Organizing committees

LOCAL COMMITTEE

Charles Manceau
Marie-Agnès Jacques
Tristan Boureau
Marion Le Saux
Perrine Portier
Armelle Darrasse
Sophie Cesbron
Sylvie Bourel

SCIENTIFIC COMMITTEE

Charles Manceau (INRA - IRHS, Angers, FRANCE)
Marie-Agnès Jacques (INRA - IRHS, Angers, FRANCE)
Matthieu Arlat (INRA-CNRS ; LIPM, Toulouse, FRANCE)
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Laurent Noël (INRA-CNRS ; LIPM, Toulouse, FRANCE)
Lionel Gagnevin (CIRAD; 3P, La Réunion, FRANCE)
Olivier Pruvost (CIRAD; 3P, La Réunion, FRANCE)
Philippe Rott (CIRAD; 3P, La Réunion, FRANCE)
Monique Royer (CIRAD; 3P, La Réunion, FRANCE)
Ralf Koebnik (IRD; GDP, Montpellier, FRANCE)
Valérie Verdier (IRD; GDP, Montpellier, FRANCE)
# Scientific Program

## Monday, July 9

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>17:00</td>
<td>Registration of participants</td>
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<tr>
<td>19:00</td>
<td>Welcome to Bon-Pasteur, Angers and introduction</td>
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<tr>
<td>19:15</td>
<td>Welcome conference</td>
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<td>Pamela Ronald: (invited speaker): The Rice XA21 Receptor Recognizes a Conserved Bacterial Signaling Molecule.</td>
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<td>20:00</td>
<td>Welcome Buffet</td>
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## Tuesday, July 10

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<tr>
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<tr>
<td>07:00</td>
<td>Breakfast</td>
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<tr>
<td></td>
<td><strong>Session I – Genome sequencing, global genomic diversity of <em>Xanthomonas spp.</em>, and molecular diagnostic tools</strong></td>
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<td><strong>Chairman: Lionel Gagnevin</strong></td>
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<td>Jan Leach: (invited speaker): The value of draft genomic sequence of rice-associated bacteria for understanding biology, diversity, and diagnosis.</td>
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<td>Joachim Vandroemme: Draft genome sequencing of <em>Xanthomonas fragariae</em> reveals reduced genome size and distinct virulence related gene content.</td>
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<td>Andreas Bühlmann: Genomics Informed Design of LAMP assays for subspecies-level phytosanitary detection of the quarantine pathogen <em>Xanthomonas arboricola pv. pruni.</em></td>
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<td>09:50</td>
<td>Prabhu B. Patil: Phylogenomic marker(s) based evolutionary studies of <em>Xanthomonas</em></td>
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<td>10:10</td>
<td>Coffee break</td>
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<tr>
<td>10:40</td>
<td>Marie-Agnès Jacques: Genome sequencing of <em>Xanthomonas axonopodis pv. phaseoli</em> CFBP4834-R reveals that flagellar motility is not a general feature of xanthomonads.</td>
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<td>Laurent Noël: How many genomes would we need to describe a <em>Xanthomonas</em> pathovar?</td>
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<td>Ralf Koebnik: Genomics-based molecular tools for studies of population structures and epidemiological surveillance of xanthomonads.</td>
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<td>12:00</td>
<td>Lunch break</td>
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<tr>
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<td><strong>Session II – Comparative and functional <em>Xanthomonas</em> genomics</strong></td>
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<td>Jeff B. Jones: (invited speaker): Comparative genomics helps explain evolution of a unique <em>Xanthomonas perforans</em> strain on pepper</td>
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<td>14:10</td>
<td>Gong-You Chen: Insights into <em>hrp</em> regulation systems of <em>Xanthomonas oryzae pv. oryzicola</em>, the causal agent of bacterial leaf streak in rice.</td>
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<td>Stéphane Cociancich: Genome mining indicates that the genus <em>Xanthomonas</em> is a promising reservoir for new bioactive non-ribosomally synthesized peptides.</td>
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<td>Cornelius Schmidtke: Genome-wide transcriptome analysis of the plant pathogen <em>Xanthomonas</em> identifies sRNAs with putative virulence function.</td>
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16:20 Jonathan Gordon: Evolutionary and Comparative Genomics of *Xanthomonas citri* pv.*citri*.
16:40 Adriana J. Bernal: Role of type three effectors from *Xanthomonas axonopodis* pv. *manihotis* in the virulence towards cassava.
17:00 Poster sessions

19:00 – Dinner

20:15 – Exhibition of collection of paintings at the Collégiade Saint Martin

**Wednesday, July 11**

07:00 – Breakfast

**Session III – Genomic plasticity and gene regulation and signaling in host-pathogen interactions.**

Chairman: Matthieu Arlat

08:00 Max Dow (invited speaker): Cyclic di-GMP signalling and virulence in *Xanthomonas*.
08:40 Chenyang He: A novel two-component system PdeKxoo/PdeRxoo regulates c-di-GMP turnover and bacterial virulence of *Xanthomonas oryzae* pv. *oryzae*.
09:00 Aileen A. O’Connell: Elucidation of the regulatory function of the Rpf/DSF system in *Xanthomonas campestris* by comparative proteomics
09:20 Daniela Büttner: Characterization of periplasmic components of the type III secretion system from *Xanthomonas campestris* pv. *vesicatoria*.
09:40 Wei Qian: Complex hybrid histidine kinase SreS in *Xanthomonas campestris* modulates expression kinetics of HPPK by positive feedback loop during stress response.

10:20 - Coffee break

**Session IV – Gene function, B. Type III effector diversity and functions**

Chairman: Tristan Boureau

10:20 Frank White (invited speaker): *Xanthomonas oryzae* assaults the host transcriptome: Ce n’est pas l’*Xanthomonas* de votre père.
11:00 Rebecca Bart: Pathogenomics of *Xanthomonas axonopodis* pv. *manihotis* reveals the core set of effector proteins conserved over space and time.
11:20 Jens Boch: A trick of the TALE.
11:40 Céline Rousseau: Comparative analysis of the XopN type III effector family in the genus *Xanthomonas*.

12:00 - Lunch Break

Chairman: Laurent Noël

13:30 Tristan Boureau: Balancing selection on promoter region is a new aspect of the multifaceted evolution of avrBs2 in *Xanthomonas axonopodis*.
13:50 Joël F. Pothier: *Xanthomonas arboricola* genomics and type three effector gene distribution.

**Session V - Gene function, C. Extracellular polysaccharides.**

14:10 Frank-Jörg Vorhölter (invited speaker): Glucose flux in *Xanthomonas campestris* towards biomass and polysaccharide biosynthesis.

**Session VI – Gene function, D. Other pathogenicity factors**

Chairwoman: Marie-Agnès Jacques

14:50 Adrian A. Vojnov (invited speaker): Roles of DSF-regulated Virulence Factors and biofilm formation in *Xanthomonas* Pathogenicity

15:30 - Tea break

16:00 Chenyang He: Functional analysis of flagellin and its glycosylation during interactions between *Xanthomonas oryzae* pv. *oryzae* and rice.
16:20 Imène Mensi: *Xanthomonas albilineans* is able to move outside of the sugarcane xylem despite its reduced genome and the absence of a Hrp type III secretion system.
16:40 Shan-Ho Chou: Structural and Functional Studies of a Type II PilZ-FimX<sup>TAL</sup> c-di-GMP Ternary Complex Essential for Xanthomonas campestris T4P Function.

Session VII – Plant defense, PTI, ETI and suppression/evasion

17:00 Erin L. Doyle (invited speaker): High throughput identification of TAL effector targets and their use in engineered resistance to *Xanthomonas*.

19:00 – Reception at Jean Lurcat museum
20:00 – Gala Dinner at “Hôtel des pénitentes”

Thursday, July 12

Chairman: Olivier Pruvost

08:00 Ulla Bonas (invited speaker): Update on the interaction between *Xanthomonas campestris* pv. *vesicatoria* and its hosts pepper and tomato

08:40 Boris Szurek: Several rice SWEET/nodulin-3 family members can function as virulence targets for *Xanthomonas* TAL effectors

09:00 Alvaro L. Pérez-Quintero: Bioinformatic strategies to predict TAL effector binding sites in plant genomes

Session VIII – Disease resistance

09:20 Thomas Lahaye (invited speaker): RNA-seq pinpoints a novel *Xanthomonas* TAL-effector activated resistance gene in a large crop genome.

10:00 - Coffee break

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10:20 Vânia Horta de Passo: Molecular genetics of interactions between *Xanthomonas campestris* and *Arabidopsis thaliana*.

10:50 Jeffrey B. Kaplan: Transgenic crops expressing dispersin B: a novel strategy for controlling *Xanthomonas* infections?

11:10 Emily J. McCallum: Characterisation of the cassava-*Xanthomonas axonopodis* pv. *manihotis* pathosystem and engineering of resistance to cassava bacterial blight.

Session IX – Roundtable Discussions

11:30 Ralf Koebnik (discussion leader)

12:30 - Lunch
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Posters Session

Posters Sessions - Genome sequencing, global genomic diversity of *Xanthomonas spp.*, and molecular diagnostic tools.

**P1** Phenotypic and Genetic Characterization of citrus bacterial canker strains in Saudi Arabia by host range, rep-PCR fingerprinting and 16S rDNA analysis.
Y.E. Ibrahim and M.A. Al-Saleh

**P2** CRISPR-associated sequence diversity within *Xanthomonas albilineans*, the causal agent of leaf scald disease of sugarcane.
Isabelle Pieretti, Mélanie Marguerettaz, Stéphanie Bolot, Sébastien Carrère, Stéphane Cociancich, Jérôme Gouzy, Philippe Rott, Monique Royer.

**P3** Genetic diversity of *Xanthomonas oryzae* pv. *oryzicola* from West Africa
I. Wonni, L. Detemmerman, S. Dao, L. Ouedraogo, S. Soungalo, O. Koita, B. Szurek, R. Koebnik, L. Triplett, B. Cottyn, V. Verdier

**P4** French Collection for Plant Associated Bacteria
Perrine Portier, Martial Briand, Géraldine Taghouti and Marion Fischer-Le Saux

**P5** Structure and Diversity of colombian populations of *Xanthomonas axonopodis* pv. *manihotis*, the causal agent of cassava bacterial blight.
Cesar A. Trujillo, Camilo E. López, Silvia Restrepo and Adriana J. Bernal.

**P6** Taxonomic re-identification of the *Xanthomonas* collection from CIRM-CFBP using partial sequencing of gyrB and rpoD
Marion Fischer-Le Saux, Martial Briand, Nadia Mhedbi-Hajri, Sophie Bonneau, Céline Fricot, Géraldine Taghouti, Marie-Agnès Jacques and Perrine Portier

**P7** *Xanthomonas arboricola* pv. *fragariae* is a doubtful pathovar
B. Cottyn, J. Vandroeme, J.F. Pothier, V. Pflüger, B. Duffy, M. Maes

**P8** Molecular diversity of the plant pathogen *Xanthomonas campestris* pv. *campestris* in a collection of isolates from four continents
Joseph M.K. Mulema, Joana G. Vicente, David A.C. Pink, Alison Jackson, Duncan O. Chacha, Lusike Wasiwila, Zakary M. Kinyua, Daniel K. Karanja, Eric B. Holub and Paul Hand

Posters Session - Genomic plasticity and gene regulation and signaling in host-pathogen interactions.

**P9** A novel c-di-GMP receptor protein PXO_00403 regulates Hrp expression from *Xanthomonas oryzae* pv. *oryzae*.
Fenghuan Yang, Fang Tian, Huamin Chen, Maosen Wu, Ching-Hong Yang and Chenyang He

**P10** Quorum sensing mediated by DSF/Rpfxoo regulates T3SS-encoded *hrp* gene expression of *Xanthomonas oryzae* pv. *oryzae*.
Huan Huo, Su Lei, Fang Tian, Huamin Chen and Chenyang He

Posters Session - Gene function, B. Type III effector diversity and functions

**P11** TAL effector RVD specificities and efficiencies
Jana Streubel, Christina Blücher, Angelika Landgraf and Jens Boch

**P12** Role of type three effectors from Xanthomonas axonopodis pv. *manihotis* in the virulence towards cassava.
Cesar A. Medina, Nathaly Montenegro, Cesar A. Trujillo, Rebecca Bart, Brian Staskawicz and Adriana J. Bernal.

**P13** Determination of the genetic and functional diversity of TAL effectors from *Xanthomonas axonopodis* pv. *manihotis*
Daniela Osorio, Luis Miguel Rodríguez, Alvaro Pérez, César Trujillo, Camilo López, Ralf Koebnik, Boris Szurek, Silvia Restrepo, Adriana Bernal.

