector (TALE)-dependent activation of specific host genes called susceptibility (*S*) genes. The number of TALEs per strain varies from 9 to 16, with African strains, which form a distinct genetic lineage, typically having fewer TALEs than Asian strains. While one or two TALEs per strain generally act as major virulence factors, the relative contributions of the other TALEs to *Xoo* pathogenicity is unclear. We sequenced the entire genomes and compared the TALE repertoires of three African *Xoo* strains. We assessed the individual contribution to pathogen virulence of 13 TALE variants represented in the three strains and identified TalB as a new, major virulence factor. RNA profiling and *in silico* prediction of TalB binding sites in rice revealed *OsTFX1*, a bZIP transcription factor previously identified as a bacterial blight *S* gene, and *OsERF#123*, which encodes a subgroup IXc AP2/ERF transcription factor, as candidate targets. Activation of *OsERF#123* using gene-specific designer TALEs restored virulence to a *talB* knockout strain, confirming *OsERF#123* as a new bacterial blight *S* gene. We have generated and are currently characterizing *OsERF#123* promoter-edited and *OsERF#123* CDS deletion lines to further probe the role of *OsERF#123* in susceptibility and to understand its function in other contexts.



## Inducers and suppressors of host innate immunity in rice-*Xanthomonas* interactions

**Ramesh V. Sonti**

National Institute of Plant Genome Research, New Delhi, India

### **Abstract**

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes bacterial blight, a serious rice disease. As part of its virulence repertoire, *Xoo* secretes a battery of cell wall degrading enzymes. Purified preparations of several of these cell wall degrading enzymes (CWDEs) can individually induce plant defense responses. By mutating genes for each of these CWDEs in the genetic background of a type 3 secretion system (T3SS) defective mutant, we demonstrate that some but not all of these CWDEs induce immune responses in a non-redundant manner during infection. In transient transfer assays, we show that 4/16 different T3SS effectors that were tested can individually suppress CWDE induced rice immune responses. A quadruple mutant that is defective in all four of these T3SS effectors, but not the single, double or triple mutants is an inducer of rice innate immune responses indicating that these four proteins function redundantly in suppression of rice innate immunity. Three of these four T3SS effectors have one or more 14-3-3 binding motifs. Each of these T3SS effectors interacts with a unique rice 14-3-3 protein and this interaction is necessary for the ability of the protein to suppress rice innate immunity. Overall, our results indicate that several different CWDEs induce rice immune responses in a non-redundant manner and that several different T3SS effectors suppress CWDE induced rice immune responses in a redundant manner.





## Session IV

### Other secretion systems

#### **Bacterial wars: Functional and structural studies on the bacteria-killing type IV-secretion system from *Xanthomonadaceae***

German Sgro<sup>1</sup>, Diorge Souza<sup>1</sup>, William Cenens<sup>1</sup>, Gabriel Oka<sup>1</sup>, Natalia Bueno<sup>1</sup>, Luciana Oliveira<sup>1</sup>, Cristina Martinez<sup>1,2</sup>, Alexandre Cassago<sup>3</sup>, Tiago Costa<sup>4</sup>, Rodrigo Portugal<sup>3</sup>, Gabriel Waksman<sup>4</sup>, Roberto Salinas<sup>1</sup>, **Chuck Farah**<sup>1</sup>

<sup>1</sup> Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, SP, Brazil

<sup>2</sup> Department of Genetics, Evolution, Microbiology and Immunology, State University of Campinas, SP, Brazil

<sup>3</sup> Laboratório Nacional de Nanotecnologia, Centro Nacional de Pesquisas em Energia e Materiais, Campinas, SP, Brazil

<sup>4</sup> Institute of Structural and Molecular Biology, Birkbeck College, London, United Kingdom

#### **Abstract**

Type IV secretion systems (T4SSs) form the most common and versatile class of secretion systems in bacteria, capable of injecting both proteins and DNA into host cells. The T4SSs from the *Xanthomonadaceae* family of bacteria present several distinguishing features, one of which is their role in the killing of bacterial rivals by injecting toxins into neighboring cells upon contact. Three purified toxins or X-Tfes (*X*anthomonadaceae-T4SS *e*ffectors) from *Xanthomonas citri* are able to degrade peptidoglycan and lyse *B. subtilis* cells and another X-Tfe is shown to possess phospholipase activity. These toxin activities are inhibited by structurally diverse cognate immunity proteins (X-Tfis) whose genes are found upstream to the X-Tfe genes. We show that X-Tfe<sup>XAC2609</sup> is secreted by *X. citri* on contact with *E. coli* cells in a T4SS-dependent manner. Using time-lapse microscopy, we observe the rapid lysis of *E. coli* cells that were in direct physical contact with *X. citri* WT cells but do not observe lysis when  $\Delta$ T4SS strains are used. We will also present a cryo-electron microscopy model of the intact *X. citri* T4SS core complex whose vast network of protein-protein interactions was functionally probed in an exhaustive mutational investigation of interface residues.



## A single cell wall degrading enzyme acts as a molecular switch for vascular and non-vascular *Xanthomonas* pathogenesis

**Jonathan M. Jacobs**<sup>1,2,3,4</sup>, Emile Gluck-Thaler<sup>1</sup>, Aude Cerutti<sup>5</sup>, Taca Vancheva<sup>4</sup>, Jillian M. Lang<sup>3</sup>, Celine Pesce<sup>1,3,6</sup>, Casey Mazzotta<sup>3</sup>, Alain Jauneau<sup>5</sup>, Valerie Verdier<sup>2</sup>, Boris Szurek<sup>2</sup>, Sébastien Cunnac<sup>2</sup>, Claude Bragard<sup>4</sup>, Gregg Beckham<sup>7</sup>, Jason Slot<sup>1</sup>, Laurent D. Noël<sup>5</sup>, Jan E. Leach<sup>3</sup>, Ralf Koebnik<sup>1</sup>

<sup>1</sup> Department of Plant Pathology, The Ohio State University, Columbus, OH, USA

<sup>2</sup> IRD-CIRAD, UMR Interactions Plantes Microorganismes Environnement, Montpellier, France

<sup>3</sup> Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

<sup>4</sup> Earth & Life Institute, Université Catholique Louvain-la-Neuve, Louvain-la-Neuve, Belgium

<sup>5</sup> LIPM, Université de Toulouse, INRA, CNRS, UPS, F-31326 Castanet-Tolosan, France

<sup>6</sup> Department of Microbiology, University of New Hampshire, Durham, NH, USA

**Keywords:** evolution, vascular, non-vascular, cell wall degrading enzymes, xylem

### Abstract

Pathogenic bacteria cause vascular and non-vascular diseases of plants. The basis of vascular and non-vascular plant pathogenesis remains unknown. Bacterial *Xanthomonas* species provide an excellent model for tissue-specific pathogenesis because this genus comprises both vascular and non-vascular bacteria of over 200 plant host species. We analyzed available *Xanthomonas* pathogen genomes and determined that a vascular pathogen-conserved cellulolytic enzyme likely played a role in adaptation to the xylem environment in this genus. This enzyme, CelA, was conserved in vascular pathogenic xanthomonads but absent in non-vascular *Xanthomonas* subgroups. We found that many nonvascular subgroups lost *celA* based on phylogenetic and genome analyses. Notably expression of CelA in nonvascular cereal pathogen *Xanthomonas translucens* pv. *undulosa* permitted xylem colonization and leaf blight symptoms similar to vascular *X. translucens* pv. *translucens* on barley. We posit that CelA acts as molecular switch for niche-specific behavior and is inactivated upon adaptation to the non-vascular environment.



## ABC transporter RaxB is required for processing and secretion of sulfated RaxX to yield a biologically active form that resembles a plant hormone peptide

**Anna Joe**<sup>1,2,3</sup>, Dee Dee Luu<sup>1,3</sup>, Yan Chen<sup>2</sup>, Ofir Bahar<sup>1</sup>, Rory Pruitt<sup>1</sup>, Leanne Chen<sup>2</sup>, Christopher Petzold<sup>2</sup>, Kelsey Long<sup>1</sup>, Cliff Adamchak<sup>1</sup>, Pamela Ronald<sup>1,2</sup>

<sup>1</sup> Department of Plant Pathology and the Genome Center, University of California, Davis, CA 95616, USA

<sup>2</sup> Feedstocks Division, Joint Bioenergy Institute, Emeryville, CA 94608, USA

<sup>3</sup> These authors contributed equally to this work

**Keywords:** XA21-mediated immunity, sulfated peptide RaxX, plant hormone mimic, type I secretion, ABC transporter, RaxB

### Abstract

The rice XA21 immune receptor is activated upon recognition of the tyrosine sulfated peptide, RaxX (required for activation of XA21-mediated immunity X) produced by *Xanthomonas oryzae* pv. *oryzae* (Xoo). RaxX shares remarkable similarity to the plant hormone PSY (plant peptide containing sulfated tyrosine) family and mimics PSY's growth promotion activity. Analyses of multiple RaxX peptides identified residues that are required for activation of immunity mediated by the rice XA21 receptor but that are not essential for root growth induced by PSY. These findings suggest that RaxX serves as a molecular mimic of PSY peptides and that XA21 has evolved the ability to recognize and respond specifically to the microbial form of the peptide.

We have hypothesized that sulfated RaxX is processed and secreted by RaxABC type I secretion system to manipulate host cellular processes. Here, we demonstrate that secretion and processing of RaxX requires the C39 proteolytic domain of the ATP-binding cassette (ABC) transporter RaxB and another predicted ABC transporter, CvaB, with 69% similarity to RaxB. We show that an Xoo strain lacking both RaxB and CvaB ( $\Delta$ raxB/ $\Delta$ cvaB) no longer secretes RaxX and cannot activate XA21. We show that although *cvaB* can partially complement the  $\Delta$ raxB/ $\Delta$ cvaB strain, RaxB alone is sufficient to proteolytically process and secrete RaxX. Our mass spectrometry analysis detected secreted RaxX peptide in a RaxB-dependent manner. We also characterized a GG-motif leader sequence that is cleaved by RaxB C39 protease. Processing of RaxX yields a small biologically active peptide that intimately resembles the host peptide hormone PSY yet is still recognized by the XA21 immune receptor.



## Bacterial outer membrane vesicles modulate the plant immune response and induce resistance to infection

Gideon Mordukhovich<sup>1,2</sup>, Leron Katsir<sup>1</sup>, Noa Sela<sup>1</sup>, **Ofir Bahar**<sup>1</sup>

<sup>1</sup> Department of Plant Pathology and Weed Research, Agricultural Research Organization, Volcani Center, Rishon LeZion, Israel

<sup>2</sup> Robert H. Smith Faculty of Agriculture, Food, and Environment, Hebrew University of Jerusalem, Rehovot, Israel

**Keywords:** outer membrane vesicles, *Xanthomonas*, plant immunity

### Abstract

Gram-negative bacteria release membrane blebs pinching off their outer membrane to the environment. These outer membrane vesicles (OMVs) are composed of membrane lipids, lipopolysaccharides, membrane and soluble proteins, nucleic acids and more. Multiple roles have been assigned to OMVs including response to stresses, delivery of virulence factors, cell-cell communication and modulation of host immunity. Surprisingly, very few studies were published on the role OMVs play in plant-microbe interactions. We recently demonstrated that *Arabidopsis* up-regulate multiple immune markers in response to OMVs purified from *Xanthomonas campestris* pv. *campestris* (Xcc) and other phytopathogen cultures. To further investigate the effects of OMVs on plants we analyzed *Arabidopsis* response to Xcc OMVs using RNA-seq and performed plant pretreatment experiments. The transcriptomic analysis revealed a large suite of immunity genes such as immune receptors and related transcription factors that are induced in response to Xcc OMVs. Additionally, *Arabidopsis* plants pretreated with Xcc OMVs became primed and were more resistant to subsequent infection with the bacterial pathogen *Pseudomonas syringae* pv. *syringae*. Several known immune elicitors, such as flagellin, EF-Tu, GroEL, were found to be associated with Xcc OMVs. However, when we tested *Arabidopsis* plants lacking the corresponding immune receptors of these elicitors (FLS2 and EFR), we did not detect a reduced immune response to OMVs. In addition to immune elicitors, various virulence factors such as type III-secretion factors and cell-wall degrading enzymes were found in association with Xcc OMVs. The possible roles of OMVs in host colonization are currently under investigation.





## Session V

### Ecology and epidemiology

#### The many cell density-dependent behaviors of *Xylella fastidiosa*: achieving disease control via pathogen confusion

**Steven Lindow**

Department of Plant and Microbial Biology, University of California, Berkeley. Berkeley, CA USA

**Keywords:** xylem, extracellular vesicles, quorum sensing, diffusible signaling factor

#### **Abstract**

The xylem-limited vascular pathogen *Xylella fastidiosa* causes lethal diseases of a wide variety of crop plants including grape, citrus, and olive by blocking water flow through the xylem and by inducing the formation of tyloses, further disrupting water movement. This pathogen has a complex lifestyle where it also colonizes the mouthparts of xylem-feeding insects which serve as obligate vectors for plant to plant spread. Expression of about 10% of the genome is influenced by accumulation of C14 and C16 *cis* 2-unsaturated fatty acids (diffusible signaling factors, DSF) encoded by *rpfF* that serve as quorum sensing signal molecules. DSF accumulation increases the adhesiveness and biofilm formation in *X. fastidiosa* required for insect colonization and transmission but reduces colonization of xylem vessels and thus disease symptoms in grape. Expression of extracellular endoglucanases and polygalacturonases as well as retractile pili required for intercellular movement are suppressed by DSF as is the release of extracellular vesicles by *X. fastidiosa* that inhibit its attachment to xylem vessels, and thus facilitate movement through the plant. Control of Pierce's disease of grape in both greenhouse and in field trials has been achieved by a process of "pathogen confusion" whereby expression of *rpfF* in plants confers constitutive production of DSF that increases the adhesiveness and reduces the active movement of *X. fastidiosa* in the plant even when cells are in low spatial density within a given xylem vessel, thereby limiting the movement of the pathogen away from the point of inoculation by insect vectors.



## What is the risk of *Xylella fastidiosa* for EU? Risk assessment and pest categorization

Ewolina Czwieniczek<sup>1</sup>, Alice Delbianco<sup>1</sup>, Giuseppe Stancanelli<sup>1</sup>, Domenico Bosco<sup>2</sup>, **Claude Bragard**<sup>3</sup>

<sup>1</sup> Animal and Plant Health Unit, EFSA, Parma, Italy

<sup>2</sup> Università di Torino, Torino, Italy

<sup>3</sup> Applied Microbiology, Earth&Life Institute, Croix du Sud 2bte L7.05.03 B1348 Louvain-la-Neuve, Belgium

**Keywords:** *Xylella fastidiosa*, insect vectors, risk assessment, host range database

### Abstract

*Xylella fastidiosa* is reported in EU since 2013, following its discovery in Puglia, Italy. Since then, it has also been reported in Corsica and Southern France, in the Balearic Islands and in Spain (Alicante Region), in Germany, not to mention several cases of interceptions. Many questions have been raised on the risks to plant health posed by the bacteria in the EU territory (EFSA, 2015). Both the EFSA plant health panel and the Animal and Plant Health EFSA unit have analysed such risk and produced urgent advice on key questions. All the disease cases reported have been listed and mapped. A host global database has also been compiled, derived from an extensive literature search, provides a list of all known host plants. An update on the current situation in the different affected areas in Europe as well as a new pest categorization document has been elaborated based on questions regarding the current distribution of *Xylella* and its different types the potential insect vectors present in EU or risk reduction options available.

### References

EFSA PLH Panel (EFSA Panel on Plant Health), 2015. Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal 2015;13 (1):3989, 262 pp., doi: 10.2903/j.efsa.2015.3989

EFSA (European Food Safety Authority), 2016. Scientific report on the update of a database of host plants of *Xylella fastidiosa*: 20 November 2015. EFSA Journal 2016;14(2):4378, 40 pp., doi:10.2903/j.efsa.2016.4378.



## Assessment of the effects of antimicrobial and quorum-sensing regulating substances on biofilm formation and cell growth of *Xylella fastidiosa* “De Donno” strain

**Massimiliano Morelli**<sup>1</sup>, Giusy D’Attoma<sup>1,2</sup>, Angelo De Stradis<sup>1</sup>, Maria Saponari<sup>1</sup>, Donato Boscia<sup>1</sup>, Stefania Zicca<sup>1</sup> and Pasquale Saldarelli<sup>1</sup>

<sup>1</sup> CNR-Istituto per la Protezione Sostenibile delle Piante (IPSP), Bari, Italy

<sup>2</sup> Università degli Studi di Bari Aldo Moro, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Bari, Italy

**Keywords:** *Xylella fastidiosa*, biofilm, quorum-sensing, *Paraburkholderia phytofirmans*, biocontrol

### Abstract

Biofilm formation is among the relevant virulence mechanisms of several plant pathogenic bacteria, including *Xylella fastidiosa*. Mainly composed of exopolysaccharides, biofilm allows bacterial cells to adhere to the surfaces and form communities protected from the hostile xylematic environment. Previous studies have shown that *X. fastidiosa* biofilm formation is closely related to quorum-sensing regulation. The alteration of this mechanism can have important consequences on the bacterial ability to colonize the host and induce symptoms. Tests have been started *in vitro* to assess the effects of exogenous substances on the cell growth of the strain “De Donno”, the causal agent of the severe disease denoted “Olive Quick Decline Syndrome”, and its capacity to form a biofilm ring adhering to the surface of glass tubes or microtiter plates.

The bacterial response was evaluated in different experimental sets, targeting diverse aspects of biofilm chemistry and regulation. One of the approaches was based on the knowledge that *X. fastidiosa* *rpfF*-gene synthesises a diffusible signalling factor (DSF) that controls biofilm formation, by testing the effect of crude extracts of an *E. coli* engineered to express “De Donno” *rpfF* gene. The possible action on biofilm synthesis, due to metabolite interference, or as a consequence of an interspecies quorum-sensing modulation, has been tested through competitive assays in presence of the rhizobacterium *Paraburkholderia phytofirmans*, recently proposed as a candidate for *X. fastidiosa* biocontrol. In addition, several natural or synthetic compounds (e.g. N-acetylcysteine, thymol, caffeic acid, etc.) have been evaluated for their action on cell growth and biofilm consistency.

Financial support for the present work was from the EU H2020-funded research project XF-ACTORS (GA 727987) and from the project “STIPXYT” funded by Regione Puglia DGR 1410/2015.



## Deciphering the emergence of *Xanthomonas vasicola* pv. *musacearum* in Eastern and Central Africa using MLVA

**Valentine Nakato**<sup>1</sup>, Sadik Muzemil<sup>2</sup>, Walter Ocimati<sup>3</sup>, Guy Blomme<sup>4</sup>, David Studholme<sup>5</sup>, Teresa Coutinho<sup>6</sup>, George Mahuku<sup>7</sup>, Emmanuel Wicker<sup>8</sup>

<sup>1</sup> International Institute of Tropical Agriculture (IITA-Uganda), Kampala, Uganda

<sup>2</sup> Southern Agricultural Research Institute (SARI), Hawassa, Ethiopia

<sup>3</sup> Bioversity, Kampala, Uganda

<sup>4</sup> Bioversity, Addis-Abeba, Ethiopia

<sup>5</sup> University of Exeter, United Kingdom

<sup>6</sup> University of Pretoria, Pretoria, South Africa

<sup>7</sup> IITA, East Africa Hub, Dars-Es-Salaam, Tanzania

<sup>8</sup> CIRAD, UMR IPME, Montpellier, France

### Abstract

Banana *Xanthomonas* wilt caused by *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) is considered an emerging disease in the Eastern and Central Africa countries. First described in Ethiopia in the 1960s, *Xvm* has caused a severe epidemics from 2001 throughout the Great Lakes region. Recent genomic analyses showed that *Xvm* is a monomorphic pathovar, further subdivided into two sublineages of supposed different origins: sublineage I from Ethiopia (SL I), sublineage II from unknown origin (SL II). Using a set of highly polymorphic markers (MLVA-19 scheme, see NAKATO et al. this conference), we addressed the population diversity and structure of *Xvm* populations in Africa.

We genotyped a collection of 335 *Xvm* strains sampled from Burundi, DRC, Ethiopia, Kenya, Rwanda, Tanzania and Uganda, collected from historical collections but also during recent surveys in 2012, 2014-2015, 2017. Twelve clusters were identified, revealing an unexpected diversity: four clusters, all specific to Ethiopia, could not be assigned to any SL. Ethiopia harbored the highest genetic diversity, highest number of private alleles, highest number of DAPC clusters, strongly suggesting that it is the most probable center of origin of *Xvm*. From population differentiation analysis, two main groups of countries were identified: (i) Ethiopia, DRC, and Rwanda; (ii) Uganda and Tanzania. Uganda, interestingly, experienced several epidemiological waves of both sublineages. Further details will be given in the presentation.

This study paves the way for future investigations related to host resistance genetics both in enset and banana, population genomics related to adaptation to host, reconstruction of invasion routes.