**P14** TAL effectors enhance virulence on diverse rice varieties when introduced individually into a TAL effector-deficient strain of *Xanthomonas oryzae*
Valérie Verdier, Lindsay R. Triplett, Aaron Hummel, Andres Cernadas, Rene Corral, Adam J. Bogdanove, and Jan E. Leach
Effect of the TAL effector protein PthB from *Xanthomonas axonopodis* pv. *manihotis* on the transcriptome of susceptible cassava host plants

Alejandra Munoz Bodnar, Alvaro Perez, Boris Szurek and Camilo Ernesto Lopez Carrascal

**Posters Sessions - Gene function, C. Extracellular polysaccharides.**

**P16** N-acetylcysteine (NAC) and copper as antimicrobial compounds by *Xanthomonas citri* subsp. *Citri.*

Simone Cristina Picchi, Marco Aurélio Takita, Marcos Antonio Machado, Alessandra Alves de Souza

**P17** Genes involved in biofilm formation of *Xanthomonas campestris* subsp. *campestris*.

Simone Cristina Picchi, Taise Lima, Marco Aurélio Takita, Marcos Antonio Machado, Alessandra Alves de Souza

**Posters Sessions - Gene function, D. Other pathogenicity factors**

**P18** Molecular analysis of the role of the bacteriophytochrome protein in the virulence of the plant pathogen *Xanthomonas campestris* pv. *campestris*.

Florence Malamud; Hernán R. Bonomi; Laila Toum; Gustavo Gudesblat; Fernando A. Goldbaum; Adrián A. Vojnov

**P19** Analysis of the putative type VI secretion systems in *Xanthomonas campestris* pv. *vesicatoria*.

Norman Adlung and Ulla Bonas

**P20** Role of chemotaxis in host specificity of xanthomonads

Arnaud Indiana, Armelle Darrasse and Marie Agnès Jacques

**P21** Structure of the PilZ-FimX<sub>EAL</sub>-c-diGMP complex responsible for the regulation of bacterial Type IV pilus biogenesis

Cristiane R. Guzzo, German Dunger, Roberto K. Salinas and Chuck S. Farah

**P22** Molecular characterization of the *Xanthomonas citri* subsp. *citri* Type IV pilus.

German Dunger, Cristiane R. Guzzo and Chuck S. Farah

**Posters Sessions - Plant defense, PTI, ETI and suppression/evasion**

**P23** Identification of a large repertoire of TAL effectors in *Xanthomonas campestris* pathovars and their plant targets.

Nicolas Denancé, Boris Szurek, Ahmed Hajri, Erin Doyle, Endrick Guy, Thomas Dugé de Bernonville, Tristan Bureauau, Stéphane Poussier, Adam Bogdanove and Laurent D. Noël

**P24** Dynamic interplay between abscisic acid, cytokinin and salicylic acid molds innate immunity of rice against the leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*.

Jing Xu, Kris Audenaert, Monica Hofte, David De Vleesschauwer

**P25** The effector HpaF from *Xanthomonas axonopodis* pv. *manihotis* is a virulence determinant that suppresses plant defense responses.


**P26** Molecular action of *Xanthomonas* effector proteins in the plant cell.

Oliver Müller, Heike Berndt, Robert Szczesny, Ulla Bonas

**Posters Sessions - Disease resistance**

**P27** Evaluation of resistance to *Xanthomonas citri* subsp. *citri* in transgenic citrus plants overexpressing AtNPR1 gene

Raquel L. Boscariol-Camargo, Barbosa-Mendes, JM; Malosso, A; Takita, MA; Machado, MA

**P28** In vitro screening to evaluate *Xanthomonas citri* subsp. *citri* in transgenic citrus plants

Raquel L. Boscariol-Camargo, Simone Picchi, Luiz Guilherme B. Fachini, Marcos A. Machado
Oral Presentations
The rice XA21 pattern recognition receptor binds a type I secreted sulfated peptide, called axY^{22}, derived from the Ax21 (activator of XA21-mediated immunity) protein. The conservation of Ax21 in all sequenced Xanthomonas spp. and closely related genera suggests that Ax21 serves a key biological function. Here we show that the predicted N-terminal sequence of Ax21 is cleaved prior to secretion outside the cell and that mature Ax21 serves as a quorum sensing (QS) factor in Xanthomonas oryzae pv. oryzae. Ax21-mediated QS controls motility, biofilm formation and virulence. We provide genetic evidence that the Xoo RaxH histidine kinase serves as the bacterial receptor for Ax21. This work establishes a critical role for small protein-mediated QS in a Gram-negative bacterium.
Genome sequencing, global genomic diversity of *Xanthomonas spp.*, and molecular diagnostic tools

Oral presentations
The value of draft genomic sequence of rice-associated bacteria for understanding biology, diversity, and diagnosis

Jan E. Leach, Lindsay Triplett, Valerie Verdier

Colorado State University, Ft. Collins, CO 80523-1177

Bacterial draft genomes, generated using high throughput, short-read sequencing technologies, provide valuable information for functional, comparative, and diagnostic information. Draft genome sequences were generated from collections of rice pathogenic bacteria as well as poorly understood strains of rice-associated bacteria to allow for development of diagnostic tools and to provide insights into evolution of genomes in different environments. Genomes were characterized from multiple diverse strains of the pathogens Xanthomonas oryzae, including variants from Asia, Africa and the United States, Burkholderia glumae and Pseudomonas fuscovaginae. In addition, we characterized the genomes of two previously uncharacterized species of non-pathogenic Xanthomonas associated with rice seed. Genomes of the weakly virulent US strains of X. oryzae were highly divergent from other studied X. oryzae pathovars. Notably, US strains lack full or partial TAL (Transcriptional Activator-Like) effectors common to other Xanthomonas pathovars. The lack of TAL effectors made the US strains a useful tool for studying roles of individual TAL effectors in diverse rice genetic backgrounds. The study provided novel insights into TAL effector functional variability among hosts and pointed to possible sources of resistance. Interesting finds from the other pathogen and nonpathogen draft genomes will also be reviewed. Finally, to expedite development of specific diagnostic primers we developed an automated pipeline that compares draft or completed genomes. Performance analysis and testing of the primers demonstrated the program to be a highly efficient means of diagnostic primer design.
Draft genome sequencing of *Xanthomonas fragariae* reveals reduced genome size and distinct virulence related gene content.

Vandroemme J\(^1\)\(^2\), Baeyen S\(^1\), Cottyn B\(^1\), De Vos P\(^2\) and M Maes\(^1\)

\(^1\)Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit - Crop Protection, Merelbeke, Belgium; 
\(^2\)Laboratory of Microbiology, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium.

*Xanthomonas fragariae* (Xf) is a bacterial strawberry pathogen and an A2 quarantine organism on strawberry planting stock in the EU. It is known for its rather weak pathogenicity, fastidious growth on artificial media, its narrow host range and its taxonomically distinct but homogenous position within the otherwise complex genus *Xanthomonas*. As part of a broader pathogenicity study of Xf, the genome of a recent Belgian isolate (LMG 25863) was sequenced and assembled to draft status, with 105 contigs of more than 200 bp long. Remarkably, the Xf draft genome (4.2 Mb) was considerably smaller than most other *Xanthomonas* genomes (~5 Mb). Compared to other plant pathogenic *Xanthomonas*, Xf appeared to miss several genes/regions with a possible relevance to pathogenicity: i) an operon involved in the breakdown of phenolic compounds, ii) one of the two Type II Secretion System coding regions in *Xanthomonas*, iii) isocitrate lyase and malate synthase, responsible for the glyoxylate shunt pathway, and iv) two genes of the *rfp* gene cluster, *rpfD* and *rpfI*. Also, only half of the cellulolytic, hemicellulolytic and pectinolytic enzyme coding genes typically present in other *Xanthomonas* genomes were found in Xf. On the other hand, we identified possible virulence-related gene regions in Xf that are not common in other *Xanthomonas* genomes, with a Type VI Secretion System coding region being the most obvious one. Also, the Type III Secretion System effector repertoire in the Xf genome included several rare variable effectors such as *xopC*, *xopE4*, *xopAF* and *xopAS*, and some putatively new effectors. The Xf draft genome further revealed a high content of externally derived DNA: i) multiple copies of several Insertion Sequence (IS) elements, comparable to *Xanthomonas oryzae*, ii) a phage coding region, iii) 18-19 kb of sequence fragments with high similarity to Plasmid III (27 kb) of *X. albilineans* GPE GP73, and iv) a CRISPR associated gene cluster of the *Yersinia pestis* subtype with 40 related spacer sequences. The draft genome sequence of LMG 25863 affirms the distinct position of Xf within the genus *Xanthomonas* and reveals a patchwork of both lost and newly acquired pathogenicity related functions. It’s pathogenicity related distinguishing features may help explain the specific, mostly endophytic association of Xf with the strawberry plant system.
Genomics Informed Design of LAMP assays for subspecies-level phytosanitary detection of the quarantine pathogen *Xanthomonas arboricola* pv. *pruni*

Andreas Bühlmann¹, Joël F. Pothier³, Jennifer A. Tomlinson², Jürg E. Frey¹, Neil Boonham², Theo H.M. Smits³, and Brion Duffy¹*

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The objective of this study was to develop a rapid, sensitive detection assay for the quarantine pathogen *Xanthomonas arboricola* pv. *pruni*, causal agent of stone fruit bacterial spot, an economically important disease of *Prunus* spp. Unique targets were identified from *X. arboricola* pv. *pruni* genomes using a comparative genomics pipeline of other *Xanthomonas* species, subspecies and pathovars, and used to identify specific diagnostic markers. Loop-mediated isothermal amplification (LAMP) was then applied to these markers to provide such a rapid, sensitive, and specific detection. The method developed showed unrivalled specificity compared to previously established techniques, distinguishing between phylogenetically close subspecies such as *X. arboricola* pv. *corylina*. The sensitivity is comparable to previously reported quantitative real-time PCR (qPCR) assay, while the unrivaled speed of LAMP technology enables a positive test in less than 15 min. The developed assay can be used with portable real-time fluorescent detectors for quantitative results as well as with DNA staining dyes to function as a simplified strategy for on-site pathogen detection.
Phylogenomic marker(s) based evolutionary studies of *Xanthomonas*

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Finest understanding of phylogenetic and taxonomic relationship of bacteria is critical for any systematic evolutionary studies on bacterial. Traditional phenotypic and sequence based markers have either greatly underestimated or overestimated the relationship of *Xanthomonas* species and pathovars further misleading the evolutionary studies. However the advent of whole genome sequencing and further genome based phylogeny and taxonomic studies are bringing in revolutionary advances in the study of bacteria. Herein we show the promise and potential of true phylogenomic marker(s) in the phylogeny and taxonomic studies of Xanthomonads and in particular plant associated ones. Further we will also explain how such insights are helping in undertaking systematic evolutionary studies of *Xanthomonas*. We successfully applied this approach on our own whole genome projects on *Xanthomonas axonopodis* pv. *punicae*, the leaf blight pathogen of Pomegranate and *Xanthomonas citri* pv. *mangiferaeindicac*, the black spot pathogen of Mango. We also show the potential of this approach of characterizing a xanthomonad upto species/pathovar level in metagenomic studies.
**Genome sequencing of Xanthomonas axonopodis pv. phaseoli CFBP4834-R reveals that flagellar motility is not a general feature of xanthomonads.**


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Xanthomonads are plant-associated bacteria that establish neutral, commensal or pathogenic relationships with plants. The list of common characteristics shared by all members of the genus *Xanthomonas* is now well established based on the entire genome sequences that are currently available and that represent various species, numerous pathovars of *X. axonopodis* (sensu Vauterin et al., 2000), *X. oryzae* and *X. campestris*, and many strains within some pathovars. These γ-proteobacteria are motile by a single polar flagellum. Motility is an important feature involved in biofilm formation, plant colonization and hence considered as a pathogenicity factor. *X. axonopodis pv. phaseoli var. fuscans* (Xapf) is one of the causal agents of common bacterial blight of bean and 4834-R is a highly aggressive strain of this pathogen that was isolated from a seed-borne epidemic in France in 1998. We obtained a high quality assembled sequence of the genome of this strain with 454-Solexa and 2X Sanger sequencing. Housekeeping functions are conserved in this genome that shares core characteristics with genomes of other xanthomonads: the six secretion systems which have been described so far in Gram negative bacteria are all present, as well as their ubiquitous substrates or effectors and a rather usual number of mobile elements. Elements devoted to the adaptation to the environment constitute an important part of the genome with a chemotaxis island and dispersed MCPs, numerous two-component systems, and numerous TonB dependent transporters. Furthermore, numerous multidrug efflux systems and functions dedicated to biofilm formation that confer resistance to stresses are also present. An intriguing feature revealed by genome analysis is a long deletion of 35 genes (33 kbp) involved in flagellar biosynthesis. This deletion is replaced by an insertion sequence called ISXapf2. Genes such as *flgB* to *flgL* and *fliC* to *fleQ* which are involved in the flagellar structure (rod, P- and L-ring, hook, cap and filament) are absent in the genome of strain 4834-R that is not motile. Primers were designed to detect this deletion by PCR in a collection of more than 300 strains representing different species and pathovars of *Xanthomonas*, and less than 5% of the tested xanthomonads strains were found non-motile because of a deletion in the flagellum gene cluster. We observed that half of the Xapf strains isolated from the same epidemic than strain 4834-R was non-motile and that this ratio was conserved in the strains colonizing the next bean seed generation. Isolation of such variants in a natural epidemic reveals that either flagellar motility is not a key function for fitness or that some complementation occurs within the bacterial population.
Walnut oozing canker: Danger of diversification/adaptation.