## Session VI

### Plant defense and resistance

#### Development of host plant resistance to *Xanthomonas campestris* pv. *musacearum*

**Leena Tripathi**

International Institute of Tropical Agriculture (IITA), Kenya

**Keywords:** Banana, *Xanthomonas campestris* pv. *musacearum*, disease resistance, transgenic

#### **Abstract**

The bacterium *Xanthomonas campestris* pv. *musacearum* (*Xcm*) is the causal agent of *Xanthomonas* wilt disease of banana and enset. The pathogen was first identified in Ethiopia more than 50 years ago on enset and later on banana. In 2001, *Xcm* was reported in Uganda and subsequently other countries in Great Lakes region of east Africa. Banana *Xanthomonas* wilt (BXW) disease is considered the most devastating disease of banana in east Africa, where it is a major staple crop produced mostly by smallholder subsistence farmers. Management of diseases in tropical perennial crops such as banana is a challenge due to continuous association of host and inoculum over a long period of time. Use of disease-resistant varieties has been an effective and economically viable strategy for management of plant diseases. However, no resistance against *Xcm* has been observed in any cultivated variety of banana except for wild-type diploid '*Musa balbisiana*'. Transgenic technology provides a cost-effective alternative to develop BXW resistant banana varieties. Transgenic bananas constitutively expressing *Hrap*, *Pflp* or *Xa21* gene demonstrated enhanced resistance against *Xcm*. We also compared the transcriptome profile of disease resistant wild type banana '*Musa balbisiana*' with the highly susceptible banana variety challenged with *Xcm* in order to understand the molecular mechanism of disease response. The information generated by transcriptome analysis will be used to develop BXW-resistant banana varieties using *Musa* genes.



## Harnessing the genome plasticity of bacterial spot species complex to guide a search for novel disease resistance in pepper

**Neha Potnis**<sup>1</sup>, Sandra Branham<sup>2</sup>, Jeffrey Jones<sup>3</sup>, and William Patrick Wechter<sup>2</sup>.

<sup>1</sup> Department of Entomology and Plant Pathology, Auburn University, Alabama, U.S.A.

<sup>2</sup> U.S. Vegetable Laboratory, USDA-ARS, Charleston, South Carolina, U.S.A.

<sup>3</sup> Department of Plant Pathology, Gainesville, Florida, U.S.A.

**Keywords:** Bacterial spot *Xanthomonas*, recessive resistance, pepper pathogenicity, GWAS

### Abstract

Host resistance has been the primary disease management strategy for managing bacterial spot of pepper caused by *Xanthomonas* species. Several resistance genes in pepper have been identified against the dominant species, *X. euvesicatoria*. Commercial pepper cultivars, containing *bs5* and/or *bs6* recessive resistance genes, are being widely utilized to provide effective control against all eleven races of *X. euvesicatoria*. However, the population structure of bacterial spot pathogens on pepper has undergone significant changes in the recent years. *X. gardneri* outbreaks in the Midwestern U.S. is of serious concern to the tomato and pepper industry due to ineffectiveness of commercial cultivars against *X. gardneri*. *X. perforans* has been a significant threat on tomato over the past two decades, and very recently a growing concern on pepper. We recently surveyed pathogen population on tomato and pepper in Alabama and identified strains belonging to emerging lineages of *X. perforans* capable of inciting disease on pepper. Commercial pepper cultivars containing *bs5* and *bs6* recessive resistance genes were ineffective in protecting against *X. gardneri*, as well as recently isolated *X. perforans* strains from Alabama. These findings indicate the need to identify novel sources of resistance against changing pathogen populations. We conducted a resistance screen of pepper germplasm using *X. gardneri* and identified 15 highly resistant pepper cultivars. Genotyping-by-sequencing, followed by association analyses have revealed novel candidate loci for disease resistance in pepper. The resistant pepper genotypes, as well as quantitative trait loci (QTL) associated with highly significant SNPs, provide important resources for breeding programs to obtain effective control against different species of bacterial spot *Xanthomonads*.



## Characterisation of resistance genes and virulence factors in the *Lolium multiflorum* – *Xanthomonas translucens* pv. *graminis* pathosystem

**Roland Kölliker**<sup>1,2</sup>, Verena Knorst<sup>1,2</sup>, Lena Hersemann<sup>2</sup>, Franco Widmer<sup>2</sup>, Bruno Studer<sup>1</sup>

<sup>1</sup> Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland

<sup>2</sup> Molecular Ecology, Agroscope, Zurich, Switzerland

**Keywords:** forage grasses, bacterial wilt, bulked segregant analysis, comparative genomics

### Abstract

Bacterial wilt, caused by *Xanthomonas translucens* pv. *graminis* (*Xtg*), is one of the most important diseases of forage grasses such as *Lolium multiflorum*, leading to substantial losses of forage quality and yield. Breeding for resistant cultivars is the only practicable means to control the disease. The overall aim of this research is to develop molecular genetic and genomic tools to benefit resistance breeding and to enable targeted resistance management. Thus, a detailed understanding of plant resistance genes and pathogen virulence factors is indispensable. In the past, a major QTL for bacterial wilt resistance was identified in the host (*L. multiflorum*) using a bi-parental mapping population. The pathogen (*Xtg*) was shown to rely on a non-canonical type III secretion system for plant infection. More recently, a number of candidate genes for bacterial wilt resistance were identified by comparing genomic sequences of resistant and susceptible parental plants and their progeny. On the other hand, comparative genomics of different *X. translucens* pathovars allowed to identify virulence traits characteristic for *Xtg*. These candidate plant resistance genes together with the bacterial virulence factors provide an invaluable resource for the development of genomics-assisted selection strategies. In addition, the well characterised plant genotypes and bacterial strains serve as an ideal model system to fully understand the complex *L. multiflorum* – *Xtg* interaction.



# POSTERS





# Session I

## Genomics and molecular diagnostics

### Poster #1

#### Comprehensive transcriptome characterization of the xanthan producer *Xanthomonas campestris* pv. *campestris* B100 using next generation RNA sequencing

**Rabeaa S. Alkhateeb**<sup>1,2</sup>, Frank-Jörg Vorhölter<sup>1,2,#</sup>, Christian Rückert<sup>3</sup>, Gerd Hublik<sup>4</sup>, Karsten Niehaus<sup>1,2</sup>, Alfred Pühler<sup>1</sup>.

<sup>1</sup> Center for Biotechnology CeBiTec, Bielefeld University, Universitätsstr. 27, 33615 Bielefeld, Germany

<sup>2</sup> Department of Proteomics and Metabolomics, Faculty of Biology, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

<sup>3</sup> Technology Platform Genomics, Center for Biotechnology CeBiTec-Bielefeld University, Universitätsstr. 27, 33615 Bielefeld, Germany

<sup>4</sup> Jungbunzlauer Austria AG, Pernhofen 1, 2064 Wulzeshofen, Austria

<sup>#</sup> Present address: MVZ Dr. Eberhard & Partner Dortmund, Brauhausstr. 4, 44137 Dortmund, Germany.

*Xanthomonas campestris* pv. *campestris* (Xcc) is a plant pathogen and the producer of xanthan, a polysaccharide with a multitude of commercial applications as a thickening agent. The presented work aims at providing a better understanding of the mechanisms that govern xanthan biosynthesis on the transcriptional level. A comprehensive study on the transcriptome of Xcc B100 was carried out applying high throughput transcription start sites sequencing and whole genome mRNA and small RNA profiling. In this context, the primary transcriptome of Xcc strain B100 was analyzed cataloguing its characteristic features at a single base pair resolution level. In addition, gene expression data were applied to enhance the accuracy of genomic feature prediction and facilitated the annotation of novel CDS and non-coding RNA genes. Using whole genome mRNA profiling, the differential transcriptome of two Xcc B100 cultures obtained during the growth and stationary phase associated with xanthan biosynthesis was globally analyzed. During the stationary phase, 40% of genes were differentially transcribed where half of these genes were up-regulated and further half was down-regulated. Nucleotide sugar precursor genes of xanthan biosynthesis exhibited a transcription pattern that did not change while genes within the *gum* gene cluster, the major player of xanthan biosynthesis were differentially transcribed. The analysis of Xcc B100 transcriptome represents a valuable foundation for genetic engineering studies aiming at enhancing the efficacy of xanthan production on industrial level and provides access to genetic blue prints of the molecular players that govern *Xanthomonas* metabolism and pathogenicity.

## **Comparative genomics of the order *Xanthomonadales*: a glimpse of bacterial adaptation to plants**

**Matthieu Arlat**<sup>1</sup>, Sébastien Carrère<sup>1</sup>, Martial Briand<sup>2</sup>, Laurent Noël<sup>1</sup>

<sup>1</sup>LIPM, Université de Toulouse, INRA, CNRS, UPS, 31326 Castanet-Tolosan, France

<sup>2</sup>IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, 49071 Beaucouzé, France

**Keywords:** virulence, type 3 secretion system, effector, cell wall degrading enzymes, CUT loci

The order *Xanthomonadales* is located at the base of the class Gammaproteobacteria, together with orders *Nevskiales* and *Cardiobacteriales*. We have undertaken a comparative genomic analysis based on these three orders to identify molecular determinants allowing bacterial adaptation to plants and to study the evolution of pathogenicity of *Xanthomonas* and *Xylella*. A total of 443 genomes covering the 3 orders have been selected for their quality and have been re-annotated, using the same pipeline. More than 25 000 groups of orthology have been defined and used to perform our comparative analyses. We have in particular studied the distribution of known virulence factors and of HrpG/HrpX regulons. We also identified genes specific to pathogenic *Xanthomonas* or *Xylella* clades which could be involved in the adaptation to plants.

## Poster #3

**Rise of a clone – Phylochronological insights into genome evolution of *Xanthomonas citri* pv. *punicae***

**Kanika Bansal**, Sanjeet Kumar, Prabhu B Patil

Bacterial Genomics and Evolution Laboratory, CSIR-Institute of Microbial Technology, Chandigarh, India.

**Keywords:** genomics, evolution, marker, effectome, clone

*Xanthomonas citri* pv. *punicae* causing bacterial leaf blight disease of pomegranate is economically important endemic pathogen in India. Since its first report in 1957, there were minor reports of its occurrence across the country. But only post-1980's, devastating affects leading 60-80% crop loss were reported, recently been observed in Turkey, South Africa, Pakistan. This sudden shift in severity of the disease prompted us to undertake chronological genomic study in order to address if multiple species or species complex are infecting host with passage of time. We collected strains of pre and post-epidemic era. We have sequenced and analyzed all available temporal strains on Illumina- MiSeq and integrated them with Nanopore long-read data, obtaining nearly complete genomes. Interestingly, genome-based typing and analysis revealed that single clone of pathogen is circulating even after half century. However, we could detect variations among isolates in single nucleotide polymorphism (SNPs), plasmid profiles, mobile elements and unique gene content correlating with virulence potential. We also identified set of genome-based unique markers (including effectome) which could be potential diagnostic tool and drug target. Such a comprehensive insight into the evolution of such a devastating pathogen would be invaluable in its surveillance and quarantine. Further, studies into structural variations, epigenomics and expression could allow to understand nature of selection in the emergence of clones of pathogenic bacteria.

## **Preliminary evaluation of bacterial diseases caused by *Xanthomonas* in Lithuania**

**Daiva Burokiene**

Nature Research Centre, Zaliuju Ezeru Str. 49, LT-08406 Vilnius, Lithuania

**Keywords:** bacterial blight, *Xanthomonas arboricola* pv. *juglandis*, walnuts, *Juglans* spp.

The studies on diversity, distribution and identification of most harmful bacterial pathogens were started only in recent years.

The climate changes from maritime in the west to continental in the east in Lithuania. It has cyclical seasonal cool springs with high possibility of frosts, warm summers, rainy autumns and rather warm winters, and sometimes weather is unpredictable. Despite these facts, walnut plants are overwintering and yielding. Walnuts are grown mainly in amateur and botanical gardens and are not of economic importance in the country. However, the first study records on the presence of bacterial walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj) on walnuts (*Juglans* spp.) were published (Burokiene, Pulawska, 2012). Xaj was isolated from *J. cinerea* and *J. mandshurica* in Lithuania.

There have been no detailed investigations about other bacterial diseases caused by *Xanthomonas* in Lithuania so far. Consequently, the survey on evaluation of bacterial disease symptoms, isolation and primary characterization of isolated strains from different host plants was started.

## Insights on *Xanthomonas arboricola* virulence: pathogenic and non-pathogenic strains

Jerson Garita-Cambronero<sup>1</sup>, **Jaime Cubero**<sup>2</sup>

<sup>1</sup> Centro de Investigación de Biocombustibles y Bioproductos. Instituto Tecnológico Agrario de Castilla y León (ITACyL), Villarejo de Órbigo, León, Spain.

<sup>2</sup> Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain.

**Keywords:** *juglandis*, *corylina*, *pruni*, virulence, non-pathogenic

*Xanthomonas arboricola* pv. *pruni* causes bacterial spot of stone fruits and together with *X. arboricola* pv. *corylina* and pv. *juglandis* are considered the most virulent pathovars of the species. In the past years, pathogenic and non-pathogenic xanthomonad strains were found living in sympatry on *Prunus* and other plant species; these strains were characterized and comparative genomic analysis allowed to identify putative factors involved in the virulence of pathovars *pruni*, *juglandis* and *corylina*. Among the variances found on them, and between pathogenic and non-pathogenic bacteria, the dissimilarity in some secretion systems was highlighted. More specifically, differences were elucidated in type three secretion system (T3SS), either in its components or effectors. These differences were clearer between pathogenic and non-pathogenic strains but also have been found when the less virulent ones were analysed. Other variants were also shown in several genomic components that may play a role in plant colonization or infection processes. Although genomics open the door to reveal the molecular mechanisms required for the disease development, functional studies are required to corroborate the actual involvement of each component identified in the bacterial genomes.

This work was funded by project RTA2014-00018-C02.

## Improving *Xylella fastidiosa* subspecies detection using Real-Time PCR and droplet digital PCR

**Enora Dupas**<sup>1,2</sup>, Sophie Cesbron<sup>1</sup>, Amandine Cunty<sup>2</sup>, Martial Briand<sup>1</sup>, Bruno Legendre<sup>2</sup>, Charles Manceau<sup>2</sup>, Marie Agnès Jacques<sup>1</sup>

<sup>1</sup> IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, 49071, Beaucouzé, France

<sup>2</sup> Anses Laboratoire de la santé des végétaux, F- 49044 Angers

**Keywords:** leaf scorch diseases, identification, Sklf, QX200™ Bio-Rad, Harper's test

*Xylella fastidiosa* is a xylem limited bacterium, which isolation from plant material remains time- consuming and poorly efficient. *X. fastidiosa* is a genetically diverse species that is currently divided into six subspecies, which display various virulence. Because management and regulations of *X. fastidiosa* outbreaks in Europe depend on the subspecies, it is of major importance to precisely identify the subspecies as early as possible when monitoring *X. fastidiosa* detection. Furthermore, some host species of *X. fastidiosa* are rich in polyphenols and polysaccharides that may act as PCR inhibitors and consequently increase detection threshold. The aim of this study was to improve *X. fastidiosa* detection by developing Real-Time PCR and droplet digital PCR (ddPCR) tests able to detect the subspecies directly from plant material. Primers and probes were designed using a bioinformatic tools based on k-mers, Sklf, to detect specific subspecies signatures from a dataset of 45 genome sequences representative of the species diversity. Primers were multiplexed in quadruplet. Simultaneously, a ddPCR test was developed with the same primers. The principle of ddPCR is to split the PCR mix in thousands of partitions. So, a PCR reaction is performed in each partition. Applying ddPCR to matrices rich in inhibitors compartmentalize target DNA but also inhibitors, and improve DNA amplification. Both methods were tested on target and non-target strains, as well as spiked samples from different host plants. Performance criteria as sensitivity and specificity were compared to those obtained with current protocol. The results are encouraging and allow *X. fastidiosa* detection in all tested matrices at low detection thresholds.

## New real-time PCR assay of *Xanthomonas campestris* pv. *campestris* based on *Zur* and *hrpF* gene detection

**Aleš Eichmeier**<sup>1</sup>, Eliška Peňázová<sup>1</sup>, Robert Pokluda<sup>2</sup>, Miroslav Baránek<sup>1</sup>, Joana G. Vicente<sup>3</sup>

<sup>1</sup> Mendel University in Brno, Mendeleum - Institute of Genetics, 691 44 Lednice, Czech Republic

<sup>2</sup> Mendel University in Brno, Department of Vegetable Science and Floriculture, 691 44 Lednice, Czech Republic

<sup>3</sup> University of Warwick, School of Life Sciences, Wellesbourne, Warwick CV35 9EF, UK

**Keywords:** real-time PCR, TaqMan<sup>®</sup> probe, brassicas, sequencing, *Zur*, *hrpF*

New real-time PCR assay based on dual labeled hydrolysis TaqMan<sup>®</sup> probe has been developed for the rapid and sensitive detection of *Xanthomonas campestris* pv. *campestris* (Xcc) and related pathovars that affect mainly *Brassicaceae* crops and ornamentals. Primers were designed to specifically amplify a 152 bp fragment of the *Zur* gene from *X. campestris* and the primers were combined with a newly designed TaqMan<sup>®</sup> probe. The developed real-time PCR assay does not detect other *Xanthomonas* species, nor other bacteria occurring on brassica tissues. To confirm the specificity of the detection, primers targeting the *hrpF* gene were used for ordinary and real-time PCR. All PCR products amplified with *Zur* and *hrpF* primers were sequenced to assess the genetic diversity of these genes in the tested isolates. Validation of the designed real-time PCR assay was carried out with thirteen Xcc strains, seven *Xanthomonas* species and pathovars and five different bacterial endophytes including *Bacillus*, *Erwinia*, *Klebsiella*, *Pantoea* and *Pseudomonas*, previously isolated from tissues of crucifers. *In silico* analysis was also carried out. The assay features were also compared with the published real-time PCR assay targeting *hrpF* gene of *X. campestris*. Combination of both real-time methods based on detection of *Zur* and *hrpF* genes was an efficient and robust assay to detect Xcc strains in brassica tissues. The protocol detects ten copies of *Zur* PCR fragment per one microliter of DNA. This method improved the specificity in relation to previously published methods as it only detected Xcc and two closely related *X. campestris* isolates.

**Whole-genome sequencing of distinct *Xanthomonas arboricola* lineages isolated from a single walnut tree host****Camila Fernandes**<sup>1,2,3</sup>, Joël F. Pothier<sup>4</sup>, Fernando Tavares<sup>1,3</sup><sup>1</sup> CIBIO-InBIO, Research Centre in Biodiversity and Genetic Resources, Universidade do Porto, Portugal<sup>2</sup> INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal<sup>3</sup> FCUP, Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Portugal<sup>4</sup> Zurich University of Applied Sciences ZHAW, Institute of Natural Resource Sciences, Environmental Genomics and Systems Biology research group, Wädenswil, Switzerland.**Keywords:** comparative genomics, virulence related genes, lineage-specific genomic features, *Xanthomonas arboricola*

*Xanthomonas arboricola* pv. *juglandis* (*Xaj*) is known as the etiological agent of important walnut diseases, leading to severe production losses and raising serious concerns about its economic impact in the future. Therefore, implementation of control and prevention measures for *Xaj* as well as advanced tools for identification of particularly virulent strains is of utmost importance. In this work we describe preliminary results of genome sequencing and comparative genomic analyses of distinct *Xanthomonas arboricola* genotypes isolated from different plant organs of a single walnut tree in Lisbon, Portugal. The genome sequences determined using 2x250bp Illumina reads resulted in genome sizes of approximately 5 Mb, a high GC content (>60%) and roughly 4,000 protein coding genes, which is in accordance to the genomic features described for other *Xanthomonas arboricola*. Genome multiple alignments between the sequenced genomes and a *Xaj* reference genome (strain CFBP 2528, NZ\_JZEF00000000.1), allowed the identification of core genomic regions and lineage-specific genomic regions. Moreover, most of these lineage-specific genomic features are associated with mobile genetic elements. Interestingly, the sequenced *X. arboricola* genomes seem to have considerable differences in the repertoires of virulence related genes, namely T2SS, T3SS, and T4SS secretion systems, type III effectors (T3Es), among others. Comprehensive genomic analysis currently under way should allow disclose lineage-specific genetic determinants and elucidate different host-specific adaptations.



**First report of the occurrence of *Xanthomonas* spp. on blueberry in Poland****Monika Kałużna**

Research Institute of Horticulture, Konstytucji 3 Maja 1/3, Skierniewice, Poland

In 2013, russet brown, irregular spots on leaves was observed on the blueberry (*Vaccinium corymbosum*) cv. Toro and Duke growing in a nursery located in Central Poland. From these leaf spots, a fluorescent and yellow bacteria were isolated. Colony morphology of yellow isolates resembling that of the *Xanthomonas* genus. Two yellow isolates obtained were positive in a PCR assay using primers X1 and X2 specific for bacteria belonging to the genus *Xanthomonas*. Further taxonomic study focused on MLSA to enable definitive classification of these isolates are being conducted. It is the first time that a bacterial disease of blueberry is caused by the *Xanthomonas* spp.

This work was financed by the National Science Centre, Poland, Grant UMO-2017/25/B/NZ9/01565.