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Walnut blight (WB) is considered as the most important biotic disease that affects walnut (Juglans regia). The causal agent, Xanthomonas arboricola pv. juglandis, causes necrosis on fruits, leaves, catkins and provokes up to 65% of fruit loss. A new disease has been observed in France since 2001. This disease is characterized by vertical cankers on trunks and branches with oozing exudates and was termed vertical oozing canker (VOC). Fluorescent-AFLP was first used for identification and characterization and clearly distinguished one cluster that grouped strains isolated from VOC. Clustering of these strains was not correlated to their geographical origins since the VOC cluster contained strains collected in both southeastern and southwestern areas. We then developed minisatellites markers, which are short nucleotidic sequences that are organized into clusters of variable number of tandem repeats (VNTR) based on the genome sequence of the X. arboricola pv. pruni strain CFBP 5530. Our objective was to develop a multilocus VNTR analysis (MLVA) scheme, a technique that is becoming increasingly popular for assessing the polymorphism in population genetics and which is a fast and easy-to-use typing method. Moreover, the selected VNTRs can be used for all pathovars of X. arboricola that makes the MLVA scheme useful for population studies of any pathovars of this species. The MLVA performed on X. arboricola pv. juglandis highlighted the genetic structure of bacterial populations. The VOC strains grouped in a single clonal complex when strains isolated from fruit, leaf and catkins form diverse singletons. This work provides performing tools to perform epidemiological studies on bacterial disease on walnut. The VOC could be due to the adaptation/selection of strains with a particular virulent trait allowing the colonization of vascular system of walnut. The elucidation of the molecular basis of this adaptation is a fascinating challenge. Type 3-effectors (T3Es) are among virulence-associated genes that are known to be implicated in evolution and adaptation. Thus we surveyed the distribution of 53 T3E genes among a collection of X. arboricola strains. Our results confirmed that T3E repertoire comprises a core set of T3E genes common to all X. arboricola and a variable set that can be correlated with pathovars and even with a genetic lineage within X. arboricola pv. juglandis: our study revealed that VOC strains harbor xopB but not xopAH whereas strains causing walnut blight have xopAH but not xopB. Further experiments remain to be developed to determine the role of these effectors in the virulence of strains that cause walnut blight and VOC in walnut.
How many genomes would we need to describe a Xanthomonas pathovar?

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Three reference genomes are publicly available for Xanthomonas campestris pv. campestris (Xcc), the causal agent of black rot of brassicas. Such a resource is more than what most of us would have dreamt of until recent times! Yet, a detailed phylogenetic analysis of Xcc using strains collected worldwide mostly on Brassica revealed an unsuspected level of genomic and phenotypic diversity. Draft genomes of 37 Xcc were obtained and revealed surprising features which would have been fully overlooked based on the 3 first Xcc genomes. Our current progress in the comparative genomics of Xcc will be presented with a particular focus on type III effector content.
Genomics-based molecular tools for studies of population structures and epidemiological surveillance of xanthomonads

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Xanthomonads are an important clade of Gram-negative bacteria infecting a plethora of plants, including rice, cereals, cassava and citrus fruits. For efficient management of plant diseases, knowledge about the pathogen’s population structure and tools for epidemiological surveillance are prerequisite. To this end, our laboratories develop new molecular typing tools based on rapidly evolving genetic loci, such as Variable Numbers of Tandem Repeats (VNTR) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). In this communication, we will present our latest typing data on several species and pathovars of Xanthomonas.
Comparative and functional *Xanthomonas* genomics
Oral presentations
Comparative genomics helps explain evolution of a unique *Xanthomonas perforans* strain on pepper

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Draft genome sequencing provides a rapid means to identify virulence factors that may help explain the complexities of host-pathogen interactions such as how species evolve to extend their host range. In Florida, prior to 1991 the bacterial spot of tomato pathogen *Xanthomonas euvesicatoria* (*Xe*) was the only species associated with tomato. At that time, *Xanthomonas perforans* (*Xp*) was identified and as of 2006 had displaced *Xe* and become the predominant *Xanthomonas* species in tomato fields. Since becoming recognized as a new pathogen in 1991, *Xp* had not been isolated from pepper and *Xe* continued to be the only xanthomonad pathogenic on pepper. *AvrXv3* present in *Xp* strains restricted host range on pepper. More recently *Xp* strains lacking a functional *avrXv3* have become predominant in Florida, but have not been associated with pepper. We recently isolated and sequenced an *Xp* strain that is able to infect pepper. *In silico* analysis of the genes present in the new pepper *Xp* strain, but absent from the previously sequenced *Xp*91-118, revealed candidate virulence factors that *Xp* might have acquired or been modified to extend its host range on pepper. *Xp* strains representative of the tomato growing regions in Florida were also sequenced. Differences in effector suites have evolved in these closely related field strains.
Insights into *hrp* regulation systems of *Xanthomonas oryzae* pv. *oryzicola*, the causal agent of bacterial leaf streak in rice

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*Xanthomonas oryzae* pv. *oryzicola*, the causal agent of bacterial leaf streak in the model plant rice, possesses a hypersensitive response and pathogenicity (*hrp*), *hrp*-conserved (*hrc*), *hrp*-associated (*hpa*) cluster (*hrp-hrc-hpa*) that encodes a type III secretion system (T3SS) through which T3SS effectors are injected into host cells to cause disease or trigger plant defenses. Mutations in this cluster usually abolish the bacterial ability to cause hypersensitive response in nonhost tobacco and pathogenicity in host rice. In *Xanthomonas* spp., these genes are generally assumed to be regulated by the key master regulators HrpG and HrpX. However, recent results in my lab have revealed novel findings: (1) HrpD6, controlled by HrpX, is a regulator for *hpa1*, *hpa2*, *hrcC*, *hrcT* and *hpaB*; (2) the expression of *hrcC*, *hrpD5*, *hrpE* and *hpa3* is HrpG- and HrpX-independent; (3) the expression of *hrcT* is HrpG-independent and HrpX-dependent; (4) HrcC, HrpE and Hpa3 are not only involved in the T3SS secretion, but also in carbon metabolism; (5) fructose-bisphosphate aldolase exhibits functional roles between carbon metabolisms and the *hrp* system of the pathogen. Thus, the unknown regulators for HrcC, HrpD5, HrpE and Hpa3 and their roles in consistently with the T3SS are undertaking in my lab.
Genome mining indicates that the genus Xanthomonas is a promising reservoir for new bioactive non-ribosomally synthesized peptides

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Xanthomonas is a large genus of Gram-negative bacteria that cause disease in hundreds of plant species. To date, the only known small molecule synthesized by non-ribosomal peptide synthesis (NRPS) in this genus is albicidin produced by Xanthomonas albilineans. The DNA gyrase inhibitor albicidin is not only an important virulence factor but also a possible lead structure for novel antibiotics. This study aims to estimate the biosynthetic potential of Xanthomonas spp. by in silico analyses of NRPS genes with unknown function recently identified in the sequenced genomes of X. albilineans and related species of Xanthomonas. We performed in silico analyses of NRPS genes present in all published genome sequences of Xanthomonas spp., as well as in unpublished draft genome sequences of Xanthomonas oryzae pv. oryzae strain BAI3 and Xanthomonas spp. strain XaS3. The most unexpected result of these analyses is that these two latter strains, together with X. albilineans strain GPE PC73 and X. oryzae pv. oryzae strains X8-1A and X11-5A, possess novel NRPS gene clusters. Furthermore, these Xanthomonas spp. strains share related NRPS-associated genes such as those required for the biosynthesis of non-proteinogenic amino acids or for the secretion of peptides. In silico prediction of peptide structures according to the NRPS architecture accounts for eight different peptides, each specific to its producing strain. Interestingly, these eight peptides cannot be assigned to any known gene cluster or related to known compounds from natural product databases. PCR screening of a collection of 94 plant pathogenic bacteria indicates that these novel NRPS gene clusters are specific to the genus Xanthomonas and are also present in Xanthomonas translucens and X. oryzae pv. oryzicola. Further genome mining revealed (i) novel NRPS genes shared by Xanthomonas spp. strains GPE PC73 and XaS3 with the plant-associated bacterium Bradyrhizobium spp. strain BTAi and (ii) novel NRPS genes specific to X. oryzae pv. oryzicola or Xanthomonas sacchari. This study revealed the significant potential of the genus Xanthomonas of producing new non-ribosomally synthesized peptides. Interestingly, this biosynthetic potential seems to be specific to strains of Xanthomonas associated with monocotyledonous plants, suggesting a putative involvement of new non-ribosomally synthesized peptides in plant-bacteria interactions.
Genome-wide transcriptome analysis of the plant pathogen *Xanthomonas* identifies sRNAs with putative virulence function

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*Xanthomonas campestris pv. vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease on pepper and tomato. In *Xcv*, essential pathogenicity and many virulence-associated genes are induced upon contact with the plant tissue by two known regulatory proteins, HrpG and HrpX. Both proteins regulate the expression of the *Xcv* type III secretion system, an essential and well-characterized pathogenicity factor. In contrast, little is known about the transcriptional landscape of *Xcv* and the role of posttranscriptional regulation in virulence. To analyze the transcriptome of *Xcv* strain 85-10 (1) we applied a differential RNA sequencing (dRNA-seq) approach based on the selective enrichment of primary transcripts (2). A fully automated analysis pipeline for sequencing data identified 1,421 transcription start sites. Genes in *Xcv* exhibit poorly conserved -10 promoter elements and no consensus ribosome binding sequence. Interestingly, 14% of the identified 5’ UTRs are leaderless, whereas 13% are unusually long. Northern analyses confirmed 16 intergenic small RNAs (sRNAs) and eight *cis*-encoded antisense RNAs in *Xcv* (2, 3). Expression of eight sRNAs was controlled by HrpG and HrpX. More detailed characterization identified candidate sX12 as a small RNA that controls virulence of *Xcv*.

The xylan utilisation system of the plant pathogen *Xanthomonas campestris* pv. *campestris* reveals common features with oligotrophic bacteria and animal gut symbionts.

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Xylan is a major structural component of plant cell wall and the second most abundant plant polysaccharide in nature. Here, by combining genomic and functional analyses, we provide a comprehensive picture of xylan utilisation by the epiphytic phytopathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*). The xylanolytic activity of *Xcc* depends on xylan-deconstruction enzymes but also on transporters. Genes of this system are specifically induced by xylo-oligosaccharides. Their regulation involves a LacI repressor, XylR, as well as HrpG and HrpX, the regulators of *Xcc* type 3 secretion system. A particularity of this system is the presence of two TonB-dependent outer membrane transporters (TBDTs) which belong to operons required for efficient growth in presence of xylo-oligosaccharides and/or for optimal survival on plant leaves.

Comparative genomics revealed that the xylanolytic machinery of *Xcc* is highly conserved among bacteria found in environments where plant material accumulates and deteriorates, including plant debris, soil, aquatic environments and the digestive tract of animals. In particular, this system displays a high degree of conservation with the xylose-regulon of the oligotrophic aquatic bacterium *Caulobacter crescentus*. Moreover, it shares common features, including the presence of TBDTs, with the xylan utilisation systems of *Bacteroides ovatus* and *Prevotella bryantii*, two gut symbionts.