**Development of a qPCR method to determine *Xanthomonas arboricola* pv. *juglandis* load in infected walnut samples**

**Leonor Martins**<sup>1,2</sup>, Camila Fernandes<sup>1,2,3</sup>, Pedro Albuquerque<sup>1,2</sup>, Fernando Tavares<sup>1,2</sup>

<sup>1</sup> CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO, Laboratório Associado, Universidade do Porto, Portugal

<sup>2</sup> FCUP, Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Portugal

<sup>3</sup> INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal

**Keywords:** *Xanthomonas arboricola* pv. *juglandis*, DNA markers, qPCR, bacteria quantification

*Xanthomonas arboricola* pv. *juglandis* (*Xaj*) is the causal agent of walnut bacterial blight (WBB), the major disease affecting *Juglans regia* (walnut), and has been implicated as the etiological agent of brown apical necrosis (BAN) and vertical oozing canker (VOC) disease. Early detection of the bacteria and assessment of infection progression are important to identify acutely virulent *Xaj* lineages for disclosing epidemiological patterns and implement suitable containment measures. Using two recently described *Xaj*-specific DNA markers, XAJ1 and XAJ6, a qPCR method was optimized to determine the bacterial load of infected walnut tissues. High qPCR specificity was confirmed by the low average Cq values for both XAJ1 (13.56 ± 0.71) and XAJ6 (13.21±0.53) markers when using several *Xaj* strains, contrary to the high Cq values recorded for non-*juglandis* *X. arboricola* pathovars (Cq>20.0) and non-*arboricola* *Xanthomonas* species (Cq>31). Walnut fruit samples spiked with 10- fold dilutions of *Xaj* (from 10<sup>8</sup> CFU/mL to 1 CFU/mL) allowed to determine qPCR calibration curves with high correlation coefficient (R<sup>2</sup>>0.99) and an efficiency of 97.6 % (±6.4) and 111.8 % (±0.5) for markers XAJ1 and XAJ6, respectively. Furthermore, the low limit of detection (LOD) of 2.7 CFUs/reaction obtained for both markers revealed a high sensitivity. Lab Infected walnut fruits were used to validate the procedure and assess the *Xaj* bacterial load of occurring infections. Ultimately, we believe that the proposed qPCR approach might inform about infection fitness of distinct *Xaj* lineages allowing identify acutely virulent *Xaj* pathotypes, and contribute to improve epidemiological surveillance of *Xaj* and phytosanitary treatment practices.

**Using genome-wide transcriptional profiling to study the carbohydrate metabolism of *Xanthomonas citri* subsp. *citri***

**Isabela Mendes Bonfim**<sup>1</sup>, Priscila Oliveira De Giuseppe<sup>1</sup>, Douglas Antonio Alvaredo Paixão<sup>1</sup>, Gabriela Felix Persinoti<sup>1</sup>, Júlia Taíssa Mazolini Gandolphi<sup>2</sup>, Mário Tyago Murakami<sup>1</sup>

<sup>1</sup> Bioethanol Science and Technology Laboratory, National Center for Research in Energy and Materials, Campinas, São Paulo, 12083-970, Brazil

<sup>2</sup> Federal University of São Carlos (UFSCar), Araras, São Paulo, 13600-970, Brazil

**Keywords:** carbohydrate metabolism, RNA-seq, plant polysaccharides, *X. citri*.

Phytopathogens such as *Xanthomonas citri* subsp. *citri* have a broad arsenal of enzymes to break down plant cell walls, which are complex and dynamic structures composed by cellulose, hemicelluloses and lignin. In some cases, the genes of these enzymes are organized in polyssacharide utilization loci (PUL) – a single genomic locus that encodes all proteins required to uptake monosaccharides from a given polysaccharide. However, other genes are scattered over the genome and it is still unclear what substrate stimulates their expression. To gain further insight into *X. citri* carbohydrate metabolism, the aim of this work is to identify the molecular machineries employed by *X. citri* to use different plant polysaccharides as carbon source. For this purpose, we combined bioinformatic and RNA sequencing approaches to evaluate the transcriptional profiling of *X. citri* in response to different plant carbohydrates. Interestingly, *X. citri* grow in minimal medium containing starch – the main storage carbohydrate of citrus leaves – or glucomannan as major carbon source, but not in minimal medium containing arabinoxylan, xyloglucan or avicel. In these cases, we replaced avicel by its subcomponent cellobiose and pre-digested arabinoxylan and xyloglucan into oligosaccharides, using endo-acting glycoside hydrolases from *X. citri* heterologously expressed in *Escherichia coli*. The growth of *X. citri* in presence of such oligosaccharides was similar to that using glucose. Total RNA was extracted from these samples and RNA-seq analysis will be presented to shed light on the molecular strategies used by *X. citri* to explore the carbohydrates of its plant host.

**Comparative genomics of *Xanthomonas oryzae* pv. *oryzae* bacteriophages**

**Ildikó Nagy**<sup>1</sup>, János Molnár<sup>1</sup>, Annamária Gazdag<sup>1</sup>, Casiana M. Vera Cruz<sup>2</sup>, Oliva Ricardo<sup>2</sup>, György Schneider<sup>3</sup>, Gábor Rákhely<sup>4,5\*</sup> and Tamás Kovács<sup>1\*</sup>

<sup>1</sup> Department of Biotechnology, Nanophagetherapy Center, Enviroinvest Corporation, Pécs, Hungary

<sup>2</sup> International Rice Research Institute, Los Banos, Philippines

<sup>3</sup> Institute of Medical Microbiology and Immunology, University of Pécs, Pécs, Hungary

<sup>4</sup> Department of Biotechnology, University of Szeged, Szeged, Hungary

<sup>5</sup> Institute of Biophysics, Biological Research Center, Szeged, Hungary

\* Address correspondence to Tamás Kovács, kovacst@enviroinvest.hu

*Xanthomonas oryzae* pv. *oryzae* (Xoo) is the causative agent of bacterial leaf blight of rice. Bacteriophages may provide an environmental friendly solution against Xoo. Isolated Xoo phages were grouped into two major groups: OP1- and OP2-like bacteriophages. In this study, 10 new OP2-like phages were isolated from Vietnam and from the Philippines, characterized and genome sequenced. Based on terminase large subunit encoding gene sequences, OP1- and OP2-like bacteriophages build a distinct phylogenetic group and OP2-like phages could be further divided into 2 major groups. Comparative full genomic analysis of the 10 newly isolated OP2-like bacteriophages revealed the phylogenetic relationship among the different isolates. A 322 bp conserved intergenic region in the OP2-like phage genomes was discovered when genome sequences of the 10 novel OP2-like phages were compared to OP2's genome. Analyzing mutation frequencies along the OP2-like phage genomes enabled us to track steps of the molecular evolution of OP2-like bacteriophages. This study provides data on the genomic characteristics, phylogenetic relationships and a route of molecular evolution of mostly novel Xoo OP2-like bacteriophages.

## Poster #13

**Evolution and dynamic pathogenicity of *Xanthomonas perforans* strains isolated from tomato and pepper in the United States**

**Eric Newberry**<sup>1</sup>, Rishi Bhandari<sup>1</sup>, Sujan Timilisina<sup>2</sup>, Gerald Minsavage<sup>2</sup>, Jeffrey Jones<sup>2</sup>, Neha Potnis<sup>1</sup>

<sup>1</sup> Department of Entomology and Plant Pathology, Auburn University, Auburn, AL

<sup>2</sup> Department of Plant Pathology, University of Florida, Gainesville, FL

**Keywords:** population genomics, host-specificity, bacterial leaf spot, *Xanthomonas perforans*

*Xanthomonas perforans* is the predominant species responsible for bacterial leaf spot of tomato in the southeast United States. The host range of this pathogen is generally considered to be restricted to tomato. However, in recent years, *X. perforans* strains have been isolated from symptomatic pepper plants, thus suggesting a recent host range expansion of the pathogen. Previously, two phylogenetic groups within *X. perforans* were described, where in the absence of effector triggered immunity induced by a single avirulence protein, displayed differing pathogenicity on pepper. To further investigate the diversity and contrasting pathogenicity of *X. perforans*, we conducted whole genome sequencing on a collection strains isolated from diseased tomato and pepper plants grown in Alabama, South Carolina, and Indiana and assessed their pathogenicity on pepper. A core genome phylogeny revealed that strains collected in Indiana and Alabama formed a distinct sub-group within *X. perforans* clade 1, which was comprised of lineages that were either pathogenic or non-pathogenic on pepper seedlings. All strains that were collected in South Carolina were pathogenic on pepper seedlings and grouped in *X. perforans* clade 2, along with other pepper pathogenic lineages. The genetic determinants of host specificity in *X. perforans* will be discussed.

**Genome-based population structure analysis of the strawberry plant pathogen *Xanthomonas fragariae* reveals two distinct groups that evolved independently before its species description**

Michael Gétaz<sup>1</sup>, Marjon Krijger<sup>2</sup>, Fabio Rezzonico<sup>1</sup>, Theo H.M. Smits<sup>1</sup>, Jan M. van der Wolf<sup>2</sup>, **Joël F. Pothier**<sup>1</sup>

<sup>1</sup> Environmental Genomics and Systems Biology Research Group, Institute of Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), CH-8820 Wädenswil, Switzerland

<sup>2</sup> Wageningen University & Research, Wageningen, the Netherlands

**Keywords:** MLVA, VNTR, CRISPR, MLSA, angular leaf spots

*Xanthomonas fragariae* is a quarantine organism in Europe, causing angular leaf spots on strawberry. It is spreading worldwide in strawberry-producing regions due to import of plant material through trade and human activities. In order to resolve the population structure at the strain level, we have employed high-resolution molecular typing tools on a comprehensive strain collection representing global and temporal distribution of the pathogen. Clustered regularly interspaced short palindromic repeat regions (CRISPRs) and variable number of tandem repeats (VNTRs) were identified within the reference genome of *X. fragariae* LMG 25863 as a potential source of variation. Strains from our collection were whole-genome sequenced and used in order to identify variable spacers and repeats for discriminative purpose. CRISPR spacer analysis and Multiple-Locus VNTR analysis (MLVA) displayed a congruent population structure, in which two major groups and a total of four subgroups were revealed. The two main groups were genetically separated before the first *X. fragariae* isolate was described, and are potentially responsible for the worldwide expansion of the bacterial disease. Three primer sets were designed on discriminating CRISPR-associated markers in order to streamline group determination of novel isolates. Overall, this study delivers typing methods to discriminate strains and monitor the pathogen population structure, more especially in the view of a new outbreak of the pathogen.

**Identification and detection of *Xanthomonas hortorum* pv. *pelargonii* in plant material using PCR method**Agnieszka Wojtania, Joanna Puławska

Research Institute of Horticulture, Skierniewice, Poland

**Keywords:** asymptomatic plants, detection, *Pelargonium*

*Xanthomonas hortorum* pv. *pelargonii* (*Xhp*) is the causal agent of bacterial blight on *Pelargonium*, leading to serious losses in crops of this plants. Bacteria isolated from *Pelargonium x hortorum* plants with the symptoms of bacterial blight, collected from different commercial greenhouse production in Poland, formed creamy colony on YNA medium. Isolated bacteria were identified as *Xhp* based on positive results of PCRs and pathogenicity tests on *Pelargonium* plants. To work out a fast and sensitive method of testing symptomless plant material for the presence of *Xhp*, two sets of primers described in literature and three different methods of plant material preparation and DNA isolation were tested. The optimal method, allowing detection of *Xhp* in different part of plants (stem, petiole and leaf blade) included the pre-incubation of plant extracts on YNA medium, DNA isolation on Genomic Mini columns or by incubation at 100°C followed by PCR with primers XcpM1/XcpM2. The elaborated method allowed for detection of *Xhp* in the sample when one leaf petiole taken from inoculated plant was mixed with 200 leaf petioles from healthy plants.

**A viability qPCR (v-qPCR) to quantify viable cell populations of *Xanthomonas citri* pv. *citri* (Xcc)**

**Isabelle Robène**, Amal Moumène, Véronique Maillot-Lebon, Claudine Boyer and Olivier Pruvost

CIRAD, UMR PVBMT Cirad-Université de la Réunion, Pôle de Protection des Plantes, 97410 Saint- Pierre, La Réunion

**Keywords:** diagnostic, PMA, real-time quantitative PCR, *Xanthomonas citri* pv. *citri*

Real-time quantitative PCR technology (qPCR) is a good alternative to quantify bacterial populations in *planta* compared to culture-dependent methods. However, PCR or qPCR amplify DNA from both dead and viable bacteria as DNA remains stable after the death of bacteria, and therefore might overestimate the size of the population that is actually efficient *in planta* and for future infections. We combined the use of propidium monoazide (PMA), a DNA-binding dye, and a real-time quantitative PCR protocol targeting a genomic region specific to Xcc, to selectively amplify the viable portion of Xcc cells. PMA penetrates only dead cells with compromised membranes and intercalates covalently into the DNA after photoactivation, subsequently interfering DNA amplification. The optimization of this v-qPCR was performed by investigating different critical parameters, especially concerning the killing treatment of the cells, the nature and the concentration of the DNA-binding dye, the light source for photoactivation and the qPCR protocol. The optimized protocol was then tested using different concentrations of killed Xcc cells and we showed its ability to exclude killed cells even for high cell concentrations. Supplementary purification steps were required when a *Citrus* plant extract matrix was added to Xcc cells. We validated this protocol *in planta* on Xcc symptoms obtained from artificially inoculated orange plants and heat treated to kill bacterial cells. This v-qPCR protocol will be further used to measure *in planta* growth rate of Xcc for different lineage/host combinations showing compatible or incompatible interactions.



**Identifying *Xylella fastidiosa* host adaptation candidate genes: The case of *X. fastidiosa* subsp. *pauca* isolates and olive trees in Italy**

**Anne Sicard**<sup>1</sup>, Maria Saponari<sup>2</sup>, Mathieu Vanhove<sup>1</sup>, Annalisa Giampetruzzi<sup>3</sup>, Giuliana Loconsole<sup>3</sup>, Pasquale Saldarelli<sup>2</sup>, Donato Boscia<sup>2</sup>, Rodrigo P.P. Almeida<sup>1</sup>

<sup>1</sup> Department of Environmental Science, Policy, and Management, UC Berkeley, Berkeley, CA, USA.

<sup>2</sup> Institute for Sustainable Plant Protection, National Research Council (CNR), Bari, Italy

<sup>3</sup> Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy

The introduction of a *Xylella fastidiosa* (*Xf*) subsp. *pauca* strain in Italy has resulted in the first disease epidemic of this pathogen in Europe. We cultured the bacterium and performed whole genome sequencing of over 70 *Xf. subsp. pauca* isolated from olive trees from 2013 to 2017 across southern Italy affected areas. We identified several genes under positive selective pressure within the Italian population; these are genes that might be involved in the adaptation of *X. fastidiosa* to olive trees. Other aspects of epidemiological relevance, such as estimating the introduction date of *X. fastidiosa* in Italy, are also being extracted from this dataset.

**Implementation of methods for the molecular detection of *Xanthomonas euvesicatoria* in pepper seeds**

Bekri Xhemali<sup>1</sup>, Davide Giovanardi<sup>2</sup>, Maja Ignjatov<sup>3</sup>, **Emilio Stefani**<sup>2</sup>

<sup>1</sup> Kosovo Institute of Agriculture, Peja, Kosovo

<sup>2</sup> Department of Life Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy

<sup>3</sup> Institute of Field and Vegetable Crops, Novi Sad, Serbia

**Keywords:** Bacterial spot of pepper, seed analysis, rep-PCR, genotyping, cluster analysis

*Xanthomonas euvesicatoria* (*Xeuv*) is a regulated bacterium affecting tomato and pepper. The organism is seed-transmitted; therefore, analysis of seeds is mandatory to ensure their phytosanitary quality. An EPPO standard has been developed for the detection of *Xeuv* and its identification and characterisation using different analytical methods, e.g. direct isolation, ELISA, PCR, genotyping. During a seasonal monitoring campaign, ELISA detected thirteen *Xeuv*-positive pepper seeds samples and their respective lots were rejected. Their sample duplicates were then re-analysed by direct isolation and simplex-PCR (following two different DNA extraction protocols), in order to compare different detection protocols and with the aim to identify the most sensitive and specific one(s). Putative *Xeuv* colonies were obtained by direct isolation and were confirmed by PCR and, then, subject to genotyping with rep-PCR, using the REP, BOX, ERIC primer sets. Eleven out of thirteen ELISA positive samples were confirmed by PCR, showing that ELISA is comparable to PCR regarding specificity and sensitivity. Direct isolation resulted in obtaining a set of different *Xeuv* isolates: these isolates were further studied and genetically compared among them and with other *Xanthomonads* affecting tomato and pepper by using four official reference strains (*Xeuv*, *X. vesicatoria*, *X. perforans* and *X. gardneri*). Results highlighted that *Xeuv* is quite a uniform population, taxonomically not so distant from *X. perforans*. *Xeuv* isolates clustered quite distant from the other two *xanthomonads*. Analysis of BOX-PCR profiles highlighted that *Xeuv* may belong to two subgroups: this might be related to a different geographical origin of seeds.

## Molecular methods for diversity assessment among xanthomonads of Bulgarian and Macedonian pepper

**Taca Vancheva**<sup>1</sup>, Mariya Stoyanova<sup>2</sup>, Elena Tasheva-Terzieva<sup>1</sup>, Nevena Bogatzevska<sup>2</sup>, Penka Moncheva<sup>1</sup>

<sup>1</sup>Faculty of Biology, Sofia University 'St. Kliment Ohridski', Sofia, Bulgaria

<sup>2</sup>Institute of Soil Science, Agrotechnologies and Plant Protection 'Nikola Pushkarov', Sofia, Bulgaria

**Keywords:** bacterial spot, RFLP, RAPD, REP-PCR, heterogeneity

Bacterial spot is an important disease of pepper in Bulgaria and Macedonia. For characterization of *Xanthomonas* species associated with bacterial spot, 161 strains were collected from various field pepper-growing regions. Among them, 131 strains were identified as *Xanthomonas euvesicatoria* and 30 as *Xanthomonas vesicatoria* using species-specific primers and RFLP-PCR on the 16S-23S ITS region. To assess the genetic diversity of the strains, two methods (RAPD-PCR and REP-PCR) with several primers were applied. Discriminatory index was calculated and analysis of molecular variance (AMOVA) was carried out.

Diversity among the isolated strains of *X. euvesicatoria* was observed by both methods with primers CUGEA-4, CUGEA-6, ERIC, and BOX A1R. Combined RAPD-PCR analysis with the two primers CUGEA-4 and CUGEA-6 had greater discriminative power (0.60) than REP-PCR, which makes this method applicable for strain diversity evaluation. Discrimination among the strains of *X. vesicatoria* was achieved by the use of REP-PCR with ERIC primers and only for the Bulgarian strains. The results demonstrated that *X. euvesicatoria* was more diverse than *X. vesicatoria* and heterogeneity was observed mainly in the Bulgarian populations. According to AMOVA, genetic variations in *X. euvesicatoria* were observed among and within populations originating from different regions, while differences based on the two countries were minor. Considerable variation among populations was achieved by RAPD primers which rather than ERIC- and BOX- primers were efficient in differentiating strains. According to the Principal Components Analysis, a relation between the climatic conditions of the regions and the genetic distance of the populations may be suggested.



## Session II

### Pathogenicity mechanisms and regulation

Poster #20

#### **Analysis of degradation pathways for phenolic compound in *Xanthomonas arboricola* pv. *juglandis* 417: A mechanism of persistence, counter-defense and virulence?**

**Renata de Almeida Barbosa Assis**<sup>1,2</sup>, Cintia H. D. Sagawa<sup>1</sup>, Leandro Marcio Moreira<sup>2,3</sup>, Abhaya M. Dandekar<sup>1</sup>

<sup>1</sup> Plant Sciences Department, University of California, Davis, CA, USA

<sup>2</sup> Center of Research in Biological Science (NUPEB), Federal University of Ouro Preto (UFOP), MG, Brazil

<sup>3</sup> Department of Biological Science (DECBI), Institute of Exact and Biological Science (ICEB), Federal University of Ouro Preto (UFOP), MG, Brazil

**Keywords:** phenolic compounds, *Xanthomonas*, adaptation, persistence, comparative genomics

*Xanthomonas arboricola* pv. *juglandis* 417 (Xaj417) is the causal agent of walnut bacterial blight, the most significant above ground disease of walnuts (*Juglans regia* L.), which are rich in phenolic compounds associated with pathogen defense. Phenolic compound degradation (PCD) pathways are required for pathogenicity of *X. campestris* and possibly for other *Xanthomonas* species. Our study focused on Xaj417 to understand how this pathogen was able to develop a counter defense to these phenolic compounds to be able to colonize this host. We identified genes related to PCD in Xaj417 and five main pathways were mapped: ketoacidate, ubiquinone and tryptophan biosynthesis, tolerance of toluene, and biosynthesis of aromatic amino acids. We investigated the ketoacidate pathway and how Xaj417 could make use of these phenolic compounds as an alternative carbon source which could explain the adaptation of this pathogen inside the plant, thereby providing new therapeutic targets to combat the virulence response of Xaj in walnuts. Our results suggest that Xaj417 not only detoxifies phenolic compounds induced by plant defense responses, but also use some intermediates as carbon source favoring their growth in plant tissue. Xaj interferes in the chorismate pathway in walnut decreasing SA production, increasing aromatic amino acid synthesis and favoring growth and siderophore production. Therefore, investigating the importance of these pathways in the interaction with compatible hosts, the elucidation of this adaptive process aid the development of strategies to interfere with the adaptation to the plant apoplast and induction of virulence that occur when the bacterium colonizes plant tissues.

***In vitro* and *in planta* transcriptomic analyses of *X. campestris* pv. *campestris***

**Alice Boulanger**<sup>1</sup>, Aude Cerutti<sup>1</sup>, Brice Roux<sup>1,2</sup>, Sébastien Carrère<sup>1</sup>, Olivier Bouchez<sup>3</sup>, Marie-Françoise Jardinaud<sup>1</sup>, Emmanuelle Lauber<sup>1</sup>, Matthieu Arlat<sup>1</sup>, Laurent Noël<sup>1</sup>

<sup>1</sup> University of Toulouse, INRA, CNRS, UPS, INP-ENSAT, Laboratory of Plant-Microorganism Interactions (LIPM), UMR 441/2594, Castanet-Tolosan, France.

<sup>2</sup> CEA, CNRS, AMU, UMR 7265, <sup>2</sup>Laboratoire de biologie du développement des plantes, Saint-Paul-lez- Durance, France.

<sup>3</sup> INRA, Genotoul Genome & Transcriptome (GeT-PlaGe), Castanet-Tolosan, France

**Keywords:** *Xanthomonas*, transcriptomic, virulence, plant adaptation

*Xanthomonas campestris* pv *campestris* (*Xcc*) is a phytopathogenic bacterium which causes black rot disease on cultivated or wild *Brassicaceae*. During its lifecycle, *Xcc* experiences changing environments such as leaf surfaces, distinct endophytic compartments (hydathode, xylem, mesophyll), seeds, plants debris. So far, genetic screens have only identified major genes important for pathogenicity. Mechanisms of pathogen entry, vascular immunity suppression, metabolic adaptation and microbial fitness in those plant environments are essentially unknown. In order to acquire a time laps picture of genes expressed during infection, deep RNA sequencing (RNAseq) experiments have been conducted on *Xcc*: the HrpG regulon *in vitro* and *Xcc* transcriptome in cauliflower hydathodes which are the first leaf organs infected by *Xcc*. A comparison of both transcriptomes will be presented.

**Coordination of virulence traits in *Xanthomonas*: a common motif with dual role****Subhadeep Chatterjee**

Centre for DNA fingerprinting and Diagnostics, Hyderabad, INDIA. 500001  
E mail: subhadeep@cdfd.org.in Tel. +91-40-24749425  
Fax: +91-40-24749448

**Keywords:** Cell-cell communication, reversible heterogeneity, iron, glucan

*Xanthomonas* group of plant pathogens causes diseases in more than 350 different plant hosts. *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Xanthomonas oryzae* pv. *oryzicola* (Xcola) are important members of the genus *Xanthomonas* which causes serious disease of rice. *Xanthomonas* coordinates the production of virulence associated function by the production and sensing of fatty acid signaling molecule known as Diffusible signal factor (DSF) by a process known as quorum sensing. We are using molecular genetics and biochemical tools to understand the quorum sensing process in Xoo and Xcola pathogens. Functional studies indicate that Xoo and Xcola regulate the production of virulence associated function in an atypical manner. We have also identified several components of the DSF mediated sensing and factors required for regulation of production of virulence associated functions. We have proposed a model in which DSF regulates functions required for entry, colonization and movement in a contrasting fashion to promote infection and spread inside host. Apart from the role of DSF in quorum sensing, we have identified a novel role of DSF in *Xanthomonas*-host interaction involving inter- kingdom signaling which will be discussed. In addition we have identified several novel functions such as a iron (Ferric binding response regulator), components of guided motility, and molecule utilize for sequestering environmental iron, which are coordinated in *Xanthomonas* to effectively utilize host iron resources and maintain iron homeostasis.

**Genetic basis of *Xanthomonas campestris* pv. *campestris* adaptation to *in vitro* and *in planta* conditions**

**Julien Luneau**<sup>1</sup>, Maël Baudin<sup>2,3</sup>, Sébastien Carrère<sup>1</sup>, Olivier Bouchez<sup>4</sup>, Marie-Françoise Jardinaud<sup>1</sup>, Jayashree Ray<sup>5</sup>, Adam Deutschbauer<sup>5</sup>, Jennifer Lewis<sup>2,3</sup>, Laurent Noël<sup>1</sup>, Emmanuelle Lauber<sup>1</sup>, Alice Boulanger<sup>1</sup>.

<sup>1</sup> University of Toulouse, INRA, CNRS, UPS, INP-ENSAT, Laboratory of Plant-Microbe Interactions, Toulouse, France

<sup>2</sup> Plant Gene Expression Center, USDA, Albany, California, USA

<sup>3</sup> Department of Plant and Microbial Biology, University of California, Berkeley, California, USA

<sup>4</sup> Genotoul Genome & Transcriptome (GeT-PlaGe), INRA, Toulouse, France

<sup>5</sup> Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA

**Keywords:** *Xanthomonas*, fitness, TnSeq, virulence, plant adaptation

*Xanthomonas campestris* pv. *campestris* (*Xcc*) is the causal agent of the black rot disease on wild and cultivated Brassicaceae worldwide. Understanding the major determinants of *Xcc* pathogenicity is crucial and has therefore been addressed during the past decades. However, little is known about genes contributing to microbial fitness at early steps of infection. Fortunately, thanks to the quick progression of Next Generation Sequencing techniques, new tools such as Transposon insertion site sequencing (TnSeq) have been developed and now allow the exploration of these aspects. We have initiated a fitness analysis of *Xcc* in both *in vitro* and *in planta* conditions based on the analysis of a bacterial mutant population by TnSeq. This fairly recent approach has been successfully used over the past few years to identify genes required for the virulence of numerous human bacterial pathogens and very recently produced interesting results in the field of plant pathology. We will explain how, by counting the relative abundance of a barcoded library of Tn-mutants, this high-throughput screening method will allow the identification of genes important and specific to key environments of the *in planta* life of the bacteria. The scope of our work takes into consideration all components of bacterial fitness (metabolic capacities, defense evasion, abiotic stress tolerance...) and focuses on four biologically relevant plant environments: Leaf surface, Hydathodes, Xylem and Mesophyll. Our progress will be presented.



**Effect of bacterial antagonists on *Xanthomonas* black rot- and fusarium wilt pathogens**

Andrea Palyzova, Katerina Svobodova, Lucie Sokolova, Jiri Novak, **Cenek Novotny**

Institute of Microbiology of the Czech Academy of Sciences, v.v.i., Videnska 1083, 142 20 Prague 4, Czech Republic

**Keywords:** biocontrol activity; *Xanthomonas campestris* pv. *campestris*, *Fusarium oxysporum*; metabolic profiling; MALDI mass spectrometry

Biocontrol agents represent an alternative to chemicals in the management of bacterial and fungal crop diseases. A siderophore obtained from *Pseudomonas aeruginosa* F60 was tested to affect growth of four *Xanthomonas campestris* pv. *campestris* (XCC) strains. Only the highest siderophore concentration of 100 µg.mL<sup>-1</sup> inhibited growth of XCC 3811 strain by slowing its doubling rate by 41% whereas in the other strains the slow down was ≤2.6%. The results showed different sensitivity of XCC strains to the growth inhibition by the pseudomonad siderophore. In the other part of the antagonistic study, *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* were used in dual agar cultures against *Fusarium oxysporum* f. sp. *conglutinans* (FOC). The metabolites produced during the microbe interactions were screened by a matrix-assisted, laser desorption/ionization (MALDI) mass spectrometry. The effect of *B. amyloliquefaciens* was mediated by a number of antimicrobial lipopeptides and siderophores and consisted in a suppression of the production of FOC's mycotoxin beauvericin. In the case of *P. aeruginosa*, the siderophores pyoverdine E/D and two rhamnolipids were produced as major bacterial metabolites. In a co-culture with FOC the production of rhamnolipids by the bacterium was blocked by the action of the fungal phytopathogen. The biocontrol of FOC by *P. aeruginosa* was weaker than that by *B. amyloliquefaciens*.

The work was supported by the projects EuroXanth COST (CA 16107), QJ1510088, and 194LO1509.

**Selenium nanoparticles as a tool for elimination of the bacterium *Xanthomonas campestris* pv. *campestris***

**Jakub Pečenka**, Aleš Eichmeier, Eliška Peňázová, Filip Gazdík, Miroslav Baránek

Mendeleum – Department of Genetics, Mendel University in Brno, Lednice, Czech Republic

**Keywords:** *Xanthomonas campestris* pv. *campestris*, selenium nanoparticles, concentration.

In this study, the inhibitory effect of commercial product based on selenium nanoparticles was investigated via application on different strains of bacterium *Xanthomonas campestris* pv. *campestris* (Xcc). Goal of the experiment was to find the most appropriate concentration of selenium nanoparticles avoiding bacterial growth. Ten concentrations of selenium nanoparticles from 2,5 to 80 ppm were used in the presence of three Xcc strains (1279A, 3811 and 3971A). Cultivation of bacteria with added nanoparticles was performed in 96-well plates and an optical density of Xcc cultures was measured using spectrophotometer. From obtained absorbance a doubling time of bacterial strains was counted.

**Use of ozone for inactivation of *Xcc* in *Brassica* seeds**

**Eliška Peňázová**<sup>1</sup>, Aleš Eichmeier<sup>1</sup>, Jakub Pečenka<sup>1</sup>, Luděk Laňar<sup>2</sup>, Miroslav Baránek<sup>1</sup>

<sup>1</sup> Mendeleum – Institute of Genetics, Mendel University in Brno, Lednice, Czech Republic

<sup>2</sup> Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy, Czech Republic

**Keywords:** ozone, *Xanthomonas campestris* pv. *campestris*, MPA

Ozone has been used in the food industry and wastewater treatment as a strong antimicrobial substance due to its oxidizing capacity. This study was focused on the use of ozone as a potential treatment of Brassica seeds against seed-borne and seed-transmitted bacterium *Xanthomonas campestris* pv. *campestris*. Ozone was produced by the Annihilator 10000 (Ozontech s.r.o., Zlín, Czech Republic) generating 10 g of ozone per hour. Samples of artificially inoculated seeds (Xcc HRIW 1279A) were exposed to the treatment for 10, 20 and 30 min with seed shaking every 5 min. The control non-inoculated variants were also examined. The concentration of ozone was set on 12840 ppm. All variants were subsequently grown on MPA with cyclohexamide, 20 seeds per Petri dish in 3 repetitions. The occurrence of Xcc colonies were daily checked and evaluated after 5 days and their identity was confirmed by PCR targeting *hrpF* gene (Berg et al., 2005). Obtained results showed the impact of different treatment time to the Xcc occurrence where increasing time correspond to the decrease of presented colonies. The best result was shown for the 30 min of treatment where only three seeds from 60 inoculated developed visible Xcc colonies on MPA medium. Moreover, the growth of other microorganisms was eliminated. The usability of this treatment was also evaluated by germination test.

**Galacturonic acid and galactose monosaccharides in EPS seems to be the target of N- acetylcysteine to decrease EPS, movement and biofilm formation in *Xanthomonas citri* subsp. *citri***

**Simone Cristina Picchi**, Laís Moreira Granato, Maria Júlia Festa Franzini, Marco Aurélio Takita, Marcos Antonio Machado, Alessandra Alves de Souza

Centro de Citricultura Sylvio Moreira/IAC, Rodovia Anhanguera Km 158, Cordeirópolis SP, Brazil, 13490-970

**Keywords:** biofilm, *xanA* gene, *Xanthomonas citri*, NAC

N-acetylcysteine (NAC) is a molecule with antibacterial properties that reduce biofilm formation and exopolysaccharides in wide range of bacteria species. However how NAC interferes on these features it is still unknown. In previous study we demonstrated that NAC also reduces biofilm formation, movement and extracellular polysaccharides in *X. citri* consequently decreasing disease symptoms. Thus, to better understand the effect of NAC on *X. citri* EPS, we generated a *xanA* mutant, a gene that encodes a phosphoglucomutase/phosphomannomutase enzyme, required for the synthesis of lipopolysaccharide and exopolysaccharide. The mutant showed a significant reduction of EPS, biofilm formation and sliding motility, confirming the role of *xanA* gene in EPS production in *X. citri*. These features in the *xanA* mutant cells were less affected by NAC with a smaller reduction of biofilm compared with the non-treated cells. In addition, similar results were obtained for sliding motility, where WT cells were not able to form a halo of motility as the untreated control. Thus, we analyzed the monosaccharides composition of the EPS produced by *X. citri* and *xanA* mutant by HPLC, to find possible targets that can be involved with NAC activity. In the presence of NAC, a reduction of mannose and glutamic acid and increased rhamnose and glucose occurred for both bacteria. The galacturonic acid and galactose monosaccharides showed different profiles in the presence of NAC, increased for the wild-type and reduced for the *xanA* mutant. The results suggest that *xanA* mutant produce a modified EPS causing the NAC to have a different behaviour.

Financial support: FAPESP 2013/01395-9 and 2013/10957-0

**Lipids profile of *Nicotiana tabacum* "Petite Havana SR1" inoculated with *X. fastidiosa* subsp. *pauca* strain De Donno**

**Valeria Scala**<sup>1</sup>, Massimo Reverberi<sup>2</sup>, Manuel Sallustri<sup>2</sup>, Nicoletta Pucci<sup>1</sup>, Vanessa Modesti<sup>1</sup>, Simone Lucchesi<sup>1</sup>, Stefania Loreti<sup>1</sup>

<sup>1</sup> Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca Difesa e Certificazione, Via C.G. Bertero, 22, 00156 Rome, Italy;

<sup>2</sup> Sapienza University, Dept. of Environmental Biology, P.le Aldo Moro 5, Roma,

**Keywords:** *X. fastidiosa* subsp. *pauca* strain De Donno

During plant-pathogen interactions, lipids might have roles in several aspects such as pathogen perception, signal transduction and downstream defence responses. Among lipids entities, amphiphilic lipids, phosphorus-free membrane lipids, free fatty acids and, their oxidized forms represent an important class of signalling molecules in the host-pathogen interaction, especially related to virulence and defence. In *Xylella fastidiosa* the role of DSF was assessed whereas poor information are currently available on the role of lipids. The lipid composition of *X. fastidiosa* subsp. *pauca* strain De Donno, associated to the olive quick decline syndrome in southern Italy, was investigated. Several lipid compounds produced in the bacterium cell as well as in the culture media were individuated and identified. The model plant *Nicotiana tabacum* Petite Havana SR1, inoculated with this bacterial pathogen shows a differential accumulation of lipid entities compared to the un-infected plants. Notably, the lipids evaluated in the study discriminate a different profile between the marginal portion of the leaf and the petioles of the infected plants. The results show that the individuated lipids entities could be involved in the interaction with the host and contribute to the bacterial lifestyle.

**Stringent response coordinates the expression of virulence and metabolic pathway genes in *Xanthomonas citri* subsp. *citri*****Yanan Zhang** and Nian Wang

Citrus Research and Education Center, Department of Microbiology and Cell Science,  
Institute of Food and Agricultural Sciences (IFAS), University of Florida, Lake Alfred, FL,  
USA

**Keywords:** RNA-seq, citrus canker, stringent response, *Xanthomonas citri* subsp. *citri*

Stringent response is critical for bacterial survival in nutrient-limited environments and contributes to bacterial infection of their hosts. This process is mainly controlled by the RNA polymerase-binding transcription factor DksA and signal molecule (p)ppGpp whose level is regulated by protein family RelA- SpoT homologue(RSH). To understand the roles of stringent response in *Xanthomonas citri* subsp. *citri* (Xcc), which is the causal agent of citrus canker, transcriptome profiling via RNA-seq was used for  $\Delta$ dksA mutant vs wild type Xcc and  $\Delta$ relA $\Delta$ spoT double mutant vs wild type Xcc. Totally, about 20% of all Xcc genes are considered as differentially expressed genes (DEGs) for each mutant strain and 482 DEGs overlapped in both mutant strains. Functional enrichment analysis showed that over-represented genes are involved in carbohydrate transport and metabolism, cell motility, inorganic ion transport and metabolism and intracellular trafficking and secretion. Further analysis and experiments revealed that stringent response inhibits expression of genes encoding stable RNA (rRNA and tRNA), ribosomal proteins, chemotaxis and flagellar components, and activates expression of genes encoding type 3 secretion system and secreted effectors, and transporters including TonB-dependent transporters and ABC transporters. In conclusion, our study showed that stringent response regulators are important pathogenicity determinants and enable the bacteria to adapt to host environment by coordinating the expression of virulence and metabolic pathway genes.

**A versatile broad-range bacterial protein expression system in *Xanthomonas oryzae***

Xiaofei Yang<sup>#</sup>, Meili Wunierbieke<sup>#</sup>, Hanmo Song, Cuiping Zhang, Xiaobin Chen, **Lifang Zou**<sup>\*</sup>, Gongyou Chen

Department of Environment and Resource, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China.

<sup>#</sup> Xiaofei Yang and Meili contributed equally in this work.

<sup>\*</sup>Corresponding author: Lifang Zou; E-mail: zoulifang202018@sjtu.edu.cn

*Xanthomonas oryzae* pv. *oryzae* (Xoo) is the causal agent of bacterial blight of rice, which is a significant threat throughout many regions of rice production. With the development of functional genomics in Xoo, the mechanisms that control virulence regulation networks have become a hot research topic. Efforts to determine the molecular mechanism of this pathogen in pathogenesis require suitable tools for genetic manipulation such as protein expression. Here we constructed different expression vectors based on the backbones of pHM1 which is a broad-host range vector widely used in Xoo strains due to high transformation efficiency. Each vector contains a DNA cassette that consists of a multiple cloning site (MCS) and a sequence encoding a C-terminal Flag or 3×Myc tag. The cassette is bound by a strong *lacI* promoter and transcriptional terminators to permit overexpression of the inserted gene or to eliminate the interference of external transcription enabling the detection of weak or accurate protein expression according to the different experiment purposes. We have demonstrated the use of these vectors in Xoo to realize the different expression levels of HrpG protein in different constructs. This new expression vector series has the potential to be used in mutant complementation and gene regulation studies in Xoo and other *Xanthomonas* spp.





## Session III

### Type III secretion and effector proteins

Poster #31

#### Co-visualization of bacteria and type III effector activity during *Xanthomonas* leaf infection

**Jules Butchacas**<sup>1,2,3</sup>, Marion Perret<sup>2</sup>, Celine Pesce<sup>2,4,5</sup>, Aude Cerutti<sup>6</sup>, Taca Vancheva<sup>1,2</sup>, Frederica Bini<sup>7</sup>, Goetz Hensel<sup>7</sup>, Ingrid Otto<sup>7</sup>, Boris Szurek<sup>2</sup>, Stephen P. Cohen<sup>3</sup>, Jan E. Leach<sup>3</sup>, Jochen Kumlehn<sup>7</sup>, Laurent D. Noel<sup>6</sup>, Claude Bragard<sup>4</sup>, Ralf Koebnik<sup>2</sup>, Jonathan M. Jacobs<sup>1,2,3,4</sup>

<sup>1</sup> Department of Plant Pathology, The Ohio State University, Columbus, OH, USA

<sup>2</sup> IRD-CIRAD, UMR Interactions Plantes Microorganismes Environnement, Montpellier, France <sup>3</sup>Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

<sup>4</sup> Earth & Life Institute, Université Catholique Louvain-la-Neuve, Louvain-la-Neuve, Belgium <sup>5</sup> Department of Microbiology, University of New Hampshire, Durham, NH, USA

<sup>6</sup> LIPM, Université de Toulouse, INRA, CNRS, UPS, F-31326 Castanet-Tolosan, France

<sup>7</sup> Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), 06466, Gatersleben, Germany.

**Keywords:** type III secretion, TAL effectors, *Xanthomonas translucens*, *Xanthomonas campestris*, microscopy

Many *Xanthomonas* pathogens require a Type III secretion system and associated virulence effector proteins to cause disease. Although Type III effector activity has been largely studied, we still lack a strong understanding about the host cells and tissues targeted by effectors during infection. Type III effectors are typically difficult to detect because they are translocated into host cells at low concentrations. We developed a novel method to detect plant cells targeted by the plant pathogenic bacteria, *Xanthomonas translucens* and *Xanthomonas campestris*. A class of Type III effectors called Transcriptional activator-like (TAL) effectors found in many *Xanthomonas* spp. including *X. translucens* and *X. campestris* induce plant gene expression by binding promoters of host genes. We created transgenic barely plants that express GFP upon infection by *X. translucens* expressing TAL effectors that target and activate the promoter of the *GFP* gene. We confirmed TAL effector-dependent GFP expression with quantitative PCR and visualization with fluorescence confocal microscopy. With co-visualization of fluorescent protein-expressing bacteria and plant cells, we overall provide evidence that major barley cell targets for *X. translucens* include both mesophyll cells and the stomata, the entry point for most major plant pathogenic foliar pathogens. To broaden the application of the effector detection, we tested TAL effector-mediated induction of an inducible GUS reporter by *X. campestris* pv. *campestris* during Arabidopsis infection. We similarly determined tissue-specific TAL effector targeting by *X. campestris* pv. *campestris*. These tools overall provide a platform to visualize and study *Xanthomonas* effector targeting of plant tissues.