These similarities and our results support an important role for TBDTs and xylan utilisation systems for bacterial adaptation in the phyllosphere, oligotrophic environments and animal guts.
Evolutionary and Comparative Genomics of *Xanthomonas citri pv. citri*

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*Xanthomonas citri pv. citri* is the causal agent of citrus canker, a serious agricultural disease spread nearly worldwide. Two pathotypes exist (A and A*) which differ in their abilities to infect different host citrus species. To examine the evolutionary history and to understand the potential mechanisms of host determination, we undertook near-complete genome sequencing for 44 strains. For analysis of the genomes, pseudomolecules were created by alignment of contigs against the complete genome of strain 306 and its plasmids. These pseudomolecules were then annotated. A table of gene presence/absence was created and phylogenetic trees were constructed from all common genes between the strains. Gene families were constructed within and across all strains which were then used to guide an analysis of the evolutionary constraints acting upon the genes in each family. SNPs were identified for each strain and were placed on different branches on the phylogeny where the information was available based on parsimony. This will allow us to identify candidate SNPs that partition between A and A* pathotypes.
Role of type three effectors from \textit{Xanthomonas axonopodis} pv. \textit{manihotis} in the virulence towards cassava.

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\textit{Xanthomonas axonopodis} pv. \textit{manihotis} (Xam) is the causal agent of cassava bacterial blight (CBB), one of the most devastating diseases in this crop. This disease can cause complete losses under the appropriate conditions. Previous studies have shown the presence of 19 type three effectors (T3E) in the genome of the model strain Xam CIO151 and these proteins, together, have a key role in the virulence of this strain. In this work we performed a systematic mutational analysis to determine the role of T3E on the virulence of this pathovar. Using the double crossing-over technique, we generated single and double mutants for a subset of genes mutants (Δ) for the T3E \textit{avrBs2}, \textit{xopN}, \textit{xopZ}, and \textit{hpaF} in the T3E repertoire of Xam. The mutants were inoculated in cassava susceptible cultivar MCOL2215. A reduction in disease symptoms was observed with single and double mutants for \textit{avrBs2}, \textit{xopZ} and \textit{hpaF} when they were compared against the wild type strains. Therefore, these effector genes have a role in the virulence of this bacterium. The mutation effects were tested on additional strains CIO303, CIO536, CIO556, CIO560, CIO650, which are representative of the diversity present in Colombia, and similar results were obtained. This underscores the importance of these proteins in the virulence of \textit{Xam} on Cassava. Results obtained in this study could be useful for the development of durable and sustainable resistance to CBB through the implementation of genes that recognize the effectors that are important for virulence.
Genomic plasticity and gene regulation and signaling in host-pathogen interactions.
Oral presentations
Cyclic di-GMP signalling and virulence in Xanthomonas

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Cyclic di-GMP is a second messenger with a role in regulation of a range of cellular functions in diverse bacteria including the virulence of pathogens. Cellular levels of cyclic di-GMP are controlled through synthesis, catalysed by the GGDEF protein domain, and degradation by EAL or HD-GYP domains. The virulence of \textit{Xanthomonas campestris pv. campestris} (Xcc) depends upon cell-cell signalling mediated by the diffusible signal molecule DSF. Synthesis of DSF is dependent on RpfF, whereas the RpfC/RpfG two-component system is implicated in DSF perception and signal transduction. RpfC is a complex sensor kinase whereas RpfG is a regulator with an HD-GYP domain, which is a cyclic di-GMP phosphodiesterase. Mutation of \textit{rpfF}, \textit{rpfG}, or \textit{rpfC} leads to a co-ordinate reduction in the synthesis of virulence factors such as extracellular enzymes, alterations in biofilm formation and reduced motility. The Rpf/DSF system controls these functions by different pathways. The transcription factor Clp acts to promote expression of genes encoding extracellular enzymes. Clp binding to promoter DNA is negatively influenced by cyclic di-GMP. This suggests a mechanism for regulation of enzyme synthesis by DSF in which the perception of DSF by RpfC leads to reduction of the level of cyclic di-GMP by RpfG allowing Clp binding to promoter sequences. By contrast, the regulation of motility by the Rpf/DSF system involves physical interaction of RpfG with two GGDEF domain-containing proteins. This interaction, which requires the GYP motif, is dependent upon DSF signalling, being reduced in the \textit{rpfF} mutant but restored by DSF addition. Loss by mutation of the two GGDEF domain proteins influences Xcc motility and virulence but has no effect on the synthesis of extracellular enzymes or biofilm formation. Further mechanism(s) serve to link Rpf/DSF signalling and cyclic di-GMP with biofilm formation, but these remain poorly understood.
A novel two-component system PdeKxoo/PdeRxoo regulates c-di-GMP turnover and bacterial virulence of *Xanthomonas oryzae* pv. *oryzae*.

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Two-component systems (TCSs) consisting of histidine kinases (HKs) and response regulators (RRs) play essential roles in bacteria to sense environmental signals and regulate multiple cell functions. One type of RR is involved in metabolism of cyclic diguanylate (c-di-GMP), a ubiquitous bacterial second messenger. Although genomic studies predicted a large number of them existing in different bacteria, only a few have been studied in details. In this work we identified and characterized a novel TCS consisting of PdeKxoo(PXO_01018)(HK)/PdeRxoo(PXO_01019)(RR) from *Xanthomonas oryzae* pv. *oryzae* (Xoo), which causes the bacterial leaf blight of rice. PdeR, a GGDEF-EAL-REC domain-containing protein was shown to have phosphodiesterase (PDE) but diguanylate cyclase (DGC) activity *in vitro* by colorimetric assays and HPLC analysis. The PDE activity of full-length PdeR needs to be triggered and activated by HK PdeKxoo but PXO_01020, another RR. Deletion of either *pdeKxoo* or *pdeRxoo* in Xoo wild type strain PXO99A had attenuated its virulence on rice. ΔpdeKxooxoo and ΔpdeR secreted less exopolysaccharide (EPS) than PXO99A, but there were no changes in terms of motility or extracellular cellulase activity. Further analysis of virulence-related gene transcripts in ΔpdeRxoo by quantitative RT-PCR (RT-qPCR) indicated EPS synthesis-related gene *gumM, gumB, gumK* and *gumD*, and type III secretion system (T3SS)-related genes *hrpG, hrpX* and *hpa1* were significantly decreased, suggesting these genes are positively regulated by PdeRxoo. Therefore, we conclude that PdeKxoo but not PXO_01020 and PdeRxoo constitute a novel TCS, which is required for the regulation of c-di-GMP turnover and full virulence in Xoo.
Elucidation of the regulatory function of the Rpf/DSF system in *Xanthomonas campestris* by comparative proteomics

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The full virulence of *Xanthomonas campestris* pv. *campestris* (Xcc) to plants depends upon cell-to-cell signalling mediated by the signal molecule DSF (for diffusable signal factor), that has been characterised as \textit{cis}-11-methyl-2-dodecenoic acid. DSF-mediated signalling regulates motility, biofilm dynamics and the synthesis of particular virulence determinants. The synthesis and perception of the DSF signal involves products of the \textit{rpf} (regulation of pathogenicity factor) gene cluster. DSF synthesis is fully dependent on \textit{RpfF}, which encodes a putative enoyl-CoA hydratase. A two-component system, comprising the complex sensor histidine kinase \textit{RpfC} and the HD-GYP domain regulator \textit{RpfG}, is implicated in DSF perception. The HD-GYP domain of \textit{RpfG} is a cyclic di-GMP phosphodiesterase and DSF perception is thereby linked to the turnover of this intracellular second messenger. The full range of regulatory influences of the Rpf/DSF system and of cyclic di-GMP in Xcc has yet to be established. In order to further characterise the Rpf/DSF regulatory network in Xcc, we used a proteomic approach to compare protein expression in wild type, \textit{rpfC}, \textit{rpfF} and \textit{rpfG} mutants. Our work confirms that the Rpf/DSF system regulates a range of biological functions associated with virulence and biofilm formation but also reveals new functions mediated by DSF. These include proteins associated with antibiotic resistance, detoxification and stress tolerance. Interestingly, we demonstrate that some proteins are regulated by either RpfC or RpfG. This suggests that RpfG and RpfC play broader roles in regulation other than perception and transduction of DSF. Furthermore qRT-PCR analysis suggests that some of the changes in protein expression in each of the selected mutants occurred post-transcriptionally. Taken together, this analysis indicates the broad and complex regulatory role of Rpf/DSF system and identifies a number of new functions some of which have recently been shown to play a role in virulence.
Characterization of periplasmic components of the type III secretion system from *Xanthomonas campestris pv. vesicatoria*

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The Gram-negative plant pathogenic bacterium *Xanthomonas campestris pv. vesicatoria* employs a type III secretion (T3S) system to translocate effector proteins into plant cells. T3S depends on the early T3S substrate HrpB2, which is essential for the assembly of the extracellular T3S pilus and is itself weakly secreted. HrpB2 interacts with the T3S substrate specificity switch protein HpaC, which prevents the efficient secretion of HrpB2 and promotes the secretion of translocon and effector proteins. The HpaC-mediated substrate specificity switch depends on the cytoplasmic domain of the inner membrane protein HrcU, which provides a substrate docking site for HrpB2. To characterize the role of HrpB2 during T3S, we performed a transposon mutagenesis, which led to the insertion of pentapeptide-encoding sequences into *hrpB2*. Complementation studies with HrpB2 mutant derivatives revealed that the C-terminal region of HrpB2 is essential for bacterial pathogenicity and T3S including secretion of HrpB2 itself. This region of HrpB2 contains a conserved VxTLxK amino acid motif that is also present in predicted inner rod proteins from animal pathogenic bacteria and is required for the contribution of HrpB2 to pilus assembly and T3S. Electron microscopy and fractionation studies revealed that HrpB2 is not a component of the extracellular pilus structure but localizes to the bacterial periplasm and the outer membrane. We therefore propose that the essential contribution of HrpB2 to T3S and pilus assembly is linked to its possible function as a periplasmic component of the T3S system at the base of the pilus.
Complex hybrid histidine kinase SreS in *Xanthomonas campestris* modulates expression kinetics of HPPK by positive feedback loop during stress response

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Among bacterial two-component signal transduction system proteins, complex hybrid histidine kinases (HyHKs) consist of multiple phosphodonsors (transmitter domains) or phosphoacceptors (receiver domains), making them multipotent and capable of involvement in complicated signaling networks. Here we identified a complex HyHK SreS (XC0730) of *Xanthomonas campestris* that regulates the bacterial stress response. SreS contains two receiver domains separated by a central transmitter domain. It is located in a polycistronic operon harboring *XC0728, XC0729, hppK* and a *cis*-encoded antisense RNA gene (*ptrA*). Although mutations in *sreS* did not eliminate the salt induction of HPPK, they led to disappearance of the HPPK expression surge observed in the wild-type. Two tandem promoters (P1 and P2) subjected to SreS regulation were found to drive the transcription of *XC0728-XC0729-sreS-hppK ptrA* operon. Of these, P1 is a strong, primary promoter that modulates the background and salt-induced transcription, whereas P2 is a weak promoter that has a special role in controlling *hppK* transcription. We propose that P1 and P2 promoters have a cooperative relationship that is associated with transcriptional interference. These results indicate that the HPPK expression kinetics, which is subtly modulated by a SreS-mediated positive feedback loop, plays an important role during bacterial stress response.
Gene function, B. Type III effector diversity and functions

Oral Presentations
Xanthomonas oryzae assaults the host transcriptome: Ce n'est pas l'Xanthomonas de votre père

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Xanthomonas oryzae pv. oryzae (Xoo) is the causal agent of bacterial blight of rice. We have conducted extensive profiling of host responses to different strains and mutants of Xoo, revealing extensive global changes in rice gene expression have been characterized during the disease process. The host transcriptional responses, which we refer to here as "transcriptional shock", are, in part, due to the large repertoire of transcription activation-like (TAL) effector genes. Thus, Xoo has adopted a somewhat different strategy for host exploitation in comparison to many of the better studied disease complexes. The question arises as to which affected genes are involved in host susceptibility. We have identified both major and minor susceptibility genes, while an extensive list of up-regulated genes await further analysis. At the same time, strains of the pathogen have a requirement for the induction of at least one member of a family of nodulin 3/SWEET genes. Susceptibility-competent members have been shown to be passive sucrose transporters implicating a role for sucrose in the disease process. Other host induced genes are predicted to function in RNA processing, phytohormone biosynthesis, abiotic stress, and gene regulation. Different strains of Xoo share common and strain specific effects on host transcription. Related strains have distinct expression profiles in comparison. On the host side, rice has adapted to TAL effector assault in a variety of ways and the identification of dominant and recessive resistance genes has revealed novel mechanisms of resistance.
Pathogenomics of *Xanthomonas axonopodis* pv. *manihotis* reveals the core set of effector proteins conserved over space and time.