**Transcription activator-like effectors are involved in the host adaptation of *Xanthomonas* strains responsible for common bacterial blight of bean**

Mylène Ruh<sup>1</sup>, Justine Foucher<sup>1</sup>, Martial Briand<sup>1</sup>, Sophie Bonneau<sup>1</sup>, Anne Prevaux<sup>1</sup>, Sébastien Carrere<sup>2</sup>, Marie-Agnès Jacques<sup>1</sup>, **Nicolas W.G. Chen<sup>1</sup>**

<sup>1</sup> IRHS, INRA, AGROCAMPUS OUEST, Université d'Angers, SFR4207 QUASAV, 42, rue Georges Morel, 49071 Beaucouzé, France.

<sup>2</sup> CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR 2594, Castanet-Tolosan, France

**Keywords:** common bacterial blight of bean, TAL effectors, horizontal gene transfer, host adaptation

Common bacterial blight is the main bacterial disease of common bean. This disease is caused by *Xanthomonas citri* pv. *fuscans* (*Xcf*) and *X. phaseoli* pv. *phaseoli* (*Xpp*). *Xcf* and *Xpp* are phylogenetically distant yet they share the ability to induce the same symptoms on common bean, which is suggestive of pathological convergence between these two pathovars. Transcription Activator-Like (TAL) effectors are type III effectors able to induce the expression of host genes to promote infection. To achieve this, TAL effectors are able to get into the nucleus of the host cells, then specifically bind to the promoter region of targeted plant genes, resulting in the overexpression of these genes. To study the role of TAL effectors in the *Xanthomonas* – common bean interaction we performed genomics and transcriptomics analyses. Single-Molecule Real-Time sequencing of 17 *Xcf* and *Xpp* genomes unveiled one to three TAL- encoding genes per strain for a total of four different *tal* genes, two of which (*tal23A* and *tal18H*) were horizontally transferred between *Xcf* and *Xpp*. These results suggest that both TAL23A and TAL18H could participate to the pathological convergence observed between the two pathovars. Combination of pathogenicity tests and transcriptomics after inoculation of a *tal18H* deletion mutant on common bean plants revealed that TAL18H was involved in symptom development and displayed a pleiotropic effect on common bean transcriptome during the interaction. Altogether, our results that TAL effectors are involved in the adaptation of *Xcf-Xpp* to common bean and highlight the role of TAL18H in *Xcf-Xpp* pathogenicity.

***Xanthomonas oryzae* pv. *oryzae* XopQ protein suppresses host innate immune responses through interaction with rice 14-3-3 proteins that are positive activators of innate immunity**

**Sohini Deb<sup>1</sup>, Mahesh K. Gupta<sup>1,2</sup>, Hitendra K. Patel<sup>1</sup>, Ramesh V. Sonti<sup>1,3</sup>**

<sup>1</sup> Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad, India

<sup>2</sup> Present address: Metahelix, Bangalore, India, <sup>3</sup>NIPGR, New Delhi, India

**Keywords:** *Xanthomonas oryzae* pv. *oryzae*, 14-3-3 protein, resistance, rice, effector

*Xanthomonas oryzae* pv. *oryzae* (Xoo) is a gram-negative bacterium which causes the serious bacterial blight disease in *Oryza sativa* (rice). During the infection process, Xoo suppresses cell wall degrading enzyme induced plant immune responses using various non-TAL effectors that are secreted through its Type- III secretion system. Four of these proteins called Xanthomonas outer protein Q (XopQ), XopN, XopX and XopZ, suppress rice innate immune responses in a redundant manner. In our work we have investigated the role of the 14-3-3 protein binding motif of the XopQ effector protein in suppression of rice innate immune responses. We have shown that phosphorylation of the 14-3-3 protein binding motif of XopQ is essential for its interaction with unique rice 14-3-3 proteins as well as for the suppression of rice innate immune responses. Also, the subcellular localization of XopQ is dependent on the phosphorylation status of this motif. Further, the target 14-3-3 proteins of XopQ are positive regulators of immune responses, which when overexpressed, confer enhanced tolerance to rice against Xoo infection.

**The role of TAL effectors in virulence of New York Isolates of *Xanthomonas campestris* pv. *campestris* on cabbage****Zoe Dubrow**

School of Integrative Plant Science, College of Agriculture & Life Sciences, Cornell University, Ithaca, NY, USA

*Xanthomonas campestris* pv. *campestris* (Xcc), the causal agent of black rot of crucifers, is one of the most important *Brassica* pathogens worldwide. Transcription activator-like effectors (TALEs) are a large family of type III secreted effectors present in some *Xanthomonas* species that play a role in bacterial colonization of host plants. The first four Xcc genomes published showed no evidence of TALEs, suggesting Xcc lacked them. However, more recent work and reclassification of *Xanthomonas campestris* pathovars have shown that many Xcc strains do contain TALEs. The role of these TALEs in virulence is currently unknown. We used PCR and western and Southern blotting to survey 124 Xcc isolates from a 10-year New York State collection and found 30% of the isolates have TALE genes and that TALE protein is expressed. The New York Xcc isolates have different TALE repertoires even among isolates found by MLSA to be closely related. To understand the role of TALEs in black rot of crucifers, we are sequencing the Xcc TALEs and determining by targeted mutagenesis whether they contribute to Xcc virulence. We will identify susceptibility (S) genes upregulated by important TALEs. These data could inform targeted resistance breeding approaches for cruciferous vegetables.

**Genome mining reveals lineages of *Xanthomonas oryzae* pv *oryzae* encoding for a variant of PthoX2 with novel targeting activity**

**Jose C. Hugueta-Tapia**<sup>1</sup>, Chonghui Ji<sup>1</sup>, Bing Yang<sup>2</sup>, Frank F. White<sup>2</sup>

<sup>1</sup> Plant Pathology Department, University of Florida, Gainesville FL USA.

<sup>2</sup> Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA

*Xanthomonas oryzae* pv *oryzae* (Xoo) is the etiological agent of bacterial blight in rice. The disease is widespread in Asia and an emerging pathogen in West Africa. The symptoms of the infection are xylem blockage and leaf senescence. Xoo strains have multiple copies of TAL effector (TALe) genes, which, due to their structure, are difficult to resolve. Single Molecule Real Time Sequencing Technology has facilitated assembly of genomes with a high content of repetitive sequences, allowing highly accurate assembly of the TALe repertoire. We conducted a pathogenicity screening and whole-genome sequencing of variant Xoo strains with regards to SWEET gene induction. Strains compatible on japonica varieties with mutations in *OsSWEET11* and *OsSWEET14* encode a variant of PthXo2, which targets *OsSWEET13* in japonica rice varieties. The PthXo2 variant contains two aberrant large repeats of 36 aa, which are predicted to provide the TALe flexible binding properties. The aberrant repeats are not present in the PthXo2 that is encoded by strain MAFF31018 and targets *OsSWEET13* in indica rice varieties. PthXo2-like strains were isolated from the Philippines and Korea and belong to different lineages based on core genome SNPs analysis. However; both groups of strains show PthoX2-like TAL effectors with similar RVDs, with one single change at the 8th position that preserves the EBE for *OsSWEET13* in japonica rice varieties.

**The *Ralstonia solanacearum* RipTAL protein Brg11 targets an effector binding element that is highly conserved across all solanaceous host plants**

Dousheng Wu<sup>1</sup>, Alvaro Perez-Quintero<sup>2</sup>, Matthias Buntru<sup>3</sup>, Tiffany Lowe-Power<sup>4</sup>, Stefan Schilberg<sup>2</sup>, Caitilyn Allen<sup>4</sup>, Boris Szurek<sup>2</sup>, **Thomas Lahaye**<sup>1</sup>

<sup>1</sup> Zentrum für Molekularbiologie der Pflanzen (ZMBP), Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 32, Tübingen D-72076, Germany

<sup>2</sup> University Montpellier 2, CIRAD, IRD, UMR Resistance Plantes Bioagresseurs, F-34394 Montpellier 5, France

<sup>3</sup> Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstr. 6, 52074 Aachen, Germany

<sup>4</sup> Department of Plant Pathology, University of Wisconsin – Madison, Madison, WI 53706, USA.

The effector protein Brg11 from the bacterial pathogen *Ralstonia solanacearum* shares structural homology to *Xanthomonas* transcription effector-like effectors (TALEs). TALEs interact with effector-binding-elements (EBEs) of plant promoters and transcriptionally activate the downstream encoded host genes to promote bacterial disease. Notably the transcription start site (TSS) of the TALE-induced (extrinsic) transcript is often distinct from the TSS of the intrinsic transcript. To identify host targets of Brg11 we used a combination of *in silico* prediction of EBEs and RNA Seq. These studies uncovered that Brg11 targets only one host gene, the products of which are involved in polyamine biosynthesis. Notably the identified EBE is conserved across all solanaceous plants. Experimental studies in tomato, tobacco and eggplant indeed showed that Brg11 transcriptionally activates the very same host gene in these three *R. solanacearum* host species. RACE studies conducted in these three species also revealed that the 5'UTR of the intrinsic transcript is about 350bp longer than the 5'UTR of the Brg11-induced extrinsic transcripts. Comparative *in vitro* and *in vivo* studies revealed that long 5'UTR of the intrinsic transcript is feedback regulated by polyamines while the short 5'UTR of the Brg11-induced transcript is not. Thus, intrinsic and extrinsic transcripts are functionally distinct resulting in high and low translation levels, respectively. We will present our latest observations on the Brg11 target genes including a model that explains how activation of this target gene promotes bacterial wilt disease.

**Biotinylation-based assay to study effector protein function in plant host cells**

**Robert Morbitzer**, Pia Lutz, Angela Dressel, Annett Strauß, Thomas Lahaye

Eberhard Karls Universität, Tübingen, Germany

**Keywords:** Avitag, biotinylation, effector proteins, *Xanthomonas euvesicatoria*-tomato pathosystem

Successful colonization of plants by bacterial pathogens requires effector-mediated suppression of defense responses and plant host reprogramming. So, bacterial effector proteins are important virulence determinants for plant pathogens. However, the study of bacterial effector function *in planta* is challenging. In order to analyse effector protein translocation and localization as well as to identify DNA and/or protein interaction partners of effectors, we adapted an assay based on Presti et al., 2017. The assay exploits the ability of the bacterial biotin ligase BirA to biotinylate pathogen effectors carrying a short peptide tag (Avitag). Stable expression of BirA in the host plant results in biotinylation of the effector proteins only upon uptake into the plant cells. We engineered *Xanthomonas euvesicatoria* strains that express T3SS effectors fused to the Avitag and are generating stable transgenic tomato plants expressing cytoplasmic and nuclear localized BirA derivatives, respectively. Streptavidin-pulldown of biotinylated effectors proteins from leaf cell extracts will not only allow us to elucidate effector localization (cytoplasmic or nuclear localization) we can further identify DNA and protein interactions *in planta*. Using this assay, we will study a number of effector proteins in the *Xanthomonas euvesicatoria*-tomato pathosystem to analyse their impact for bacterial virulence.

**The *Xanthomonas* effector protein Xopl suppresses the stomatal immunity of tomato****Oliver A. Nagel** and Ulla Bonas

Department of Plant Genetics, Martin Luther University Halle-Wittenberg, D-06120 Halle, Germany

**Keywords:** type III effectors, tomato, stomatal immunity, plant defense

Our laboratory studies the interaction between *Xanthomonas campestris* pv. *vesicatoria* (Xcv) and its host plants pepper and tomato. Essential for pathogenicity is the type III secretion system (T3SS) which translocates more than 30 effector proteins (T3Es) into the plant cell cytoplasm. Here, T3Es suppress the plant innate immunity and alter the plant metabolism to the pathogen's advantage. Due to a eukaryotic F-box motif in the N-terminal region, the T3E Xopl is supposed to integrate into plant SCF (Skp1-Cullin-F-box protein) complexes which target proteins for ubiquitination. Interaction studies in yeast showed that Xopl specifically interacts with one out of 21 *Arabidopsis thaliana* Skp1-like proteins (ASK), suggesting that upon infection, Xopl integrates into particular SCF. A yeast-two-hybrid screen with Xopl as bait identified five proteins, that presumably are involved in the regulation of stomatal movement. Silencing of two of these potential interactors confirmed that they mediate stomatal closure after PAMP treatment in *Nicotiana benthamiana*. In tomato plants, virulence of Xcv85-10Δ*xopI* strains is dramatically reduced. The stomatal aperture is as well reduced, suggesting that Xopl is essential for Xcv entry into the host plant apoplast. Stomata assays with stable *xopI* transgenic *N. benthamiana* lines showed, that Xopl suppresses stomatal closure induced by different treatments, suggesting that Xopl maybe affects different pathways of stomatal immunity.



**Modular cloning of the type III secretion gene cluster from the tomato and pepper pathogen *Xanthomonas campestris* pv. *vesicatoria***

**Christian Otten**<sup>1</sup>, Jens Hausner<sup>2</sup>, Sylvestre Marillonnet<sup>3</sup>, Michael Jordan<sup>4</sup> and Daniela Büttner<sup>1</sup>

<sup>1</sup> Martin Luther University Halle-Wittenberg, Institute of Biology, Department of Genetics, Halle (Saale), Germany

<sup>2</sup> Present address: Icon Genetics GmbH, Halle (Saale), Germany

<sup>3</sup> Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany

<sup>4</sup> Present address: Labor Blumenstrasse, Erfurt, Germany

**Keywords:** type III secretion, *Xanthomonas campestris* pv. *vesicatoria*, modular cloning system

*Xanthomonas campestris* pv. *vesicatoria* is the causal agent of bacterial spot disease on tomato and pepper plants. Pathogenicity of *X. campestris* pv. *vesicatoria* depends on a type III secretion (T3S) system, which translocates effector proteins into plant cells. The T3S system is encoded by a chromosomal 23-kb *hrp* (hypersensitive response and pathogenicity) gene cluster, which is organized in eight transcriptional units and contains 25 genes. Additional accessory genes are located in the flanking region of the *hrp* gene cluster. The analysis of individual genomic deletion mutants as well as biochemical approaches has led to the functional characterization of several components and control proteins of the T3S system. To facilitate genetic manipulations and to allow the rapid introduction of multiple mutations into the T3S gene cluster, we generated a multi-gene construct for the expression of T3S genes using the Golden Gate-based modular cloning (MoClo) system. The use of a set of MoClo vectors with alternating sites for type II restriction enzymes allowed the stepwise assembly of single operons and the insertion of reporter genes into the final expression construct, which encodes components, regulatory and accessory proteins of the T3S system. Here, we used the modular T3S gene cluster to analyse the contribution of single and multiple T3S genes to the localization of a cytosolic ring component of the T3S system, which was analysed as fusion to sfGFP (super folder green fluorescent protein) by fluorescence microscopy.

## A survey of TAL effector diversity reveals extensive gene conversion as a driver of functional specialization

**Alvaro L Perez-Quintero**<sup>1,2</sup>, Boris Szurek<sup>1</sup>

<sup>1</sup> Institut de Recherche pour le Développement, 911 Avenue Agropolis, 34000 Montpellier.

<sup>2</sup> Institut de Biologie de l'École Normale Supérieure, 46 Rue D'Ulm, 75005 Paris.

**Keywords:** *Xanthomonas*, TAL effectors, recombination

TAL effectors (aka TALEs) are repeat containing proteins found in the genus of phytopathogenic bacteria *Xanthomonas*. They are able to bind DNA through their repeat region and induce genes in the host plant. Thus variation in repeat number and order can lead to diversification of virulence functions and evasion of recognition by the host. Variation in TAL effector repeats and repeat sequence between different taxonomic groups has remained largely unexplored. In this study we describe the diversity found in a large set (957) of TAL effector sequences from 19 *Xanthomonas* pathovars and 3 groups of organisms containing TALE-like sequences, and we use this information to infer evolutionary mechanisms for TAL effectors. For this, we analyzed variation at three levels of organization in TAL effectors sequences: 1) the individual repeat level, 2) the TAL effector sequence level and 3) the repeat “motifs” level. The results of these analyses showed loss of repeat sequence diversity through the *Xanthomonas* genus. The “homogenization” of repeat sequences seems to favor high rates of recombination between repeats as evidenced by patterns of repeat substitution and insertion/deletions in TAL effector sequences. To assess the extent of recombination, particularly gene conversion, between TAL effectors, we designed the program RecTAL to identify shared motifs of repeats. This program identified multiple motifs in our dataset, leading us to propose that the swapping of repeat blocks between TAL effectors is a motor for TAL effector specialization that allows for fast functional diversification through the acquisition of new targets in host plants.

## The *Xanthomonas* type-III effector XopS interferes with proteasomal turnover of a WRKY transcription factor to dampen the induction of plant defense responses

**Margot Raffener**<sup>1</sup>, Tiziana Guerra<sup>1</sup>, Suayib Üstün<sup>1\*</sup>, Frederik Börnke<sup>1,2</sup>

<sup>1</sup>Leibniz-Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany

<sup>2</sup>Institute for Biochemistry and Biology, University of Potsdam, Germany

\*present address: Swedish University of Agricultural Sciences, Uppsala, Sweden

**Keywords:** *Xanthomonas campestris*, pepper, type III effectors, ubiquitination

In plants, WRKY transcription factors play important roles as positive and negative regulators of defense gene expression. Many Gram-negative plant pathogenic bacteria rely on so called type-III effector (T3E) proteins as virulence factors to interfere with the induction of defense responses at different levels. We show here that the T3E XopS of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper, interacts inside the plant cell nucleus with a protein pair consisting of the transcription factor WRKY40 and an E3-ubiquitin ligase. The E3 ligase interacts with WRKY40 *in vitro* and *in planta* and ubiquitinates the transcription factor *in vitro*. Accordingly, transient expression in leaves of *Nicotiana benthamiana* suggests that WRKY40 undergoes rapid proteasomal protein turnover. However, WRKY40 protein strongly accumulates upon co-expression with XopS, indicating that XopS interferes with proteasomal turnover of WRKY40 and thus could interfere with defense gene induction that requires degradation of this negative regulator of plant defense. WRKY40 target genes in pepper have not yet been identified; however, inferred from the information available for *Arabidopsis* WRKY40 target genes we have analyzed the expression of *EDS1* and *JAZ8* during infection of pepper with *Xanthomonas* wild type or a *xopS* deletion strain. A significant increase in mRNA level of both genes could be observed in tissue infected with the XopS deletion strain, suggesting that this T3E is required to prevent the induction of WRKY40 target genes during *Xanthomonas* infection of susceptible pepper plants. A possible mechanism by which XopS interferes with proteasomal turnover of WRKY40 is discussed.

## **Knockout lines and a counteracting paralog provide clues to the role of *OsSULTR3;6* in rice susceptibility to bacterial leaf streak**

**Andrew C. Read**<sup>1</sup>, Mathilde Hutin<sup>1</sup>, Joseph J. Belanto<sup>2</sup>, Daniel F. Voytas<sup>2</sup>, Matthew R. Willman<sup>3</sup>, and Adam J. Bogdanove<sup>1</sup>

<sup>1</sup> Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853 USA

<sup>2</sup> Department of Genetics, Cell Biology and Development and Center for Genome Engineering, 321 Church St SE, University of Minnesota, Minneapolis, MN 55455 USA

<sup>3</sup> Plant Transformation Facility, Cornell University, Ithaca, NY 14853 USA

**Keywords:** TAL effector, bacterial leaf streak of rice, *Xanthomonas oryzae*, genome editing, susceptibility

*Xanthomonas oryzae* pv. *oryzicola* (Xoc), which causes bacterial leaf streak of rice, typically encodes more than two dozen type III secreted transcription activator-like effectors (TALEs). Once delivered to the host cell, TALEs bind specific DNA sequences and upregulate expression of nearby genes. All characterized Xoc isolates encode a TALE that activates a predicted sulfate transporter gene, *OsSULTR3;6*, and in studied cases, this activation increases disease susceptibility. We used TALEN and CRISPR/Cas genome editing technologies to disrupt activation of *OsSULTR3;6* by altering the TAL effector binding site as well as by deleting the gene sequence. We measured the impact of edits on disease severity using homozygous, transgene-free plants. Separately, in wild-type plants, we observed that activation of the *OsSULTR3;6* paralog *OsSULTR3;2* with designer TAL effectors results in a robust decrease in virulence. Functional characterization of these related genes with very different impacts on disease progression is underway and will yield important insights into the functional basis for the role of *OsSULTR3;6* in susceptibility and an understanding of its function in other contexts.

## Identification of putative susceptibility genes to citrus canker mediated by PthA4 effector in different varieties of sweet orange

**Reinaldo Rodrigues de Souza-Neto**<sup>1,2</sup>, Celso Eduardo Benedetti<sup>3</sup>, Marco Aurélio Takita<sup>2</sup>, Alessandra Alves de Souza<sup>2</sup>

<sup>1</sup> Centro APTA Citros Sylvio Moreira, Instituto Agronômico de Campinas, Cordeirópolis, Brazil.

<sup>2</sup> Departamento de Genética, Evolução e Bioagentes, Instituto de Biologia, Universidade Estadual de Campinas, Brazil

<sup>3</sup> Laboratório Nacional de Biociências, Centro Nacional de Pesquisa em Energia e Materiais, Campinas Brazil.

eMail: reinaldo@ccsm.br

**Keywords:** *Xanthomonas citri*, TALE, citrus, effectors

The pathogenicity of *Xanthomonas citri* is associated with the secretion of effectors such as PthA, which belong to a class of genes called Transcription Activator-Like Effectors (TALEs). These effectors bind to specific region on promotor of susceptibility genes named Effector Binding Element (EBE). Lateral Organ Boundaries 1 (LOB1) has been described as susceptibility gene regulated by *pthA4*. However, previous studies showed that other genes are also regulated by *pthA4* and the regulation can differ among varieties. Thus, to identify new putative susceptibility genes regulated by PthA4 in three different sweet orange varieties, 17 genes were previously selected by RNA-seq and microarray. EBE region correspondent to PthA4 was searched on promotor region of each gene and nine genes were selected. The gene expression was evaluated 48h after *X. citri* (WT) and *pthA4* mutant ( $\Delta pthA4$ ) inoculation. The results showed up-regulation of 7 genes in all varieties inoculated with WT, what indicates that PthA4 has a conserved role among them. However, the level of expression varied according to the sweet orange variety. Expansin, for instance, showed very high expression in all varieties but in Pineapple the induction was at least twice as higher as the other two varieties. LOB1 on the other hand presented an induction at least seven times higher in Hamlin than in the Pineapple and Valencia. The same is observed for the other genes tested (encoding cyclin, LPT and pectate lyase). The plant response to WT compared to  $\Delta pthA4$  reveals that they are possible susceptibility genes differentially regulated by *pthA4*.

Financial Support: FAPESP.

**Code-cracking TAL effector function and evolution in the rice-*Xanthomonas oryzae* system**

Alvaro L Pérez-Quintero, Mathilde Hutin, Tran Tuan Tu, Alexis Dereeper, Sébastien Cunnac, Lionel Gagnevin, **Boris Szurek**

<sup>1</sup> IRD, CIRAD, Université Montpellier, IPME, Montpellier, France

**Keywords:** TAL effectors, evolution, Africa, *Xanthomonas oryzae* pv. *oryzae*

Transcription Activator-Like (TAL) effectors from *Xanthomonas* plant pathogenic bacteria can bind to the promoter region of plant genes and induce their expression. DNA-binding specificity is governed by a central domain made of nearly identical repeats, each determining the recognition of one base pair via two amino acid residues. Inferring functional and evolutionary relationships between TAL effectors is often challenging due to their repetitive nature. We thus developed the suite QueTAL to offer tailored tools for comparison of TAL effector genes: The program DisTAL can be used to align repetitive regions of TAL effectors and assess events of recombination between repeats. And the program FuncTAL is aimed at finding TAL effectors with similar DNA-binding capabilities. The programs accurately represented phylogenetic and functional relationships between TAL effectors using either simulated or literature-curated data. We used these programs to infer evolutionary patterns of these effectors in African strains from *X. oryzae* pv *oryzae* (*Xoo*), the causing agent of bacterial leaf blight in rice, this revealed different mechanisms of TAL effector evolution and highlighted the importance of recombination for the generation of new TAL effector variants. Finally, we used RNAseq to understand how different variants can contribute to virulence, this revealed some novel features of TAL effector activity that can be used to further improve predictions and functional analyses.

**A conserved motif required for efficient export of XopB from *Xanthomonas campestris* pv. *vesicatoria***

Heike Prochaska<sup>1</sup>, **Sabine Thieme**<sup>1</sup>, Sebastian Daum<sup>2</sup>, Magnus Hallensleben<sup>1</sup>, Kirsten Bacia<sup>2</sup> and Ulla Bonas<sup>1</sup>

<sup>1</sup> Institute for Biology, Dept. of Genetics, Martin-Luther University Halle-Wittenberg, Halle, Germany

<sup>2</sup> Institute for Chemistry, Dept. of Biophysical Chemistry, Martin-Luther University Halle-Wittenberg, Halle, Germany

**Keywords:** type III effector, translocation signal, lipid binding

The type III-secretion (T3S) system is an essential pathogenicity factor of most Gram-negative plant-pathogenic bacteria and injects bacterial effector proteins directly into the plant cell cytosol. T3E export requires an N-terminal secretion signal. The signal is also present in extracellular components of the T3S system, termed non-effectors, which are secreted by the T3S core apparatus but not translocated into the plant cell. How the T3S system discriminates between T3Es and non-effectors is still enigmatic. Previously, we described a conserved translocation motif (TrM) in a number of T3Es from *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*). Recently, we analyzed the putative TrM of the *Xcv* effector XopB in more detail. XopB contributes to *Xcv* virulence, suppresses defense responses triggered by several T3Es and induces cell death in *Nicotiana benthamiana* and *Arabidopsis thaliana*. Amino acid substitutions in XopB revealed that the TrM is required for efficient type III-dependent secretion and translocation. The motif determines dependency of XopB export on the general T3S chaperone HpaB. Furthermore, the TrM mediates specific binding of XopB to one of the major lipid components in *Xanthomonas* membranes, suggesting that association to the bacterial membrane prior to secretion might support type III-dependent transport.

**TALE neighborhood: A genomic approach to picture worldwide TALome diversity of *Xanthomonas phaseoli* pv. *manihotis***

**Carlos Zárate**<sup>1,2,3</sup>; Leidy Rache<sup>2</sup>; Christian Vernière<sup>4</sup>; Lionel Gagnevin<sup>3</sup>; Adriana Bernal<sup>1</sup>; Boris Szurek<sup>3</sup>

<sup>1</sup> Laboratorio de Interacciones Moleculares de Microorganismos Agrícolas (LIMMA), Universidad de los Andes, Bogotá, Colombia

<sup>2</sup> Laboratorio de micología y fitopatología de la Universidad de los Andes (LAMFU), Universidad de los Andes, Bogotá Colombia

<sup>3</sup> IRD, CIRAD, Université Montpellier, IPME, Montpellier, France

<sup>4</sup> Cirad, UMR BGPI, F-34398 Montpellier, France

**Keywords:** cassava bacterial blight, *Xanthomonas phaseoli* pv. *manihotis*, TAL effectors, diversity, evolution

*Xanthomonas phaseoli* pv. *manihotis* (*Xpm*), the causal agent of Cassava Bacterial Blight (CBB), is one of the major threats for cassava production worldwide. As in many *Xanthomonas* spp., pathogenicity of *Xpm* relies on type-III secreted Transcription Activator-Like Effectors (TALEs), a group of proteins that interact with host DNA and induce the transcription of target genes (TGs), including disease-susceptibility genes. So far, only two *Xpm* TALEs, TAL14 and TAL20, have been described as major virulence factors, as a result of the induction of an unknown TG and the sugar transporter encoding gene *MeSWEET10a*, respectively. Moreover, information about diversity, distribution, function and evolution of *Xpm* TALomes is limited. This work aimed at screening, sequencing and comparing *Xpm* TALEs and their genomic context, from a panel of strains representing worldwide diversity of this pathogen. We profiled TALomes using PCR and took advantage of geographical and MLVA data from strains isolated from South-American, African and Southern-Asian cassava fields, leading to the selection of a subset of 24 strains. Genomes of these strains were sequenced using the Single Molecule Real Time (SMRT) technology of Pacific Biosciences; then assembled, annotated, and TALEs and their genomic context were compared and analyzed. A detailed analysis of TAL effectors distribution in a genomic context will be presented, with the aim of shedding light on the genetic mechanisms that shape the evolution of TALomes in *Xpm*.



## Session IV

### Other secretion systems

Poster #47

#### Characterization of the *Xanthomonas citri* subsp. *citri* type VI Secretion System

Ethel Bayer dos Santos<sup>1\*</sup>, Lídia dos Passos Lima<sup>2\*</sup>, Lucas de Moraes Ceseti<sup>2</sup>, Camila Yuri Ratagami<sup>2</sup>, Eliane Silva de Santana<sup>2</sup>, Shaker Chuck Farah<sup>1</sup>, Cristina E. Alvarez-Martinez<sup>2</sup>

<sup>1</sup> Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brasil

<sup>2</sup> Departamento de Genética, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brasil

\*authors contributed equally to this work

**Keywords:** secretion system, extracytoplasmic function sigma factors

Bacterial type VI secretion systems (T6SS) are trans-envelope machines responsible for translocation of effector proteins into target cells or to the extracellular milieu. T6SS play important roles in several aspects of bacterial physiology, including virulence, interbacterial competition and nutrient uptake. In this work, we describe the first functional characterization of the *Xanthomonas citri* subsp. *citri* T6SS. We show that the *X. citri* T6SS is required for increased resistance to predation by the amoeba *Dictyostelium discoideum*. Induction of T6SS gene expression in response to incubation with amoeba requires the extracytoplasmic function sigma factor EcfK. Phenotypic analysis of an *ecfK* mutant strain showed sensitivity to amoeba predation. Interestingly, *ecfK* forms a putative operon with a gene encoding a eukaryotic-like Ser/Thr kinase, which was named *pknS*. To investigate a possible mechanism of EcfK activation by phosphorylation, we introduced phosphomimetic substitutions on conserved Ser/Thr residues of EcfK. Overexpression of the phosphomimetic mutant *ecfK*<sup>T51E</sup> caused induction of the T6SS gene expression in the absence of amoeba, as shown by qRT-PCR analysis. Phenotypic analysis of a *pknS* mutant strain showed that PknS is also required for induction of T6SS gene expression and resistance to amoeba predation. Overexpression of the phosphomimetic mutant *ecfK*<sup>T51E</sup> fully restored the wild-type phenotype of the  $\Delta pknS$  strain, showing that PknS functions through activation of EcfK, leading to induction of T6SS gene expression.

**Analysis of type II and type IV secretion systems from *Xanthomonas campestris* pv. *vesicatoria***

**Sabine Drehkopf**, Ingeborg Schütz, Felix Scheibner and Daniela Büttner

Martin Luther University Halle-Wittenberg, Institute of Biology, Department of Genetics, Halle (Saale), Germany

**Keywords:** Type II secretion, type IV secretion, extracellular enzymes

*Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease on pepper and tomato plants. Essential for pathogenicity of *Xcv* is the type III secretion (T3S) system, which translocates bacterial effector proteins into eukaryotic cells. In addition to the T3S system, the efficient interaction of *Xcv* with its host plants depends on the Xps-type II secretion (T2S) system, which secretes degradative enzymes into the extracellular milieu. Xps-T2S substrates from *Xcv* include proteases, xylanases and a lipase, which contribute to bacterial virulence. Notably, in the absence of functional T2S systems, secretion of T2S substrates from *Xcv* is not completely abolished, suggesting that they are targeted to alternative transport routes. In agreement with this observation, we detected T2S substrates in outer membrane vesicles of *Xcv*.

*Xcv* also possesses two type IV secretion (T4S) systems, which resemble the VirB/D4-T4S system from *Agrobacterium tumefaciens* and the Icm/Dot-T4S system from the animal-pathogenic bacterium *Legionella pneumophila*, respectively. *Xcv* is the only known plant-pathogenic bacterium, which contains an Icm/Dot-like T4S system. To investigate a potential role of both T4S systems in bacterial virulence, we performed infection studies with T4S-deficient *Xcv* mutant strains. Furthermore, we analysed the secretion and possible translocation of candidate T4S substrates. Our results suggest that T4S systems do not play a major role during the interaction of *Xcv* with its host plants.

**Structural insights into a bacterial killing type IV secretion system core complex using cryo-EM**

**Germán G. Sgro**<sup>1,2</sup>, Tiago R. D. Costa<sup>2</sup>, William Cenens<sup>1</sup>, Diorge P. Souza<sup>1</sup>, Alexandre Cassago<sup>3</sup>, Luciana C. Oliveira<sup>1</sup>, Roberto K. Salinas<sup>1</sup>, Rodrigo V. Portugal<sup>3</sup>, Gabriel Waksman<sup>2</sup> and Chuck S. Farah<sup>1</sup>

<sup>1</sup> Instituto de Química, Universidade de São Paulo, São Paulo, 05508-000, Brazil

<sup>2</sup> Institute of Structural and Molecular Biology, Birkbeck College, London, WC1E7HX, United Kingdom

<sup>3</sup> Laboratório Nacional de Nanotecnologia, Centro Nacional de Pesquisas em Energia e Materiais, Campinas, 13083-970, Brazil

**Keywords:** cryo-EM, single-particle reconstruction, type IV secretion system core complex, *Xanthomonas citri*

Type IV Secretion Systems (T4SSs) are multiprotein complexes involved in the transport of DNA and proteins from bacterial and archaeal cells. Recently, our group described a new function for T4SSs from the Xanthomonadaceae family of bacteria, the secretion of antibacterial effectors that target and kill competing bacteria. T4SSs are typically composed of 12 components that form two major assemblies: the inner membrane complex embedded in the inner membrane and the core complex embedded in both the inner and outer membranes. Although progress is steadily being made to decipher the structural and molecular basis of T4SSs function, it has been hampered by the lack of high-resolution structures. We heterogously expressed and purified the intact T4SS core complex from *Xanthomonas citri* (Xac), a member of this family of bacteria. The sample was submitted to cryo-electron microscopy single-particle reconstruction, from which a 3.3-Å-resolution model could be built. The vast network of protein-protein interactions in this 1.13 MDa assembly was functionally probed in an exhaustive mutational investigation of interface residues. This unprecedented structure significantly expands our knowledge of the molecular details of T4SS organization and assembly, allowed the identification of specific interactions that could potentially be used as rational drug design targets and helps us understand how these systems have evolved to inject toxins into target cells.



## Session V

### Ecology and epidemiology

Poster #50

#### Can the microbial ecology toolbox help the fight against *Xylella fastidiosa* in Europe?

**Alexandre Barretto de Menezes**

National University of Ireland Galway, Galway, Ireland

**Keywords:** microbial ecology, microbiome; symbionts; *Xylella fastidiosa* ecology

Predicting and controlling the spread of *Xylella fastidiosa* in Europe demands a thorough understanding of the biotic and abiotic variables that may influence this pathogen's invasion ecology. The symbionts sharing plant host and vector habitats with *X. fastidiosa* are likely to influence infection and disease manifestation, which calls for a detailed characterization of known and potential host and vector microbiomes. Recent improvements in methods for extracting and processing microbial nucleic acids from host environments can enable efficient characterization of the symbiotic bacterial and fungal communities that may affect *X. fastidiosa* colonization of European ecosystems. Such symbiont datasets would be a valuable resource for the prediction and mitigation of *X. fastidiosa* spread in Europe. For example, microbial co-occurrence analysis can reveal microorganisms showing positive or negative associations with *X. fastidiosa*, which could be used as potential indicators of the likelihood of spread (including into new areas and onto novel plant hosts). Microorganisms showing negative associations with *X. fastidiosa* could be further explored as potential biocontrol agents, and microbiome and shotgun metagenomic sequencing may reveal the mechanisms associated with asymptomatic versus symptomatic infections in susceptible hosts. This presentation will highlight the potential advantages, as well as limitations, of the use of the modern molecular ecological toolbox to aid in efforts to understand *X. fastidiosa* ecology in the European continent. The aim is to inspire stakeholders to include the wider plant and vector microbiome in their efforts to control and mitigate *X. fastidiosa* invasion in Europe.

**Improving the typing of *Xylella fastidiosa* directly from plant material**

**Sophie Cesbron**, Quentin Beaupere, Coralie Marais, Martial Briand, Marie-Agnès Jacques

INRA, UMR1345 Institut de Recherche en Horticulture et Semences, SFR4207 QUASAV, F-49071 Beaucouzé, France

**Keywords:** nested-MLST, quarantine disease

Different sequence types of *Xylella fastidiosa* were identified in France based on direct MultiLocus Sequence Typing (MLST). However, direct typing on DNA is partly efficient (from 55 to 85% of the samples). In order to improve the sensitivity of this identification, we developed a direct nested-MLST on CTAB extracted DNA. This method was performed based on a largely used scheme targeting seven loci (*cysG*, *gltT*, *holC*, *leuA*, *malF*, *nuoL*, *petC*). Nested primers were designed from multi-sequence alignments of 38 genomes representing all subspecies. Sequences obtained were long enough to be used for BLAST comparison in PubMLST database. No nonspecific amplification product were observed in these samples. Efficiency of the nested MLST was tested on CTAB extracted DNA from 106 symptomatic samples (37 species) collected in June 2017 in Corsica in comparison with RT-PCR Harper's test. Using our nested-MLST assay : complete profiles were obtained for eight samples, partial profiles were obtained for 66 samples tested undetermined and 18 tested negative with Harper RT-PCR. Only one complete profile was obtained using conventional PCRs for MLST. Using the nested approach, the limit threshold was lowered (minimum DNA concentration for *cysG* detection : 50 fg/μl or 20 copies/μl of genomic DNA) compared to conventional PCR. Using nested-MLST assay on CTAB extracted DNA, plants that were not yet considered hosts tested positive and revealed novel STs in France. The nested-MLST PCR is a sensitive and culture-independent typing method that can be used directly on plant material. This method will be used for epidemiosurveillance purposes.

## Poster #52

**Persistence and dynamics of *Xanthomonas campestris* pv. *campestris* in a natural soil environment after the previous highly infected cabbage production**

**Filip Gazdík**, Jakub Pečenka, Aleš Eichmeier, Miroslav Baránek

Mendeleum, Department of Genetics, Mendel University in Brno, Lednice, Czech Republic

**Keywords:** *Xanthomonas campestris* pv. *campestris*, persistence, dynamics, soil environment, fluctuation

*Xanthomonas campestris* pv. *campestris* (Xcc) is a seed-borne bacterium that causes great economic losses of brassicas worldwide. Beside the infection originating from seeds, it can survive in soil and weed's phyllosphere, where it can serve as a reservoir for new infections. The presented study is focused on the Xcc's persistence and dynamics in a natural soil environment. The experiment was established on a field where a highly infected cabbage production was held in 2016 (Lednice na Moravě, Czech Republic). It consisted of 9 sampling points evenly spread over an area of 4 m<sup>2</sup>. The soil samples were taken from the depth of 5 to 10 cm in two-month intervals from October 2016 to May 2018. Ten samplings had been done in total. The presence of Xcc in the soil samples was tested by standard PCR targeting the *hrpF* gene (Berg et al., 2005) and visualized by standard electrophoresis filled with 1.2 % agarose gel. The obtained results were also confirmed by sensitive nested real-time PCR targeting the *Zur* gene. The results of this research indicate, that the real-time PCR is more suitable and accurate technique for detection of the Xcc in soil, as it can detect low concentrations of the pathogen, which are not detectable by the standard PCR. Regarding the persistence and dynamics of Xcc in the soil, we can state, that it's concentration did not decrease in sampling period, but it showed a certain fluctuating tendencies during the year, depending on the season of the year.

**Studies on the etiology and control of brown apical necrosis (BAN) and bacterial blight of walnut caused by *Xanthomonas arboricola* pv. *juglandis* in Turkey**

**Hatice Özaktan**, Senem Akat

University of Ege Faculty of Agriculture Department of Plant Protection, 35100, Bornova-İzmir/Turkey,  
email: hatice.ozaktan@ege.edu.tr

Turkey is the fourth country in respect to walnut production all around the World after China, U.S.A. and Iran with the production of 200.000 tons per year. There are three major disease problems of walnut orchards in Turkey such as anthracnose caused by *Gnomonia leptostyla*, Walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj), and brown apical necrosis (BAN) on walnut fruits caused by Xaj in association with some fungi. Bacterial blight of walnut, caused by Xaj is present in all main areas of walnut production in the Western part of Turkey. Copper based compounds have been the only means of control for more than 40 years. Data indicates that copper resistant strains of the walnut blight pathogen are not killed by standard copper applications under field conditions. 19 walnut (*J. regia*) cultivars/selected clones in Turkey were investigated for their resistance to the disease by immature nuts and seedling tests. According to the test results on nuts and seedlings of different varieties of walnuts, the commercial cultivars Chandler, Hartley and local cultivar Şebin were recorded as highly susceptible to Xaj, while cv. Franquette was classified as less susceptible, this was followed by cv. Pedro. First observations on BAN in Turkey was realized within the scope of EU FP7 COST Action 873 in 2008. The etiology of BAN was determined by monitoring the changing of the population of causal agents which were responsible for the disease. Symptoms were first visible in the first half of June and since then, the microorganisms associated with BAN has started to cause remarkable yield reduction because of premature walnut fruit drop. The disease severity increasingly continued until the mid of August. In this study, it was indicated that the major causal agent of BAN was Xaj, and fungal agents seemed to be involved in the induction of BAN, causing secondary infections, and growing as saprophytes on bacterial-infected tissues, thus enhancing disease symptoms and severity. At the end of the field observations, it was indicated that Hartley was the most susceptible variety to the disease. The result of this study indicated that Copper oxychloride was the most effective chemical against to the main causal agents of BAN.