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Cassava Bacterial Blight (CBB), incited by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), negatively affects beleaguered farmers in tropical regions of the world who rely on cassava as a major food source. We report Illumina based-high quality draft genomes for 65 *Xam* strains and deduce the phylogenetic relatedness of *Xam* across all areas where cassava is grown. Using an extensive database of type three effector proteins from animal and plant pathogens, we identify the complete effector repertoire for each sequenced strain. While effector repertoire size only loosely correlated with pathogen virulence levels, our data identified the core components of the *Xam* virulence arsenal which have been maintained over 12 countries, 3 continents and 70 years of evolution. We predict that resistance strategies targeting these highly conserved effectors will result in the most durable resistance phenotypes in the field.
A trick of the TALE.

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Transcription activator-like effectors (TALEs) are key molecular weapons of most, but not all plant-pathogenic xanthomonads. *Xanthomonas* spp. deliver TALEs via a type III secretion system into plant cells where they localize to the nucleus to activate expression of target plant genes. The target plant genes are termed susceptibility genes if they favor bacterial virulence and termed resistance genes if their induction restricts bacterial proliferation. The best studied example of susceptibility genes are sucrose efflux transporter (SWEETs) which are targeted by several TALEs from rice-pathogenic *Xanthomonas oryzae* pv. *oryzae* and which might support bacterial nutrition in the apoplast.

TALEs have attracted a widespread attention, because of the modular and predictable way they bind to DNA. They employ a number of tandem 34-amino acid repeats. The repeats are near-identical with variations in two adjacent amino acids, termed repeat-variable diresidues (RVDs). We discovered that each repeat binds to one base of the DNA and the RVDs specify which base is bound. In nature approx. 23 RVD combinations can be found, but not all specificities are known, so far.

We developed a modular cloning method termed "Golden TAL Technology" to assemble TALEs with any given combination of RVDs and thus any desired DNA-binding specificity. We used this cloning method to build artificial TALEs and study novel RVD specificities with transient reporter assays. Surprisingly, RVDs differ not only in their specificity, but also in their efficiency. Key RVDs are absolutely required to facilitate overall TALE functionality. The number and positioning of these key RVDs for efficient TALE design will be discussed. Natural DNA-boxes are rarely perfect matches to the inherent RVD specificities of the corresponding TALEs. We have studied the effect of positional mismatches on TALE activity using our established reporter assay. The exceptionally predictable TALE DNA-specificity also allows a computational prediction of possible susceptibility targets in sequenced genomes of host plants which will greatly aid in understanding the role of TALEs for *Xanthomonas* spp. virulence.
Comparative analysis of the XopN type III effector family in the genus *Xanthomonas*.

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Type III effectors (T3E) are bacterial proteins injected in the plant cell via the type III secretion system to suppress plant defenses. However, T3E or their action may be recognized by plants, leading to the onset of a strong defense response (HR). Thus, T3E are good candidate to undergo selective pressures imposed by the host. In the species *Xanthomonas axonopodis* (Vauterin et al. 1995), numerous pathovars were described. Therefore, this species allows studies of selective pressures imposed by the host.

XopN is a ubiquitous T3E in the genus *Xanthomonas*. Nucleotidic sequences analysis of *xopN* of 127 strains belonging to the species *X. axonopodis* allowed to identify selective pressures acting on this T3E. PAML analysis highlighted two sites under diversifying selection in *xopN* sequence. Most interestingly, comparative analysis of nucleotidic sequences of *xopN* highlighted the existence of several variants of *xopN* among sequenced strains belonging to several species of *Xanthomonas* (*X. axonopodis, X. oryzae, X.campestris*). Indeed, in *X. axonopodis*, only 5’- and 3’-ends of *xopN* display strong sequence similarity with *xopAB*, a T3E only present in *X. oryzae*. In contrast, the central part of *xopN* is present with other 5’- and 3’-ends at another locus in *X. oryzae, X. campestris* and *P. syringae*. This suggests that *xopN* emerged due to the insertion of a genetic element inside *xopAB* in *X. axonopodis*. The main function of XopN described in the literature is that it interacts with the tomato protein TARK-1 (Kim et al. 2009). Our sequence analysis shows that the interaction site with TARK-1 is polymorphic in the genus *Xanthomonas*. In *X. axonopodis*, this interaction site is encoded in the 5’-end similar to *xopAB*. This suggests that XopAB in *X. oryzae* may interact with a TARK-1 homolog in rice. The fact that *xopN* in *X. campestris* and *X. oryzae* harbor 5’-end different than in *X. axonopodis*, suggests that XopN could have different functions according to the species. Phenotyping of Δ*xopN* deletion mutant on bean by measuring chlorophyll fluorescence is underway.
Balancing selection on promoter region is a new aspect of the multifaceted evolution of \textit{avrBs2} in \textit{Xanthomonas axonopodis}.

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Type three effectors (T3Es) are major virulence factors for Gram-negative bacterial pathogens. In plant pathogenic bacteria, T3Es play a dual role in the interaction as they promote virulence on susceptible hosts, whereas they trigger specific defenses in resistant plants. Consequently, this results in a coevolutionary conflict between hosts and pathogens, where successful pathogens escape plant recognition by evolving their repertoire of T3Es.

In the present study, we analyzed the molecular evolution of the \textit{avrBs2} T3E gene in a collection of 65 strains of the bacterial species \textit{Xanthomonas axonopodis}. We chose the T3E gene \textit{avrBs2} since it is present in all strains of \textit{X. axonopodis}. The collection used encompassed strains representative of 17 distinct pathovars. Each pathovar groups strains displaying pathogenicity on the same host plants. Thus the dataset allowed to test whether host-imposed selection occurred on \textit{avrBs2}. We analyzed the polymorphism and constructed the genealogy of \textit{avrBs2}. We then aimed at detecting selective pressures acting on both promoter and coding regions of \textit{avrBs2}. Indeed, selective pressures acting on promoter regions are mostly overlooked, even though such pressures may affect the level of expression of the coding region. We provided evidence that balancing selection was acting on the promoter region, and that this correlated with a differential expression of \textit{avrBs2}. To our knowledge, these results are the first evidences that selection acting on promoter sequences may play a role in the evolution of T3Es. We also showed that strong purifying selection acted on the coding region of \textit{avrBs2}. Finally we detected lateral transfer events that involved the non-coding and coding regions of \textit{avrBs2} between strains sharing a mutual ecological niche. These latter results illustrate the primacy of the ecological environment in the evolution of bacterial virulence. Altogether, our data highlight the importance of considering both promoter and coding region to understand the selective forces shaping virulence-associated genes.
**Xanthomonas arboricola** genomics and type three effector gene distribution

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*Xanthomonas arboricola pv. pruni* (Xap) causes bacterial spot on commercial, ornamental and forest *Prunus* species. Outbreaks can result in severe economic losses to fruit quality and yield, branch/tree dieback, and orchard devastation, particularly on peach, apricot, nectarine and plum. *This stone* is endemic in the USA, NZ and is a quarantine regulated pathogen in Europe and elsewhere, compounding economic loss with eradication and regulatory expenses. Wide host range and climate change patterns portend potential increased invasion risk of this pathogen. Despite the regulatory and economic significance of Xap, almost nothing is known about the genetics of Xap compared to other xanthomonads. To fill this gap, a genome sequencing and comparative genomics approach were undertaken.

We have sequenced the complete genome of a genotypic-representative Xap strain from Europe (Italy, CFBP 5530). This is the first complete genome sequence for this species. Paired-end 454-pyrosequencing and primer walking on a fosmid library gave 3 contigs. The chromosome is 4.85 Mb with 65.6% GC ratio and 3,912 predicted CDS. Xap has a unique 41.2 kb plasmid with a 62.3% GC ratio and 45 CDS. Comparative genomics with other *Xanthomonads* was used to identify *X. arboricola*-specific genes indicating features relevant to differential host specificity and virulence mechanisms. Among those, composition and role of type III secretion system effectors repertoires was surveyed within all six *X. arboricola* pathovars. Applied genomics has identified over 90 VNTR, several of which are currently being used to examine biodiversity and epidemiology of Xap and related pathovars, and design of improved diagnostics.

This first complete genome sequence was also successfully used as a template to perform genomic assembly via mapping of short reads of several isolates from related pathovars. For example, the genome of a Xap isolate GBBC 2038 from the ornamental *P. laurocerasus* was assembled into 93 contigs. These two genomes show very high identity, the main difference being the presence of 10 kb genomic island in the ornamental isolate.

Applied and comparative genomics improved our knowledge on host specificity and biodiversity of Xap, and allowed the development of efficient methods for detection of this plant disease. Overall, the availability of the first genome of *X. arboricola* constitutes a precious knowledge base for better comprehension of all other *X. arboricola* pathovars.
Gene function, C. Extracellular polysaccharides.
Oral Presentations
Glucose flux in *Xanthomonas campestris* towards biomass and polysaccharide biosynthesis.

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Xanthomonads utilize carbohydrates like glucose as a source for energy and building blocks. As plant pathogens, xanthomonads can salvage sugars that originate from breaching plant cell walls at a fairly early phase of virulent interactions and adjust their gene expression accordingly. Both, building blocks and energy derived from imported carbohydrates facilitate the growth of *Xanthomonas* cultures, where they are ultimately turned into biomass. A substantial amount of the imported glucose can be used to synthesize the exopolysaccharide xanthan. Hence, *X. campestris* strains, but also other species like *X. arboricola*, are efficient producers of this thickening agent. Xanthan is involved in pathogenicity and produced industrially in large scale. Now we employed systems biology techniques to analyse the carbohydrate metabolism of *X. campestris pv. campestris*. Mainly by means of the CARMEN and CellDesigner software, a large-scale stoichiometric network of the metabolism was reconstructed from the complete genome sequence of *X. campestris pv. campestris* B100. This metabolic model was suitable for flux-balance analysis (FBA). The model could be used to predict the effects of gene deletions or enzyme inhibition. Metabolic key reactions predicted to affect culture growth at different degrees as well as a reaction specific for xanthan biosynthesis were analysed in detail. When the effects predicted for deleting such reactions were compared to the results obtained by deleting the genes in the wet lab, the stoichiometric model turned out to be a reliable tool for metabolic analyses in *X. campestris pv. campestris*. To validate and refine the FBA model in more detail, we have now started to analyse the metabolic fluxes in *X. campestris pv. campestris* B100 by metabolic flux analysis of bacterial cultures grown with \textsuperscript{13}C-labeled glucose.
Gene function, D. Other pathogenicity factors
Oral Presentations
Roles of DSF-regulated Virulence Factors and biofilm formation in Xanthomonas Pathogenicity

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Xanthomonas campestris pv. campestris and Xanthomonas citri subsp. citri are the causative agents of black rot and citrus canker diseases, in cruciferous and citrus plants, respectively. Cell–cell signalling systems encoded by genes within the rpf cluster are required for the full virulence of both plant pathogens. Those systems have been implicated in regulation of production of extracellular enzymes, cyclic glucan and the exopolysaccharide xanthan and in the regulation of biofilm formation. Cell–cell communication is mediated by the diffusible signal factor (DSF), an unsaturated fatty acid. We have made progress in understanding the roles of xanthan, cyclic glucan and biofilm development in the interaction of X. Campestris and X. citri with plants and of the mechanistic basis of regulation of these processes by DSF. New roles for xanthan and cyclic glucan in disease through suppression of plant immune responses have been uncovered. We have also revealed the capacity of X. campestris to modulate stomatal aperture as other bacterial strategy of defence suppression by a DSF-regulated factor with unknown structure. Xanthan induces susceptibility to X. campestris in Arabidopsis thaliana and Nicotiana benthamiana by suppressing basal defences such as callose deposition. Unlike xanthan, which acts only locally, the effects of cyclic glucan on plant defense suppression and callose deposition occur in a systemic fashion. Crystal violet staining and confocal laser scanning microscopy analysis of the bacteria expressing the green fluorescent protein were used to evaluate attachment and biofilm formation on abiotic and biotic (leaf) surfaces. The extracellular polysaccharide xanthan (EPS) played a key role in the biofilm maturation, survival on leaf surfaces and virulence. By generation of two mutants: X. citri fliC (flagelin gene) and X. citri flgE (hook gene), both involved in the flagellar structure, we demonstrated that biofilm formation is a flagellar-dependent process in X. citri being important in the formation of mushroom-shaped structures and water channels, and in the dispersion of pioneer cells from the mature biofilm. The absence of flagellin produced a slight reduction in X. citri pathogenicity and this reduction was more severe when the complete flagellum structure was absent.
**Functional analysis of flagellin and its glycosylation during interactions between *Xanthomonas oryzae pv. oryzae* and rice.**

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Flagellin, the major component of bacterial flagellum, can elicit innate immune response in plants. Posttranslational modification of flagellin, such as glycosylation plays an important role in determining the consequence of this interaction. *Xanthomonas oryzae pv. oryzae* (Xoo), the causal agent of bacterial blight disease of rice, possesses a single polar flagellum. The genomic sequence of Xoo strain PXO99A has revealed an island in the central part of flagellar regulon, comprising of 10 genes putatively involved in glycosylation modification, which implied that the flagellin of Xoo might be modified by glycosylation. We were interested in elucidating the role of flagellin and its posttranslational modification during the interactions between Xoo and plants. In this study, we generated a fliC gene mutant in wild-type PXO99A and characterized its phenotypes. Compared with wild-type strain PXO99A, the fliC mutant lost flagellum and motility. Pathogenicity assays showed the fliC mutant was slightly impaired in its ability to cause disease on rice plant. Crude extracts containing Xoo flagellin were obtained and tested in rice cell suspension. The results showed no alkalization, \( \text{H}_2\text{O}_2 \) generation or cell death was triggered, suggesting that Xoo flagellin might not elicit defense response in host rice cells. We suspected that the flagellin might be glycosylated, which prevented it from being recognized by the host cells. Western blot analysis demonstrated the size of FliC was smaller in the mutant defective in rfbC, a gene encoding O-antigen biosynthesis protein in the glycosylation island. This result was consistent with our hypothesis. More experiments to decipher the glycosylation mechanism of Xoo flagellin, and its influence on the interactions between flagellin and plant cells are underway.
**Xanthomonas albilineans** is able to move outside of the sugarcane xylem despite its reduced genome and the absence of a Hrp type III secretion system.

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*Xanthomonas albilineans*, the causal agent of leaf scald disease of sugarcane, is a pathogen that experienced genome reduction during its speciation. Additionally, this xanthomonad is notably missing the Hrp type III secretion system and the xanthan gene cluster that are commonly found in pathogenic *Xanthomonas* species. *X. albilineans* was up to now considered as limited to the xylem of sugarcane. However, recently published studies suggested that *X. albilineans* was able to invade tissues other than the xylem of sugarcane leaves but the occurrence of *X. albilineans* outside the xylem has not been clearly proven. In this study, we used confocal microscopy and transmission electron microscopy to investigate the localization of this pathogen in diseased leaves and stalks of sugarcane. Three sugarcane cultivars with different levels of resistance to leaf scald were inoculated with the green fluorescent protein labelled *X. albilineans* strains XaFL07-1 (from Florida) and GPE PC73 (from Guadeloupe). Sections of sugarcane leaves and stalks were examined 8-60 days after inoculation in order to localize *X. albilineans* in the different plant tissues. Confocal microscopy observation of symptomatic leaves confirmed the presence of the pathogen in the protoxylem and the metaxylem, however, *X. albilineans* was also observed in the phloem, the parenchyma and the bulliform cells of the leaves. Similarly, the protoxylem and the metaxylem of infected sugarcane stalks were invaded by *X. albilineans*. Surprisingly, the pathogen was also observed in apparently intact storage cells of the stalk and in the intercellular spaces between these cells. Several of these observations made by confocal microscopy have been confirmed by transmission electron microscopy. *X. albilineans* can therefore no longer be considered as a xylem-limited pathogen. To our knowledge, this is the first description of a plant pathogenic bacterium invading apparently intact non-vascular plant tissue and multiplying in parenchyma cells. The mechanisms and virulence factors used by *X. albilineans* to enter and invade different tissues of sugarcane remain to be identified.
**Structural and Functional Studies of a Type II PilZ-FimX\textsuperscript{EAL} - c-di-GMP Ternary Complex Essential for *Xanthomonas campestris* T4P Function**

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Bacterial type IV pilus (T4P) is a non-flagellar machinery mediating diverse cellular functions, such as surface motility, biofilm formation and pathogenicity. Recent data indicate that T4P biogenesis is initiated via interaction of a non-canonical type II PilZ protein with the FimX and PilB ATPase under high c-di-GMP concentration. However, molecular details of such interactions remain to be elucidated. We now report the hetero-complex crystal structure between a type II PilZ\textsubscript{1028} protein and a FimX\textsuperscript{EAL} from *Xcc* (*Xanthomonas campestris* pv. *campestris*) in the presence of c-di-GMP. We demonstrate that c-di-GMP is indispensable for the stable formation of type II *Xcc* PilZ\textsubscript{1028} - *Xcc* FimX\textsuperscript{EAL} complex, which is evidenced by a variety of biophysical methods including ITC and gel filtration chromatography. We also show that binding of type II *Xcc* PilZ\textsubscript{1028} protein induces dimerization of *Xcc* FimX\textsuperscript{EAL} domains via a N-terminal helix swapping to form a (*Xcc* PilZ\textsubscript{1028})\textsubscript{2} - (*Xcc* FimX\textsuperscript{EAL} - c-di-GMP)\textsubscript{2} hetero-tetramer complex. In addition, we also observed considerable flexibility for c-di-GMP, which is demonstrated by the discovery of two novel monomeric structures for c-di-GMP — a "bulged" form in the *Xcc* FimX\textsuperscript{EAL} - c-di-GMP complex and an "extended but twisted" form in the *Xcc* PilZ\textsubscript{1028} - *Xcc* FimX\textsuperscript{EAL} - c-di-GMP complex. Extensive *in vitro* and *in vivo* studies were further carried out using a series of *Xcc* PilZ\textsubscript{1028} and *Xcc* FimX\textsuperscript{EAL} variants to confirm the unique binding modes of c-di-GMP and the importance of the *Xcc* PilZ\textsubscript{1028} - *Xcc* FimX\textsuperscript{EAL} - c-di-GMP complex in controlling *Xanthomonas campestris* T4P-initiated motility. Altogether, the results represent an important step toward understanding how bacterial T4P function is controlled by c-di-GMP in molecular level.
Plant defense, PTI, ETI and suppression/evasion
Oral Presentations
High throughput identification of TAL effector targets and their use in engineered resistance to *Xanthomonas*

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Transcription activator-like (TAL) effectors from *Xanthomonas* are type III secreted DNA binding proteins that activate effector-specific host genes for disease susceptibility or resistance. Each TAL effector’s central repeat region determines its specific DNA binding site. In each repeat, amino acids 12 and 13 (the repeat variable diresidue, RVD) together specify a single binding site nucleotide through base-specific contacts of residue 13 with the plus strand of the DNA. Discovery of this RVD-nucleotide binding code has enabled prediction of previously unknown TAL effector targets as well as the design of custom TAL effector-based fusion proteins to target novel sequences. *Xanthomonas oryzae* pathovars oryzae (*Xoo*) and oryzicola (*Xoc*) cause rice bacterial blight (BB) and bacterial leaf streak (BLS), respectively. Representative strains we sequenced encode 15 and 26 TAL effectors respectively, yet most have unknown targets. Using a scoring system based on RVD-nucleotide association frequencies for known TAL effector-target pairs, we scanned the rice promoterome for candidate binding sites for *Xoo* and *Xoc* TAL effectors. Cross-referencing these lists with microarray data, we identified 65+ candidate target genes, and subsequently verified 19, including at least one that plays a major role in BLS. Comparison of validated and non-validated predictions provided a set of characteristics useful for predicting or engineering functional binding sites. Our approach, combined with much-needed technologies to rapidly inventory TAL effector sequences in regional *Xanthomonas* populations, can identify candidate TAL effector targets in a high-throughput way. Identifying conserved and important targets opens up the possibility of engineering host resistance to *Xanthomonas* by placing TAL effector binding site elements (EBEs) in the promoters of executor resistance genes. Using this approach, we added six distinct EBEs for *Xoo* and *Xoc* TAL effectors to the promoter of the *Xa27* gene for BB resistance, resulting in broadened specificity for additional *Xoo* strains and all tested *Xoc* strains. However, the added EBEs contain sequences apparently under selection in rice promoters, suggesting they may act as endogenous regulators and could activate the cell death-inducing resistance gene inappropriately under some conditions. Therefore, caution is warranted, and promoter engineered plant lines should undergo extensive testing prior to commercial deployment.
Update on the interaction between *Xanthomonas campestris pv. vesicatoria* and its hosts pepper and tomato

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We study *Xanthomonas campestris pv. vesicatoria* (Xcv), the causal agent of bacterial spot disease on pepper and tomato. Essential for bacterial pathogenicity is the type III secretion system that delivers more than 25 different effector proteins (Xops, *Xanthomonas* outer proteins) into the plant cell cytoplasm. Little is known about the role of individual effector proteins in susceptible plants. In contrast, resistant tomato and pepper lines specifically recognize certain "injected" effector proteins, designated “avirulence” (Avr) proteins. Effector recognition in many cases results in induction of the hypersensitive reaction (HR). The HR is a rapid, localized cell death reaction of the infected plant tissue that leads to a halt of bacterial proliferation. A well-studied effector is AvrBs3, the founding member of a large family of type III effector proteins, also termed TAL (transcription activator-like). AvrBs3 is a transcription factor in the plant cell and induces hypertrophy (cell enlargement) of mesophyll cells in susceptible host plants as well as other solanaceous plants. In resistant ECW-30R pepper plants that carry the dominant resistance gene Bs3, AvrBs3 induces the HR. AvrBs3 activities depend on a central region of 17.5 nearly-identical 34-aa repeats that represent a novel DNA binding motif, nuclear localization and the presence of an acidic activation domain. Recent data on AvrBs3 and other type III effectors will be presented.
Several rice SWEET/nodulin-3 family members can function as virulence targets for *Xanthomonas* TAL effectors

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Bacterial plant pathogenic Xanthomonads translocate TAL effectors into plant cells to function as specific plant transcription factors via a novel programmable DNA-binding domain. Several TAL effectors from rice-pathogenic *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains constitute important virulence factors, but the role of target induced genes is poorly understood. The *Xoo* TAL effectors TalC and AvrXa7, from strains PXO86 and BAI3 that originate from the Philippines and Burkina Faso respectively, direct expression of the rice gene *Os11N3* as a common target. The induction of *Os11N3* or its homolog *Os8N3* is essential for disease development and it was recently shown that both genes encode sugar transporters, which might play a role in the nutrition of pathogens. Presumably, *Xoo* TALs upregulate these genes in parenchyma vascular cells, thus leading to an increase of sugar concentration in the xylem which is the ecological niche of *Xoo*. Both of these susceptibility genes belong to the SWEET/nodulin-3 gene family, which is conserved in plants, humans, nematodes and insects.

We identified Tal5 as a novel TAL effector from African *Xoo* strain MAI1 isolated in Mali, which also induces expression of rice *Os11N3*, as shown by Q-PCR, GUS reporter and pathogenicity assays. Interestingly, Tal5 DNA target box differs from those of TalC and AvrXa7, illustrating a case of functional convergence for the induction of *Os11N3* by distant *Xoo* strains and lineages. To further validate this plant virulence target, we constructed artificial TAL effectors (ArtTALs) that recognize different *Os11N3* DNA sequences than the three natural TAL effectors. These ArtTALs efficiently complemented an *Xoo talC* mutant demonstrating that induced expression of this particular plant gene is crucial to support *Xoo* virulence. To further analyze if other members of the rice SWEET/nodulin-3 family can function as potential virulence targets, we constructed a series of ArtTALs that specifically target individual members of the rice SWEET/nodulin-3 family. Our results identified several novel TAL effector virulence targets and validated the importance of the rice SWEET/nodulin-3 family for *Xanthomonas* virulence.
Bioinformatic strategies to predict TAL effector binding sites in plant genomes

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TAL effectors are proteins from the genus \textit{Xanthomonas} that upon translocation into plant cells via the type III secretion system bind to promoter regions of plant genes and induce their expression. The code for TAL-DNA binding specificity was recently discovered and the structures of TAL-DNA complexes have been also described. A pair of two amino acids within tandemly repeated protein modules, called RVD (repeat variable diresidues), binds specifically to one base pair of the DNA molecule. As more and more \textit{Xanthomonas} TAL effectors are being identified in different species and pathovars of \textit{Xanthomonas}, there is a need for fast and customizable software for TAL binding site prediction. We developed two bioinformatics strategies to search for candidate TAL binding sites in plant genomes. Both algorithms use a positional weight matrix (PMW) to translate a set of RVDs into possible binding sequences. This matrix was carefully constructed using knowledge from published specificities for different RVDs and information from structural data. One strategy, named Storytaller, uses the sequences generated with the PMWs to construct a hidden markov model that is then used to scan promoter regions using HMMER. The other strategy, named Talvez, uses a log-likelihood scoring system to identify possible binding sites according to the PMWs. Both programs were tested against a set of experimentally validated positive (increased gene transcription) and negative TAL-DNA interactions. Both algorithms accurately scored positive interactions significantly higher than negative interactions, with Talvez performing better than Storytaller, and they both performed better than other published similar strategies. Both programs were also able to identify binding sites in promoter regions of genes shown to be induced in response to TAL effectors in different microarray data. The screening of known TAL binding sites using these programs also allowed to postulate a position-based scoring model for TAL-DNA interactions. Altogether, these strategies represent a valuable tool for researchers to identify pathogenicity targets for TALs in any plant genome.
RNA-seq pinpoints a novel *Xanthomonas* TAL-effector activated resistance gene in a large crop genome.