***Xanthomonas arboricola* pv. *pruni* the causal agent of bacterial spot disease of stone fruits in the Republic of Srpska****Biljana Radusin Sopić**<sup>1</sup>, Biljana Lolić<sup>1</sup>, Gordana Đurić<sup>1,2</sup><sup>1</sup> University of Banja Luka, Institute of Genetic Resources, Banja Luka, Bosnia and Herzegovina<sup>2</sup> University of Banja Luka, Faculty of Agriculture, Banja Luka, Bosnia and Herzegovina**Keywords:** quarantine bacterium, stone fruits, orchards, nurseries

*Xanthomonas arboricola* pv. *pruni* (Xap) the causal agent of bacterial spot disease of stone fruits and almond is regulated as quarantine pathogen in the European Union and the European and Mediterranean Plant Protection Organization (EPPO, A2 list). This bacterium cause disease of stone fruits and almond worldwide and produce severe yield losses. The aim of this work was to determine presence and distribution of this pathogen on territory of the Republic of Srpska since there is no confirmed information about it. Monitoring was conducted during 2017. in commercial orchards and nurseries. Leaves, twigs and branches of hosts (peach, nectarine, plum, apricot, cherry and almond) were inspected and samples were taken for laboratory analysis. Detection and identification were done according to EPPO diagnostic protocols PM 7/64(1) and PM 7/100 (1), with slight modifications. Freeze dried bacteria CFBP 2535 (producer CIRM, France) were used as reference material. Out of 124 analyzed samples, 2 samples confirmed as positive and both originated from plum orchards. Further studies on *Xanthomonas arboricola* pvs. are planned to be conducted within national project of Ministry of Agriculture, forestry and waters in 2018.

Acknowledgement: This project was supported by Ministry of Agriculture, forestry and waters and Faculty of Agriculture, through Special surveillance program for the presence of quarantine pest organisms in stone fruits on theritory of the Republic of Srpska in 2017, Grant No. 12.03.3-330-2281/17.

**Black rot of brassicas (*Xanthomonas campestris* pv. *campestris*): seed transmission, spread and standards**

**Steven J Roberts**

Plant Health Solutions, 20 Beauchamp Road, Warwick CV34 5NU, UK

**Keywords:** *Xanthomonas*, seed health, testing, epidemiology, modelling

*Xanthomonas campestris* pv. *campestris* is well known as an important seed-borne pathogen of brassicas. Seed health assays should be designed to have a high probability of detecting unacceptable seed lots; there has been much discussion over the years recent of the value of the most sensitive detection assays and the tolerance standards required to achieve satisfactory control. Mathematical models have been developed to describe transmission of the pathogen from seed to seedling, subsequent spread in module-raised brassica transplants, and spread in the field. The transmission model relates the probability of transmission to the mean dose of bacteria per seed and the spread model relates the proportion of plants contaminated to the distance from the primary infector. Using these models, with different initial parameters, the potential for development of disease epidemics can be explored for negative results obtained by seed health assays with different sensitivities (detection limits) and tolerance standards. Examples of different scenarios will be presented, and suggest that the greatest risk arises when negative test results are obtained from seed lots with a relatively high proportion of infested seeds but low number of bacteria per seed.

***Stenotrophomonas maltophilia* - a rising pathogen of local varieties of tomatoes in Bulgaria?****Mariya Stoyanova**<sup>1</sup>, Daniela Ganeva<sup>2</sup>, Nevena Bogatzevska<sup>1</sup><sup>1</sup> Department of Phytopathology, Institute of Soil Science, Agrotechnologies and Plant Protection “Nikola Pushkarov”, Sofia, Bulgaria<sup>2</sup> Department of Breeding, Variety Maintenance and Introduction, Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria**Keywords:** *Stenotrophomonas maltophilia*, seeds, tomato, pathogen

From 2014 an unusual appearance of tomato seeds (*Solanum lycopersicum*) has been observed. The seeds were produced by local genotypes without visible symptoms of disease from the region of Plovdiv in Bulgaria. The seeds exhibited large brown-grey sections on the surface which prolonged in depth. The affected seeds lacked germinating ability or were unable to produce normal seedlings. Bacteria were derived from exudates, seed coat, endosperm and germ. Some of the strains induced hypersensitive reaction in tobacco leaves. Several tests were carried out to investigate pathogenicity - inoculation of leaves, seeds and plants. All isolates were identified as *Stenotrophomonas maltophilia* by Biolog system and sequencing of the 16S rRNA gene region. *Stenotrophomonas* was recovered also from damaged flowers alone or in association with *Xanthomonas* sp.

**A MLVA scheme for the analysis of population genetic diversity and structure of *Xanthomonas vasicola* pv. *musacearum***

Valentine Nakato<sup>1</sup>, Juan Luis Fuentes Rojas<sup>2</sup>, Christian Verniere<sup>3</sup>, Laurence Blondin<sup>3</sup>, **Emmanuel Wicker**<sup>4</sup>

<sup>1</sup> IITA, Kampala, Uganda

<sup>2</sup> Univ. Montpellier-CIRAD, UMR IPME, Montpellier, France

<sup>3</sup> CIRAD, UMR BGPI, Montpellier, France

<sup>4</sup> CIRAD, UMR IPME, Montpellier, France

We describe the development of a new genotyping method on *Xanthomonas vasicola* pv *musacearum* (*Xvm*), the causing agent of Ensete/banana Xanthomonas Wilt, based on Multi Locus variable number of tandem repeats Analysis (MLVA), to understand its population structure and diversity at different time and spatial scales. Mining nine publicly available *Xvm* genomes, the POLLOC-V pipeline (L.-M. Rodriguez-R, R. Koebnik) detected 36 microsatellite loci, among which 21 were selected for primer design. Based on typeability, reproducibility, and polymorphism, 19 markers were retained and multiplexed into 5 mixes. This MLVA-19 scheme was then applied on a 335-strain *Xvm* collection (detailed in Nakato et al, this conference), giving 280 haplotypes. Most loci had a high typeability, with 18 of the 19 loci amplified in more than 97% of strains. Specificity was clear to *Xvm* and *X.vasicola*: seven loci were amplified within the two other pathovars of *X.vasicola*, but gave *Xvm*-specific allele sizes. We checked by a Genotype Accumulation Curve that the number of markers we used was sufficient for further diversity analyses. Using three complementary approaches, we found that MLVA-19 and Wasukira's SNPs (2012) gave congruent results: sublineages I and II were identified by both methods, while MLVA-19 also highlighted a third cluster. MLVA-19 was clearly more discriminative than Wasukira's SNP-derived SNPs (HGDI= 1.00 and 0.75 respectively), and was resolvent enough to discriminate several haplotypes from a single field in Uganda. This MLVA scheme is a tool of choice for molecular epidemiology of the banana- and enset-affecting *Xvm* populations.

## Session VI

### Plant defense and resistance

Poster #58

#### Flexibility in TALEs – an adaption to variable virulence targets and plant resistance

**Sebastian Becker**<sup>1,2,\*</sup>, Annekatrin Richter<sup>2</sup>, Stefanie Mücke<sup>1</sup>, Jana Streubel<sup>1,2</sup> and Jens Boch<sup>1,2</sup>.

<sup>1</sup> Leibniz University Hannover, Institute of Plant Genetics, 30419 Hannover, Germany

<sup>2</sup> Martin-Luther-University Halle-Wittenberg, Institute of Biology, 06120 Halle (Saale), Germany

\* Contact: sebastian.becker@genetik.uni-hannover.de

**Keywords:** transcription activator-like effectors (TALEs), aberrant repeats, *Xanthomonas oryzae*, rice

Plant pathogenic *Xanthomonas* spp. bacteria specifically induce the transcription of target genes by translocating transcription activator-like effectors (TALEs) into host cells. TALEs contain highly conserved 34aa-repeats for the binding of DNA in a simple one repeat to one nucleotide manner. Rare repeat-variants harbor small deletions or insertions, resulting in repeats that differ from the usual repeat consensus. These aberrant repeats enable the TALE to conditionally recognize target sequences with a -1 nucleotide frameshift. Likely, this mechanism is based on the aberrant repeat looping out upon encountering a sequence with a frameshift, whereas the repeats can perfectly align into the repeat array at sequences without a frameshift.

We analyzed naturally occurring aberrant repeat variants in unprecedented detail, showing the impact and limits of multiple aberrant repeats in a single TALE. The experiments demonstrated that it is possible for a TALE not only to tolerate multiple aberrant repeats but to bind sequences with larger deletions by looping out several repeats simultaneously. Furthermore, we will present several new repeat variants - naturally occurring as well as artificially generated - and show that not all aberrant repeats allow a frameshift binding or result in a functional TALE. We also analyzed the first known natural TALE harboring two aberrant repeats and identified its target to be the well known virulence target *OsSWEET13* from rice. As an adaptive mechanism in the molecular arms race between pathogen and host, aberrant repeats can help TALEs to overcome INDEL-based resistance mechanisms.

## Rice bacterial blight resistance at high temperature suppresses the abiotic response

**Stephen Cohen**, Jan Leach

Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

**Keywords:** Plant stress, climate change, *Xanthomonas oryzae*, rice, transcriptome

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), results in significant losses to global rice production. BB is more severe during periods of high temperature, but little is known about the mechanisms underlying this phenomenon. We conducted a transcriptomics experiment to better understand how *Xa7*, a rice BB resistance gene that unusually functions better at high temperature, directs the host response during simultaneous temperature stress and pathogen attack. Intriguingly, the salicylic acid response, an important pathway for rice defense to Xoo, was down-regulated at high temperatures in both susceptible and resistant interactions, suggesting that enhanced *Xa7*-mediated resistance at high temperature functions independently of the well-characterized salicylic acid-mediated defense pathway. Plants exhibiting resistance at high temperature significantly down-regulated the signaling pathway downstream of the plant hormone abscisic acid (ABA), while plants in a susceptible interaction up-regulated this pathway. Because ABA regulates host adaptation to abiotic stresses and increased ABA signaling enhances rice susceptibility to Xoo in normal temperature interactions, these results suggest that plants exhibiting *Xa7*-mediated resistance prioritize response to pathogen over abiotic stress. ABA is likely an important node for cross-talk among abiotic and biotic response pathways in rice. This ongoing study provides insights into how to optimize the development of rice varieties for use in a changing climate.

## Evaluation of the effect of *Xylella fastidiosa* on leaf ionome and calcium-related gene expression profiles of infected olive trees

**Giusy D'Attoma**<sup>1,2</sup>, Leonardo De La Fuente<sup>3</sup>, Pasquale Saldarelli<sup>2</sup>, Raied Abou Kubaa<sup>2</sup>, Massimiliano Morelli<sup>2</sup>, Annalisa Giampetruzzi<sup>1</sup>, Donato Boscia<sup>2</sup>, Vito Nicola Savino<sup>1</sup>, Paul Cobine<sup>4</sup>.

<sup>1</sup> Università degli Studi di Bari, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Bari, Italy.

<sup>2</sup> Consiglio Nazionale delle Ricerche, Istituto per la Protezione Sostenibile delle Piante, Sede Secondaria di Bari, Bari, Italy.

<sup>3</sup> Auburn University, Department of Entomology and Plant Pathology, Auburn, Alabama, United States of America.

<sup>4</sup> Auburn University, Department of Biological Sciences, Auburn, Alabama, United States of America

**Keywords:** *Xylella fastidiosa*, ionome, calcium-dependent protein kinase, signaling, olive

*Xylella fastidiosa* (Xf) is a xylem-limited bacterial plant pathogen that is responsible for Olive Quick Decline Syndrome (OQDS), a devastating disease reported in Salento (Apulia, Italy). Field observations show that olive cv. 'Leccino' show milder symptoms when compared to the highly susceptible cv. 'Ogliarola'. A prior transcriptome analysis of 'Leccino' and 'Ogliarola' cultivars in response to infection by *X. fastidiosa* 'De Donno' strain has revealed that a Calcium-Dependent Protein Kinase (CDPK1) gene is upregulated in Ogliarola infected leaves. Moreover Ca accumulation in leaves have been associated with symptomatic tobacco, blueberry, grapes and pecan plants infected with Xf. Calcium is a critical second messenger and its cellular distribution can trigger diverse physiological processes including stress response and plant defence. Based on these observations we pursued a study of the ionome and CDPK1 gene expression profiles of symptomatic and asymptomatic infected leaves of cv. Leccino and Ogliarola from three orchards. Comparison between the two cultivars reveals changes in ionome and CDPK1 gene expression. 'Leccino' symptomatic leaves had significant ( $p < 0.01$ ) higher Ca concentration as compared to asymptomatic leaves, while differences for the susceptible cv. "Ogliarola" were non-significant ( $p > 0.2$ ). In addition sodium levels were higher in symptomatic leaves of both varieties. qRT-PCR confirmed that CDPK1 expression in 'Ogliarola' is significantly increased relatively to 'Leccino' trees grown in the same field and the increase is higher in symptomatic leaves. The impact of these changes on disease progression will be discussed.

The present work was done in the framework of an STM granted by the EU COST Action CA16107 and with the financial and scientific support of the EU H2020 research projects POnTE (GA 635646).

**OsSWEET13 and 14, key susceptibility factors for rice blight, are not essential for phloem loading****Joon-Seob Eom**<sup>1</sup>, Jungil Yang<sup>1</sup>, Bing Yang<sup>2</sup> and Wolf B. Frommer<sup>1,3</sup><sup>1</sup> Institute for Molecular Physiology, Heinrich Heine Universität Düsseldorf and Max Planck Institute for Plant Breeding Research, Köln, Germany<sup>2</sup> Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011, USA<sup>3</sup> Institute of Transformative Bio-Molecules (ITbM), Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8602, Japan**Keywords:** OsSWEETs, sucrose transporter, Xoo, Tal effector, phloem loading

*Xanthomonas oryzae* pv. *oryzae* (Xoo) secret type III TAL (transcriptional activator-like) effectors to induce host gene expression. Xa25/OsSWEET13 and Os11N3/OsSWEET14 are one of the target gene of various tal effector. PthXo2 target promoter region of OsSWEET13 and AvrXa7, Pthxo3, Tal5 and Talc target promoter region of OsSWEET14. Despite the induction of OsSWEET13 and OsSWEET14 is key factor for bacterial virulence, little is known for their physiological function in rice development. In Arabidopsis and Maize, a close homolog genes, AtSWEET11,12 and ZmSWEET13a,b,c play an important role for sucrose phloem loading pathway. It was suggested that SWEETs mediated sucrose export from the vascular parenchyma cell to the phloem apoplasm is the key step for phloem sucrose loading into the sieve element/companion cell(SECC) by sucrose/proton symporter, AtSUC2 and ZmSUT1. To test potential role of OsSWEET13 and 14 for phloem loading pathway, here we generate reporter fusion lines and CrispR/Cas9 mediated mutants for OsSWEET13 and 14. OsSWEET13 and 14 both localized phloem tissues throughout the leaf blade and sheath tissues in translational GUS reporter fusion lines. OsSWEET13 and 14 shows sucrose transporting activity in heterologous HEK293 cell system. But, unlike Arabidopsis or maize, both single and double mutants of OsSWEET13 and 14 does not show remarkably phenotype in both greenhouse and field condition. It is conceivable that rice may not utilize the apoplasmic phloem loading pathway in normal conditions.



**Comparative transcriptomics profiling of resistant and susceptible common bean genotypes in response to *Xanthomonas phaseoli* pv. *phaseoli***

**Justine Foucher**<sup>1</sup>, Mylène Ruh<sup>1</sup>, Anne Prevaux<sup>1</sup>, Sébastien Carrere<sup>2</sup>, Martial Briand<sup>1</sup>, Marie-Agnès Jacques<sup>1</sup>, Nicolas W.G. Chen<sup>1</sup>.

<sup>1</sup> IRHS, INRA, AGROCAMPUS OUEST, Université d'Angers, SFR4207 QUASAV, 42, rue Georges Morel, 49071 Beaucouzé, France.

<sup>2</sup> CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR 2594, Castanet-Tolosan, France

**Keywords:** Common bean, transcriptomics, resistance

Common bean (*Phaseolus vulgaris*) is a major legume crop consumed worldwide. Two main gene pools have been identified for cultivated common bean in South America (Andean gene pool) and in Mexico and Central America (Mesoamerican gene pool). Common bacterial blight affects common bean crops everywhere where beans are cultivated and causes up to 40% yield loss in the most severe cases. *Xanthomonas phaseoli* pv. *phaseoli* is one of the agents responsible for this disease. In order to investigate its impact on common beans originating from the two major gene pools, we inoculated plants from genotypes BAT93 (Mesoamerican) and JaloEEP558 (Andean) with *X. phaseoli* pv. *phaseoli* strain CFBP6546R. Pathogenicity tests revealed that BAT93 was resistant to *X. phaseoli* pv. *phaseoli* while JaloEEP558 was susceptible. To characterize the genes differentially expressed during the interaction with *X. phaseoli* pv. *phaseoli*, we performed RNAseq experiments 48h after inoculation with strain CFBP6546R on these two common bean genotypes. First, to describe the general expression pattern of common bean during the interaction, we analyzed the core transcriptome of these two genotypes. Then, we searched for genes specifically induced or repressed in resistant and susceptible background. Our study provides the first description of common bean transcriptomes after inoculation with *Xanthomonas*, and brings preliminary information potentially important for further management of common bacterial blight of bean.

**CRK7 is required for rice resistance against *Xanthomonas oryzae* pv. *oryzae* infection**

Chunlian Wang, Zhiyuan Ji, Mingwei Zhang, Rezwan Tariq, Feifei Xu, Yongchao Tang, Kaili Zheng, Kaijun Zhao

National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China

**Keywords:** OsCRK7, bacterial blight, resistance, rice, *Xoo*

R5024, a near-isogenic line derived by cross and backcross between Y238 (an accession of *Oryzae. rufipogon* Griff) and the cultivated rice variety IR24, shows a broad spectrum of resistance against bacterial blight. RNA-sequencing reveals that expression of the *OsCRK7* gene in R5024 was highly induced upon *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) infection. The lesion length of *OsCRK7*-overexpression plant leaves inoculated with PXO99<sup>A</sup> was much shorter compared with the wild type, while the knockout plants showed increased susceptibility to *Xoo*. To reveal the mechanism of *OsCRK7*-mediated disease resistance, we have constructed a cDNA library from R5024 challenged with PXO99<sup>A</sup>, and identified 3 candidates of *OsCRK7*-interacting proteins by yeast two-hybrid screening.

**Dual RNA-seq of tomato leaves after *Xanthomonas* infection****Jan Niklas Kemna**<sup>1</sup>, Jan Grau<sup>2</sup>, Cornelius Schmidtke<sup>1</sup> and Ulla Bonas<sup>1</sup><sup>1</sup> Institute for Biology, Dept. of Genetics, Martin Luther University Halle-Wittenberg, Halle, Germany<sup>2</sup> Institute of Computer Science, Martin Luther University Halle-Wittenberg, Halle, Germany

Successful infection of pepper and tomato by *Xanthomonas campestris* pv. *vesicatoria* (Xcv) depends on a type III secretion system (T3SS) and the translocation of type III effector proteins into plant cells. Effector proteins interfere with host cellular pathways and ultimately enable bacterial multiplication in the plant tissue. We performed a dual-RNA-sequencing approach after tomato (cultivar Heinz) infection with Xcv to elucidate potential roles of tomato microRNAs (miRNAs) during the infection process. For this, we isolated total leaf RNA at four different time points after infection with two different Xcv strains. Sequencing reads from mRNA and miRNA libraries were mapped to both the Xcv and the tomato genome and statistically analyzed with a focus on differential accumulation of mRNAs and known and novel tomato miRNAs. Furthermore, we combined the mRNA- and miRNA-Seq data to identify potential miRNA targets. Overall, we identified five differentially expressed miRNAs and predicted a novel miRNA-mRNA interaction.

**Functional analysis of TAL15, a novel avirulence gene candidate in the *Xanthomonas phaseoli* pv. *manihotis* - Cassava pathosystem****Edilene Ramírez**<sup>1,2</sup>, Emilie Thomas<sup>2</sup>, Boris Szurek<sup>2</sup>, Camilo Ernesto López Carrascal<sup>1</sup><sup>1</sup> Universidad Nacional de Colombia, FPM, Bogotá, Colombia<sup>2</sup> IRD, CIRAD, Université Montpellier, IPME, Montpellier, France**Keywords:** cassava bacterial blight, *Xanthomonas phaseoli* pv. *manihotis*, TAL effectors, avirulence

*Xanthomonas phaseoli* pv. *manihotis* (*Xpm*) is the causal agent of cassava bacterial blight (CBB) which is heavily threatening cassava production in the tropical belt. As many other *Xanthomonas* species, *Xpm* relies on transcription activator-like (TAL) effectors for virulence. TAL effectors are type-III secreted proteins acting as bona fide plant transcription factors. Once translocated into host plant cells, TAL effectors bind to the promoter of specific host genes and induce their expression to the benefit of the pathogen. To counter-act this virulence mechanism, plants have co-opted TAL effector transcriptional activation strategy by embedding TAL effector binding elements (EBEs) in the promoter of so-called executor *E* genes which expression leads to host resistance. In this study, we report on the identification and functional characterization of TAL15, a novel *Xpm* TAL effector with avirulence activity. We will also present our attempt to identify the *E* gene candidates regulated by TAL15 using an RNAseq-based transcriptomic approach.