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Transcription activator-like effector (TALE) proteins of the plant pathogenic bacterium *Xanthomonas* bind to and transcriptionally activate host susceptibility genes, promoting disease. Immune systems in mono- and dicot plants take advantage of this mechanism, having convergently evolved TALE binding boxes upstream of resistance (R) genes that direct their transcriptional activation by matching TALEs as exemplified by the pepper *Bs3* and rice *Xa27* R genes. We postulated, therefore, that next-generation sequencing based transcriptome profiling (RNA-seq) could generally replace the laborious positional cloning approach in the cloning of TALE-specific R genes. In a proof-of-principle experiment RNA-seq identified a candidate for the novel pepper R gene *Bs4C* that mediates recognition of the *Xanthomonas* TALE protein AvrBs4. Genetic mapping and complementation studies confirmed that the candidate transcript indeed corresponds to the pepper *Bs4C* gene. These findings demonstrate that TALE-specific R genes can be cloned from large-genome crop species with a highly-efficient RNA-seq approach.
Molecular genetics of interactions between *Xanthomonas campestris* and *Arabidopsis thaliana*

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*Xanthomonas campestris* is a bacterial pathogen in vegetable brassicas (*e.g.* cabbage, broccoli and kale), ornamentals (*e.g.* wallflower and garden stocks) and closely related wild species including *Arabidopsis thaliana*. Black rot and leaf spot are distinct diseases caused by different pathovars named *X. campestris pv. campestris* (*Xcc*) and *X. campestris pv. raphani* (*Xcr*), respectively, which can result in significant crop losses. Our aim has been to improve an understanding of innate defense against *Xanthomonas* using *A. thaliana* as a model for molecular genetic investigation. Natural phenotypic variation was observed among 20 *A. thaliana* accessions, from a worldwide distribution, following inoculations with isolates representing two common races of *Xcc* and three races of *Xcr*. Several accessions were identified which can replicate the pattern of reactions amongst these races in the original brassica host differential. Fine mapping of a gene that confers resistance to *Xcr* in *A. thaliana* Columbia (RXCR1) has defined a 268-kilobase interval on chromosome 3. Bacterial genomics will also be investigated using whole genome sequence of fourteen isolates representing most of the known races of *Xcc* and *Xcr*. Comparative genome analysis will be used to investigate presence/absence and DNA sequence variation in pathogenicity-related genes.
Transgenic crops expressing dispersin B: a novel strategy for controlling Xanthomonas infections?

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Many members of the Proteobacteria produce poly-N-acetylglucosamine (PNAG), an extracellular polysaccharide that mediates intercellular adhesion, tissue attachment, biofilm formation and immune evasion. Among the xanthomonads, genes homologous to the PNAG biosynthetic genes are present in the genomes of X. citri, X. oryzae, X. oryzicola, X. musacearum, X. vasculorum, X. malvacearum, X. gardneri and X. vesicatoria. Dispersin B (DspB) is a bacterial enzyme that degrades PNAG. Among proteobacterial phytopathogens, DspB has been shown to inhibit biofilm formation by Ralstonia solanacearum and to disaggregate highly aggregated clumps of X. citri. DspB also inhibits biofilm formation by Pseudomonas fluorescens and Pectobacterium carotovorum subsp. carotovorum. Transgenic tobacco plants expressing DspB are resistant to P. carotovorum subsp. carotovorum infection in vivo. Since many xanthomonads produce PNAG, engineering crops to express DspB may be a useful strategy for controlling infections caused by Xanthomonas spp. in a range of food crops such as banana, rice and others. Since the DspB transgene strategy targets biofilm formation as opposed to bacterial growth, it is less likely to promote resistance and may provide a long-term infection control strategy.
Characterisation of the cassava-Xanthomonas axonopodis pv. manihotis pathosystem and engineering of resistance to cassava bacterial blight.

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Cassava (Manihot esculenta) is used as carbohydrate source by nearly a billion people in developing countries and has an increasing importance for industrial starch and biofuel production in Asia. Crops affected by cassava bacterial blight (CBB, caused by Xanthomonas axonopodis pv. manihotis or Xam), the major bacterial disease affecting cassava growing regions across South America, Africa and Asia, can suffer yield losses up to 100%. We have established an in vitro systemic inoculation procedure and tested model African cultivar TMS 60444 plantlets with over 30 geographically and temporally diverse Xam strains. Our results revealed that African strains show higher levels of virulence than South American strains, suggesting co-evolution and adaptation of Xam strains for maximum virulence against regional cassava varieties. Susceptibility of additional varieties from Africa and South America to a subset of these Xam strains is currently being evaluated to further characterise this hypothesis, results of which will be presented. Matrix analysis of Xam virulence and the presence/absence of type III effectors identified by Illumina sequencing of more than 60 Xam strain genomes revealed several effectors that are likely to play a role in virulence. In order to determine the relative contribution of various defense pathway responses to pathogen infection in cassava, results of targeted qRT-PCR analysis of compatible and noncompatible pathogen interactions will be presented, as will analysis of defense responses caused by high and low virulence strains in susceptible and more tolerant varieties. To date, no cassava variety has been shown to be resistant to infection with any tested Xam strain; therefore, it is likely that transgenic approaches will be necessary in order to generate cassava varieties that display strong, durable resistance to CBB. Preliminary results from several transgenic strategies undertaken to engineer CBB resistance, including expression of pathogen recognition receptors EFR (Arabidopsis thaliana) and Xa21 (Oryza sativa) and the R-gene Bs2 (Capsicum chacoense), will be presented.
Poster Presentations
Phenotypic and Genetic Characterization of citrus bacterial canker strains in Saudi Arabia by host range, rep-PCR fingerprinting and 16S rDNA analysis.

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A total of 34 strains of Xanthomonas citri subsp citri (Xcc) isolated from Saudi Arabia were examined for their pathogenicity on leaves of Mexican lime (Citrus aurantifolia) and grapefruit (C. paradisi). These strains were grouped into two types (A and A*) based on symptoms induced on leaves of the two citrus species. There were 22 (64.7%) strains belonging to type A which induced typical erumpent canker lesions with water-soaked margin on leaves of all citrus species. Whereas strain in the other type A* caused atypical symptoms on citrus leaves of grapefruit. Strain in type A* which induced typical erumpent canker lesions with water-soaked margin on Mexican lime, but induced flat necrotic lesions with water-soaked margin on grapefruit. Based on physiological, biochemical and genetic characterizations including NaCl tolerance, hydrolysis of gelatin, oxidation of carbon sources, polymerase chain reactions with primers specific to Xcc, rep-PCR and DNA sequence of 16SrDNA strains causing citrus canker in Saudi Arabia belong to the A and A* types of Xcc.
CRISPR-associated sequence diversity within *Xanthomonas albilineans*, the causal agent of leaf scald disease of sugarcane.

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*Xanthomonas albilineans* is a xylem-invading pathogen that causes leaf scald, a lethal disease of sugarcane. Unlike other xanthomonads, *X. albilineans* exhibits a large intra-species variability which was previously observed with different genetic markers (PFGE for Pulsed Field Gel Electrophoresis and MLSA for Multi Locus Sequence Analysis). The CRISPR systems (Clustered Regularly Interspaced Short Palindromic Repeats) are repetitive structures in bacteria and Archaea composed of exact 24- to 48-bp repeated sequences (or "repeats") separated by unique sequences of similar length (or “spacers”). Over 40 gene families, which are found nowhere except near these repeats, have been designated collectively as CRISPR-associated (cas) genes. CRISPR/cas systems participate in an antiviral response, probably by an RNA interference-like mechanism. Analysis of the variability of CRISPR spacers is currently used to perform diversity or epidemiological studies in bacteria. The genome sequence of *X. albilineans* revealed the occurrence of two different CRISPR/cas systems in this pathogen. The first system, called CRISPR-1, is associated with seven cas genes and contains repeats of 31 base pairs. It is similar to the CRISPR system found in several sequenced species of *Xanthomonas*. The second system, called CRISPR-2, is associated with six cas genes and contains repeats of 28 base pairs. There is only one *Xanthomonas* pathovar that is known to contain a similar CRISPR-2 system, namely *X. campestris* pv. *raphani*. In this study, we analyzed the polymorphism of the two different CRISPR/cas systems among 21 strains spanning the genetic diversity of *X. albilineans*. We have either sequenced PCR products resulting from amplification of spacers or cas genes, or used sequences from draft genome sequences. Whereas CRISPR-2 is ubiquitous within the 21 strains, CRISPR-1 is absent in three strains. The loss of CRISPR-1 by a common ancestor of these three strains is in accordance with the MLSA phylogeny. As described in other bacteria, we observed a variability of the CRISPR spacers, not only between phylogenetically distant strains, but also between closely related strains (acquisition of new spacers at the 5’ leader-proximal end of CRISPR and deletion or replacement of some spacers in the central region). This polymorphism within *X. albilineans*, which is congruent with previous MLSA and PFGE results, provides a better resolution of the phylogeny of *X. albilineans* strains.
Genetic diversity of *Xanthomonas oryzae* pv. *oryzicola* from West Africa

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Bacterial Leaf Streak (BLS) caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) was first reported in Africa in the 1980s. Following the recent expansion of rice cultivation in West Africa, a substantial reemergence of BLS was observed in Burkina Faso and Mali. *Xoc* strains were isolated from cultivated and wild rice varieties showing BLS symptoms. Samples were collected at various sites in four and three different regions of Burkina Faso and Mali, respectively. A collection of 58 *Xoc* strains was evaluated for virulence on rice varieties. African *Xoc* strains showed high variation in lesion length on susceptible cultivars. A set of strains was further characterized using a Multi Locus Sequence Analysis using seven housekeeping genes. Dendrograms generated for the data sets obtained from MLSA clearly separated different groups among African *Xoc*. RFLP analysis was performed using the TALE *avrXa7* as a probe, resulting in the identification of twenty one haplotypes. PCR-based analyses of two conserved TTSS (*avrRxo1* and *xopW*) also differentiated the strains into distinct groups. *avrRxo1* was detected in only 30% of African *Xoc* strains. Functionality of *avrRxo1* was confirmed by leaf infiltration on rice Kitaake *Rxo1* lines. Sequence analysis of *xopW* revealed three distinct groups among Asian and African *Xoc* strains. Together, our results demonstrate that African *Xoc* strains, while differentiable from the Asian strains, are highly diverse and rapidly evolving.
Genetic resources are a key element for biological research, and being able to count on reliable resources is a crucial point.

The French Collection for Plant Associated Bacteria (CFBP), created in 1973, is a public collection, a biological resource center which missions are to preserve the biodiversity and associated data and to provide these resources to the international scientific community. CFBP is specialized in plant-pathogenic bacteria, and among more than 6000 accession, holds about 1200 Xanthomonas strains representative of the whole known diversity of this genus.

ISO9001 certification guarantees continuous improvement of the service, transparency, traceability and respect of regulations related to strains distribution.

CFBP is part of the CIRM (International Center for Microbial Resources), a French network of 5 microbial collections who aims in improving strains conservation.

In order to improve the quality of resources, CFBP strains are being re-identified on a molecular basis. Two housekeeping genes are sequenced for each strain. These data will permit to i) ensure identity of each strain, ii) construct a database associated to CFBP resources, iii) complete PhyloSearch database, iv) acquire a technique permitting identification of each species and genus present in CFBP, and v) provide data for future research projects.

This project is achieved for all Xanthomonas present in CFBP, and results are visible on the on-line catalog by a label « recommended » or « not recommended » by CFBP.