***Arabidopsis thaliana* BSK7 and BSK8 are involved in PTI signaling****Guy Sobol**, Bharat Bhusan Majhi, Guido Sessa

School of Plant Sciences and Food Security, Tel-Aviv University, 69978 Tel-Aviv, Israel

**Keywords:** Brassinosteroid signaling kinase, pattern-triggered immunity, signal transduction, receptor-like cytoplasmic kinase, *Arabidopsis thaliana*

Receptor-like cytoplasmic kinases (RLCKs) have been shown to participate in PTI signaling and to interact with plant pathogen recognition receptors (PRRs). Members of the *Arabidopsis thaliana* brassinosteroid signaling kinase (BSK) family of RLCKs were previously implicated in brassinosteroid signaling and immunity. Here, we show that BSK7 and BSK8 interacted with the PRR FLS2 in yeast and *in planta*. Accordingly, *bsk7* and *bsk8* mutant plants displayed altered PTI responses, including flg22-induced production of reactive oxygen species, callose deposition at the cell wall, and expression of the defense-related gene *PR1*. Conversely, in both mutants, flg22-induced MAPK activation and expression of the *FRK1* and *WRKY29* genes was similar to wild-type plants. Both mutants displayed enhanced susceptibility to *Botrytis cinerea*, whereas *bsk7* plants, but not *bsk8*, displayed enhanced susceptibility to *Pseudomonas syringae* pv. *tomato* DC3000. In parallel research, we started to examine whether type III effectors of the *Xanthomonas* bacterial pathogen may target the BSK8 homolog (BSK830) of its host plant *Solanum lycopersicum*. Among 35 *Xanthomonas* effectors tested, seven interacted with BSK830 in a split luciferase complementation assay performed in *Nicotiana benthamiana* plants. Together, these results suggest that BSK7 and BSK8 (or BSK830) are components of PTI signaling that may be targeted by bacterial effectors.

**Towards the map-based isolation of the pepper *R* gene *Bs7* mediating recognition of the *Xanthomonas* effector protein AvrBs7**

**Annett Strauß**<sup>1</sup>, Adnane Boualem<sup>2</sup>, Angela Dressel<sup>1</sup>, Jerry Minsavage<sup>3</sup>, Abdelhafid Bendahmane<sup>2</sup>, Jeff Jones<sup>3</sup>, Thomas Lahaye<sup>1</sup>

<sup>1</sup> Zentrum für Molekularbiologie der Pflanzen (ZMBP), Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 32, Tübingen D-72076, Germany

<sup>2</sup> INRA-CNRS, UMR1165, Unite de Recherche en Genomique Vegetale, Evry, France

<sup>3</sup> Department of Plant Pathology, University of Florida, Gainesville, FL, USA

**Keywords:** XopH, HopAO1, Bs7, AvrBs7

Plant resistance (*R*) genes mediate recognition of microbial effectors. Molecular isolation of plant *R* genes is a key step in order to elucidate the mechanistic basis of how the given *R* protein mediates detection of the matching microbial effector. Often plant *R* proteins recognize not the effector protein itself but the changes that the microbial effector induces on a given host target protein. Thus, identification of plant *R* proteins provides not only insights into recognition but also the virulence function of a given microbial effector. In this context we aim to isolate the pepper *R* gene *Bs7* that mediates recognition of the *Xanthomonas gardneri* effector protein AvrBs7 as well as the corresponding *X. euvesicatoria* effector XopH (Potnis et al., 2012). Recent studies revealed that XopH dephosphorylates phytate (inositol hexakisphosphate [IP6]) exclusively at its C1 carbon position resulting in IP5 (Blüher et al., 2017). Accordingly, XopH is 1-phytase. Based on the biochemical function of XopH it seems conceivable that Bs7 perceives either the reduction in IP6 or the increase in IP5 levels. We have genetically mapped pepper *Bs7* and aim to positionally clone *Bs7*. Progress towards the map-based isolation of *Bs7* will be presented.

## Using natural elicitors for integrated plant disease management

**Abdel Rahman Al-Tawaha**

Department of Biological sciences, Al-Hussein bin Talal University, Maan, Jordan

Field experiments were conducted to determine if isoflavone concentration of mature soybean seeds could be increased using elicitor compounds. The effects on soybean seed isoflavone concentrations following foliar applications actinomycetes spores (*Streptomyces melanosporofaciens* strain EF-76) at different concentrations and growth stages were evaluated. Combined chitosan seed treatment and foliar applications were also evaluated. Concentrations of daidzein, genistein, glycitein, and total isoflavones were determined by HPLC. Foliar applications actinomycetes caused a marked increase in individual and total isoflavone concentration. Results indicate that elicitors hold promise as a way of increasing isoflavone concentration of mature soybean seeds.

**Comparative transcriptomic analysis of disease resistant and susceptible banana genotypes challenged with *Xanthomonas campestris* pv. *musacearum***

**Jaindra N. Tripathi**<sup>1</sup>, Trushar Shah<sup>1</sup>, Kariuki S. Muiruri<sup>1</sup>, Manpreet Katari<sup>2</sup>, Leena Tripathi<sup>1</sup>

<sup>1</sup> International Institute of Tropical Agriculture (IITA), P.O. Box 30709-00100, Nairobi, Kenya

<sup>2</sup> Department of Biology, New York University, New York, United States

**Keywords:** Banana *Xanthomonas* wilt, *Xanthomonas campestris* pathovar *musacearum*. transcriptome analysis, *Musa balbisiana*, Pisang Awak

Banana *Xanthomonas* Wilt (BXW), caused by bacterial pathogen *Xanthomonas campestris* pathovar *musacearum* (Xcm), is one of the most damaging disease of banana in East and Central African countries. The disease can be managed in the field by cultural practices like using clean planting materials, disinfecting farming tools and removal of infected plants from fields or replacing with alternative crops. There is no disease resistance available in any of the cultivated variety of banana except for wild-type diploid '*Musa balbisiana*' (genome BB). To understand the defence mechanism of resistance and susceptibility of banana genotypes, the transcriptome analysis of disease resistant wild type banana '*Musa balbisiana*' and highly susceptible banana cultivar 'Pisang Awak' challenged with Xcm was performed through RNA sequencing using Illumina HiSeqTM 2500. The number of differentially expressed genes (DEGs) were higher in '*Musa balbisiana*' in comparison to 'Pisang Awak' at both 12 h and 48 h post inoculation. The DEGs were further mapped to the biotic stress pathways. Several genes related to biotic stresses including transcription factors, hormone responses, plant signaling, disease resistance, oxidoreductase activity and cell wall enforcement were differentially expressed in both genotypes. This study offers the transcriptomic profile of the resistant and susceptible banana genotype during early infection with Xcm.



**OsSWEET11, a host disease-susceptibility gene for bacterial blight, is a key player in seed filling in rice****Jungil Yang**<sup>1,2</sup>, Dangping Luo<sup>4</sup>, Bing Yang<sup>4</sup>, Wolf B. Frommer<sup>1,2,3</sup>, and Joon-Seob Eom<sup>1,2</sup><sup>1</sup> Institute for Molecular Physiology, Heinrich Heine Universität Düsseldorf and Max Planck Institute for Plant Breeding Research, Köln, Germany<sup>2</sup> Department of Plant Biology, Carnegie Science, 260 Panama St., Stanford, CA 94305<sup>3</sup> Institute of Transformative Bio-Molecules (ITbM), Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8602, Japan<sup>4</sup> Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011, USA

Rice is a major staple food for over half of the world's population. Thus, its productivity must be improved to meet this growing demand. Bacterial blight, *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a major disease of rice in most of the rice growing countries and severely diminishes the quality and yield. Rice SWEET gene family play roles in the interaction of rice and *Xanthomonas oryzae* pv. *oryzae* (Xoo), creating susceptibility (Chu *et al.*, 2006, Antony *et al.*, 2010, Chen *et al.*, 2010). OsSWEET11 is transcriptionally activated by PthXo1 (transcription factor-like effectors) injected into the host cells after Xoo infection. It has been suggested that these proteins move sugar from cells to provide carbon and energy to Xoo. However it remains to be determined what the biochemical functions of SWEET proteins in physiological process or whether sucrose transport activity is necessary for susceptibility. OsSWEET11 and 15 showed all hallmarks of being responsible for seed filling with sucrose efflux function at the nucellar projection and transfer across the nucellar epidermis/aleurone interface, delineating two major steps for apoplastic seed filling. It will be important to ensure that genome editing of the promoter of OsSWEET11 with the purpose of engineering resistance does not impact proper OsSWEET11 expression in seeds, to ensure that resistant lines do not carry a yield penalty.



# List of Participants

<b>ALKHATEEB</b>	Rabeaa	Germany	alkhateb@cebitec.uni-bielefeld.de
<b>ALVAREZ-MARTINEZ</b>	Cristina	Brazil	ceamarti@unicamp.br
<b>ARLAT</b>	Matthieu	France	matthieu.arlat@inra.fr
<b>ASSIS</b>	Renata de Almeida Barbosa	USA	redab@ucdavis.edu
<b>BAHAR</b>	Ofir	Israel	ofirb@agri.gov.il
<b>BANSAL</b>	Kanika	India	kanikabansal@imtech.res.in
<b>BECKER</b>	Sebastian	Germany	sebastian.becker@genetik.uni-hannover.de
<b>BERAN</b>	Pavel	Czech Republic	pavel.beran@centrum.cz
<b>BOCH</b>	Jens	Germany	jens.boch@genetik.uni-hannover.de
<b>BOGDANOVE</b>	Adam	USA	ajb7@cornell.edu
<b>BONAS</b>	Ulla	Germany	ulla.bonas@genetik.uni-halle.de
<b>BÖRNKE</b>	Frederick	Germany	boernke@igzev.de
<b>BOULANGER</b>	Alice	France	alice.boulanger@inra.fr
<b>BRAGARD</b>	Claude	Belgium	claud.bragard@uclouvain.be
<b>BURDMAN</b>	Saul	Israel	saul.burdman@mail.huji.ac.il
<b>BUROKIENE</b>	Daiva	Lithuania	daiva.burokiene@botanika.lt
<b>BUTCHACAS</b>	Jules	USA	julesbutchacas@gmail.com
<b>BÜTTNER</b>	Daniela	Germany	daniela.buettner@genetik.uni-halle.de
<b>CATARA</b>	Vittoria	Italy	vcatara@unict.it
<b>CESBRON</b>	Sophie	France	sophie.cesbron@inra.fr
<b>CHATTERJEE</b>	Subhadeep	India	subhadeep@cdfd.org.in
<b>CHEN</b>	Nicolas	France	nicolas.chen@agrocampus-ouest.fr
<b>CHEN</b>	Gongyou	China	gyouchen@sjtu.edu.cn
<b>COHEN</b>	Stephen	USA	stephen.cohen@colostate.edu
<b>COSTA</b>	Joana	Portugal	jcdcosta@ipn.pt
<b>COTTYN</b>	Bart	Belgium	bart.cottyn@ilvo.vlaanderen.be
<b>CUBERO</b>	Jaime	Spain	cubero@inia.es
<b>D'ATTOMA</b>	Giusy	Italy	giusy.dattoma@ipsp.cnr.it
<b>DE MENEZES</b>	Alexandre Barretto	Ireland	alexandre.demenezes@nuigalway.ie
<b>DEB</b>	Sohini	India	sohinideb@ccmb.res.in
<b>DERMIĆ</b>	Edyta	Croatia	edermic@agr.hr
<b>DREHKOPF</b>	Sabine	Germany	sabine.haensch@online.de
<b>DUBROW</b>	Zoe	USA	zed6@cornell.edu
<b>DUPAS</b>	Enora	France	enora.dupas@inra.fr
<b>EICHMEIER</b>	Ales	Czech Republic	ales.eichmeier@mendelu.cz
<b>EOM</b>	Joon-Seob	Germany	eom@mpipz.mpg.de
<b>ERICKSON</b>	Jessica	Germany	jessica.erickson2525@gmail.com
<b>FARAH</b>	Chuck	Brazil	chsfarah@iq.usp.br
<b>FERNANDES</b>	Camila	Portugal	camilafernandes@msn.com

<b>FISCHER-LE SAUX</b>	Marion	France	marion.le-saux@inra.fr
<b>FOUCHER</b>	Justine	France	justine.foucher@inra.fr
<b>FROMMER</b>	Wolf B	Germany	frommew@hhu.de
<b>GADEA</b>	Jose	Spain	lgadeav@ibmcp.upv.es
<b>GAGNEVIN</b>	Lionel	France	lionel.gagnevin@cirad.fr
<b>GASIC</b>	Katarina	Serbia	gasickatarina@yahoo.com
<b>GAZDÍK</b>	Filip	Czech Republic	ldfilipg@gmail.com
<b>GRAU</b>	Jan	Germany	grau@informatik.uni-halle.de
<b>HAINAUX</b>	Thomas	Belgium	thomas.hainaux@uclouvain.be
<b>HE</b>	Chenyang	China	hechenyang@caas.cn
<b>HUGUET-TAPIA</b>	Jose	USA	jhuguet@ufl.edu
<b>HULIN</b>	Michele	UK	michellehulin@hotmail.com
<b>HUTIN</b>	Mathilde	USA	mathildehutin1@gmail.com
<b>JACOBS</b>	Jonathan	USA	jmjacobs2@gmail.com
<b>JACQUES</b>	Marie-Agnès	France	Marie-Agnes.Jacques@inra.fr
<b>JI</b>	Zhiyuan	China	jizhiyuan@caas.cn
<b>JOE</b>	Anna	USA	ajoe@ucdavis.edu
<b>JONES</b>	Jeffrey	USA	jbjones@ufl.edu
<b>KALUŻNA</b>	Monika	Poland	monika.kaluzna@inhort.pl
<b>KEMNA</b>	Jan Niklas	Germany	jan.kemna@genetik.uni-halle.de
<b>KOEBNIK</b>	Ralf	France	ralf.koebnik@ird.fr
<b>KÖLLIKER</b>	Roland	Switzerland	roland.koelliker@usys.ethz.ch
<b>KOVACS</b>	Tamas	Hungary	kovacst@enviroinvest.hu
<b>LAHAYE</b>	Thomas	Germany	thomas.lahaye@zmbp.uni-tuebingen.de
<b>LEACH</b>	Jan	USA	jan.leach@colostate.edu
<b>LINDOW</b>	Steven	USA	icelab@berkeley.edu
<b>LUNEAU</b>	Julien	France	julien.luneau2@inra.fr
<b>MARTINS</b>	Paula	Brazil	pmmm@outlook.com.br
<b>MARTINS</b>	Leonor	Portugal	leonor.martins@fc.up.pt
<b>MENDES BONFIM</b>	Isabela	Brazil	isabela.bonfim@ctbe.cnpem.br
<b>MORBITZER</b>	Robert	Germany	robert.morbitzer@zmbp.uni-tuebingen.de
<b>MORELLI</b>	Massimiliano	Italy	massimiliano.morelli@ipsp.cnr.it
<b>MÜCKE</b>	Stefanie	Germany	muecke@genetik.uni-hannover.de
<b>NAGEL</b>	Oliver	Germany	oliver.nagel@genetik.uni-halle.de
<b>NAGY</b>	Ildikó	Hungary	dr.nagy.ildiko.katalin@enviroinvest.hu
<b>NAKATO</b>	Valentine	Uganda	V.Nakato@cgiar.org
<b>NEWBERRY</b>	Eric	USA	ean0018@auburn.edu
<b>NEWMAN</b>	Marie-Anne	Denmark	mari@plen.ku.dk
<b>NOEL</b>	Laurent	France	laurent.noel@inra.fr
<b>NOVOTNY</b>	Cenek	Czech Republic	novotny@biomed.cas.cz
<b>OLIVA</b>	Ricardo	Philippines	r.oliva@irri.org
<b>OTTEN</b>	Christian	Germany	ch.otten@gmx.de
<b>ÖZAKTAN</b>	Hatice	Turkey	hatice.ozaktan@ege.edu.tr
<b>PEČENKA</b>	Jakub	Czech Republic	jakubpecenka@gmail.com

<b>PEŇÁZOVÁ</b>	Eliška	Czech Republic	penazova.e@gmail.com
<b>PEREZ QUINTERO</b>	Alvaro L.	France	alperezqui@gmail.com
<b>PICCHI</b>	Simone	Brazil	scpicchi@gmail.com
<b>POTHIER</b>	Joël F.	Switzerland	joel.pothier@zhaw.ch
<b>POTNIS</b>	Neha	USA	nzp0024@auburn.edu
<b>PUŁAWSKA</b>	Joanna	Poland	joanna.pulawska@inhort.pl
<b>RADUSIN SOPIC</b>	Biljana	Bosnia Herzegovia	biljana.radusin.sopic@igr.unibl.org
<b>RAFFEINER</b>	Margot	Germany	raffeiner@igzev.de
<b>RAMIREZ VARGAS</b>	Edilene	Colombia	eramirezv@unal.edu.co
<b>READ</b>	Andrew	USA	acr242@cornell.edu
<b>ROBENE</b>	Isabelle	France	isabelle.robene@cirad.fr
<b>ROBERTS</b>	Steven	UK	s.roberts@planthealth.co.uk
<b>RODRIGUES DE SOUZA NETO</b>	Reinaldo	Brazil	reinaldo@ccsm.br
<b>SALOMSKIENE</b>	Joana	Lithuania	joana.salomskiene@ktu.lt
<b>SCALA</b>	Valeria	Italy	valeria.scala@crea.gov.it
<b>SCHMIDT</b>	Sarah	Germany	sarah.schmidt.2@hhu.de
<b>SCHMIDTKE</b>	Cornelius	Germany	cornelius.schmidtke@genetik.uni-halle.de
<b>SESSA</b>	Guido	Israel	guidos@tauex.tau.ac.il
<b>SGRO</b>	Germán	Brazil	ggsgro@iq.usp.br
<b>SICARD</b>	Anne	USA	anne.sicard@berkeley.edu
<b>SOBOL</b>	Guy	Israel	guysobol@mail.tau.ac.il
<b>SONTI</b>	Ramesh	India	sonti@ccmb.res.in
<b>STEFANI</b>	Emilio	Italy	emilio.stefani@unimore.it
<b>STOYANOVA</b>	Mariya	Bulgaria	mimka@gbg.bg
<b>STRAUSS</b>	Annett	Germany	annett.strauss@zmbp.uni-tuebingen.de
<b>STREUBEL</b>	Jana	Germany	jana.streubel@genetik.uni-hannover.de
<b>SZUREK</b>	Boris	France	boris.szurek@ird.fr
<b>TAVARES</b>	Fernando	Portugal	ftavares@cibio.up.pt
<b>AL-TAWAHA</b>	Abdel Rahman	Jordan	abdeltawaha74@gmail.com
<b>THIEME</b>	Sabine	Germany	sabine.thieme@genetik.uni-halle.de
<b>TRIPATHI</b>	Leena	Kenya	L.Tripathi@cgiar.org
<b>TRIPATHI</b>	Jaindra	Kenya	j.tripathi@cgiar.org
<b>TSUGE</b>	Seiji	Japan	s_tsuge@kpu.ac.jp
<b>ÜSTÜN</b>	Suayib	Sweden	suayib.ustun@slu.se
<b>VAN DEN HOORN</b>	Renier	UK	renier.vanderhoorn@plants.ox.ac.uk
<b>VANCHEVA</b>	Taca	UK	tacavancheva@gmail.com
<b>VICENTE</b>	Joana	UK	joana.vicente@warwick.ac.uk
<b>WANG</b>	Nian	USA	nianwang@ufl.edu
<b>WHITE</b>	Frank	USA	ffwhite@ufl.edu
<b>WICKER</b>	Emmanuel	France	emmanuel.wicker@cirad.fr
<b>YANG</b>	Bing	USA	byang@iastate.edu
<b>YANG</b>	Jungil	Germany	jyang@mpipz.mpg.de

<b>ZÁRATE</b>	Carlos	France	ca.zarate10@uniandes.edu.co
<b>ZHANG</b>	Yanan	USA	yananzh@ufl.edu
<b>ZHAO</b>	Kaijun	China	zhaokaijun@caas.cn
<b>ZOU</b>	Lifang	China	zoulifang202018@sjtu.edu.cn

This brochure is based upon work from COST Action CA16107 EuroXanth, supported by COST (European Cooperation in Science and Technology).

COST (European Cooperation in Science and Technology) is a pan-European intergovernmental framework. Its mission is to enable break-through scientific and technological developments leading to new concepts and products and thereby contribute to strengthening Europe's research and innovation capacities. [www.cost.eu](http://www.cost.eu)



Funded by the Horizon 2020 Framework Programme  
of the European Union