CFBP website provides access to the on-line catalog and to PhyloSearch, a web-based tool to help identification of bacteria: http://www.angers.inra.fr/cfbp/index_e.html

We encourage researchers to deposit their biological resources in a biological resource center such as CFBP in order to insure permanence of their resources and reliability of their research work.
Cassava bacterial blight, produced by \textit{Xanthomonas axonopodis} pv. manihotis (Xam), is the most important bacterial disease in this crop. Fifteen years ago, migration process and a complex population structure of the pathogen were detected among different agroecological regions in Colombia. Aiming to establish the current status of the population structure of \textit{Xam} in Colombia, three cassava-cropping regions were sampled in the Caribbean region in the country. Approximately, 200 isolates of \textit{Xam} were characterized using AFLPs with four combinations of selective primers. Isolates clustered with 75\% of similarity were designated as a haplotype. Population structure was assessed for each region and subsequently contrasted among regions. In addition, virulence-associated genes were sequenced to determine their potential use as a tool for \textit{Xam} population studies. The results showed structured populations in both Eastern plains and the Caribbean region. However, populations in the Caribbean region presented a higher diversity index compared with those in the Eastern plains. Additionally, migratory processes of the pathogen between different regions could be detected in Colombia. In contrast to AFLP data, a low variation was detected in the Type Three effector (T3E) genes, however, point mutation in \textit{xopE1} and \textit{xopQ} were correlated with AFLP results. This study shows the current condition of populations of \textit{Xam} in Colombia and it contributes to improve the existing bacterial blight control practices.
Taxonomic re-identification of the *Xanthomonas* collection from CIRM-CFBP using partial sequencing of gyrB and rpoD

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The CIRM-CFBP is a Biological Resource Center that holds more than 6000 bacterial strains available to the scientific community (http://www.angers.inra.fr/cfbp/). Most of them are plant pathogenic bacteria. The collection is almost forty years old and many strains were acquired decades ago. At that time, bacterial identification was essentially based on limited phenotypic data and on host of origin. In addition, since then, new taxa have been proposed based on discovery of new micro-organisms or on taxa revision. Within the genus *Xanthomonas*, numerous taxa revisions lead to the description of new species and pathovars (Bull et al. 2010 and 2012). To be able to use the new proposed names in the catalog of CFBP strains, it is necessary to apply a method that discriminates strains at least at the species level. Thus, a priority action of CIRM-CFBP is to re-identify all the accessions with molecular tools, in order to validate their species-identity or to reclassify them. We started with the genus *Xanthomonas*, the second best represented genus within the collection with 1200 accessions. Partial sequencing of housekeeping genes is becoming a gold standard for species identification in many genera. Within the genus *Xanthomonas dnaK, fyuA, gyrB* and *rpoD* were successfully used for species delineation (Parkinson et al. 2007, Young et al. 2008). At CIRM-CFBP we chose partial sequencing of *gyrB* and *rpoD*. Using two loci is a mean to avoid the pitfall of single locus-based identification that could lead to mis-identification due to inter-species homologous recombination. It is also a mean to increase the discrimination level.

Using this approach, we confirmed the identity of most of the strains. Some of them were reclassified in new taxa according to new classification schemes. Results of this study are available in the online catalog. Strains phylogenetically related to the corresponding type or pathotype strain are labeled “recommended by CFBP” and the taxa identified by *gyrB* and *rpoD* is mentioned. The sequences feed a database used by the web-tool Phylosearch which performs phylogenetic trees to help with the identification of unknown isolates.

Bull et al. 2010, J. Plant Pathol. 92(3):551-592
Xanthomonas arboricola pv. fragariae is a doubtful pathovar

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The whole genome sequences of two Xanthomonas arboricola pv. fragariae (Xaf) strains with different geographic origin, pathotype strain LMG 19145 and strain LMG 19146, were drafted and compared. The sequence divergence found in their phylogenetic marker genes \textit{gyrB}, \textit{rpoD}, \textit{dnaK} and \textit{fyuA} suggested that the two Xaf strains were different genomovars of \textit{X. arboricola}. Moreover, type three secretion system (TTSS) genes were found in the genome of strain LMG 19146, whereas none of these were present in the genome of the pathotype strain LMG 19145.

Another ten Xaf culture collection strains were additionally analyzed by 16S rDNA and \textit{gyrB} sequencing and MALDI-TOF MS. The strains were confirmed as \textit{X. arboricola}, however, genetically diverse and with different affiliations to the pathotype strains of other \textit{X. arboricola} pathovars. Specific PCRs for \textit{hrcQ} and \textit{avrBs2} revealed presence of these TTSS genes in only three strains.

Moreover, the Xaf collection strains showed a doubtful pathogenicity in strawberry inoculation tests. Our sequence based analyses and the unclear pathogenic potential question the criteria that are applied up to now for classifying strains as Xaf but also the validity of Xaf as a separate pathovar of \textit{X. arboricola}. 
Molecular diversity of the plant pathogen *Xanthomonas campestris* pv. *campestris* in a collection of isolates from four continents

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A study was conducted to determine the molecular diversity among a collection of 110 *Xanthomonas campestris* pv. *campestris* isolates collected from *Brassica oleracea* (subspecies *acephala* (kale), *botrytis* (cauliflower), *capitata* (cabbage), *gongylodes* (kohlrabi), *italica* (broccoli) and *tronchuda* (Portuguese kale)), *Brassica napobrassica*, *Brassica rapa*, *Iberis* sp. and *Sinapis arvensis*. The collection of isolates represented a global distribution of the most predominant races (1 and 4) in addition to representatives of races 2, 3, 5, 6 and 7. The analysis was based on three genes, the housekeeping gene *gyraseB* and two effector genes, *avrXccC* and *xopXccN*. Sequence typing with *gyraseB* did not discriminate the isolates suggesting it is not a good method of studying phylogenetic differences between isolates belonging to the same pathovar. *XopXccN* offered more discriminatory power than *avrXccC*. It was highly variable with 14 haplotypes and a preponderance of non-synonymous over synonymous mutations observed in the sequenced isolates in comparison to the only six haplotypes observed for *avrXccC*. None of the observed non-synonymous mutations resulted in a non-functional protein because no early stop codons were observed. This suggested that *xopXccN* has possibly been modified by diversifying selection to generate diverse protein products for the purpose of escaping recognition by proteins encoded by host resistance genes. Correlation between race and host subspecies was not observed in either of the phylogenies generated from effector gene analysis. This is possibly due the few effector genes analysed, which do not reflect all of host resistance genes available in the *Brassica* population.
A novel c-di-GMP receptor protein PXO_00403 regulates Hrp expression from *Xanthomonas oryzae pv. oryzae*.

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*Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of bacterial leaf blight of rice, has been an important model to decipher the molecular mechanisms of bacterial pathogen-plant interactions. The genome of Xoo strain PXO99A contains about 26 genes encoding GGDEF, EAL or HD-GYP domain proteins, which are potentially involved in the c-di-GMP signaling pathway. However, their biochemical features and functions are largely unknown. To elucidate the c-di-GMP signaling pathway and virulence regulation in Xoo, we began with investigating the functions of each of these proteins. Here, we identified and characterized one of them, PXO_00403, which possesses GGDEF, EAL, PAS and REC domains. Deletion of *PXO_00403* in PXO99A attenuated the bacterial pathogenicity on the susceptible rice plants, and caused a delayed hypersensitive response (HR) on nonhost tobacco plants. No significant changes in EPS production, flagellar motility and biofilm formation were found in ∆*PXO_00403* compared with PXO99A. Reverse transcriptase quantitative PCR (RT-qPCR) analysis showed type III secretion system (T3SS)-related regulatory genes *hrpG*, *hrpX* and harpin gene *hpa1* were largely down-regulated in ∆*PXO_00403*. Isothermal titration calorimetry demonstrated that PXO_00403 protein binds c-di-GMP with high affinity as a stoichiometry of 1:1 in *vitro*. In addition, no diguanylate cyclase (DGC) or phosphodiesterase (PDE) activity of PXO_00403 was detected, which is consistent with *in silico* analysis showing that its GGDEF and EAL domains were degenerate. Therefore, we concluded that PXO_00403 acts as a c-di-GMP receptor in PXO99A to regulate bacterial pathogenicity and induction of HR in plants. More experiments to decipher the mechanisms by which PXO_00403 is a signal receptor and regulator of virulence gene expression in Xoo are underway.
Quorum sensing mediated by DSF/Rpfxoo regulates T3SS-encoded hrp gene expression of Xanthomonas oryzae pv. oryzae.

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It has been previously shown that bacterial virulence of phytopathogenic Xanthomonas spp. is regulated by quorum sensing (QS), which is mediated by diffusible signal factor (DSF) and signal proteins RpfF, RpfC and RpfG. To further elucidate the regulatory mechanisms in the bacterial blight pathogen of rice X. oryzae pv. oryzae (Xoo), structural features of QS-encoded rpfxoo genes was identified and virulence function were characterized through the bioinformatics and gene mutation analysis, bacterial population of Δrpfxoo mutants in the infacted leaf tissues of rice was detected, and transcription levels of type III secretion system (T3SS)-encoded hrp genes were measured via RT-qPCR assays in this study. Results showed that rpfxoo genes were found to be highly conserved in plant-pathogenic Xanthomonas spp. RpfFxoo was one of enoyl-CoA hydratase family members, RpfCxoo was structurally featured in N-terminal histidine kinase domain and C-terminal receiver (REC) domain, while RpfGxoo contained REC and HD-GYP domains. Δrpfxoo, the gene deletion mutants were generated from the wild-type strain PXO99A. DSF production was deficient in ΔrpfFxoo, ΔrpfF+Cxoo and ΔrpfF+Gxoo, while DSF was overproduced in ΔrpfCxoo and reduced in ΔrpfGxoo. DSF production of ΔrpfFxc, ΔrpfCxc and ΔrpfGcc, the mutants of X. campestris pv. campestris can be restored as the wild-type strain XC1, by in trans complementation of rpfxoo, rpfcxoo and rpfgxoo. All the mutants except Δrpfxoo were remarkably deficient in extracellular polysaccharide (EPS) production. All the mutants significantly exhibited the reduced virulence, population and spread in the leaf tissues rice compared with PXO99A. Transcription of hrp genes (hrpG, hrpX, hpa1and hrpB) was significantly regulated by QS. Expression of rpfxoo genes was regulated by Rpfxoo proteins and bacterial density-dependent. Therefore, it might be suggested that QS regulates T3SS expression via the DSF/c-di-GMP-mediated signaling pathway(s).
Phytopathogenic bacteria of the genus *Xanthomonas* inject type III effectors directly into the cytoplasm of the host plant cell. A special group of type III effectors is the TAL effector family. After injection TAL effectors enter the nucleus where they bind to specific promoter sequences and activate transcription of downstream genes. To mediate specific binding to their DNA target sequence TAL effectors contain a central repeat region that consists of tandemly arranged repeats of a 34 aa motif and functions as a novel DNA binding domain. The amino-acid sequence of the repeats is highly conserved and mainly differs at position 12 and 13, the so called repeat variable diresidue (RVD). Two independent studies deciphered that the DNA specificity is encoded by the RVD composition of the TAL effector in which each RVD is specific for one or more nucleotides (e.g. HD=C, NN=A/G, NI=A, NG=T, NS=A/C/G/T). Recent crystal studies demonstrated that only amino-acid position 13 directly interacts with the nucleotide whereas amino-acid 12 mediates stabilizing contacts with the protein backbone. The modular and predictable binding to DNA target sequences spurred the interest to use TAL effectors as a tool for targeted genome editing and gene expression. Several new assembly methods that include the generation of user defined TAL effector derivatives fused to catalytic domains for example nuclease domains allow the use of TAL effectors in a wide range of applications including different model systems (e.g. plant, mouse, human, zebrafish). Each application relies on highly specific and active TAL effector proteins. The four key RVDs NI, HD, NG and NN confer high specificity but only little is known about the influence of the RVD composition on the overall activity of a TAL protein. In our study we found that RVDs differ in their efficiencies and thereby influence the overall activity of the TAL effector which is an important extension to the known code. Furthermore we analyzed the specificity of new RVDs including NH that is highly specific for G. We compared the efficiencies of the G specific RVDs NN, NK and NH. On the basis of these results we derived rules that help to ensure high specificity and activity of TAL proteins.
Role of type three effectors from *Xanthomonas axonopodis* pv. *manihotis* in the virulence towards cassava.

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*Xanthomonas axonopodis* pv. *manihotis* (*Xam*) is the causal agent of cassava bacterial blight (CBB), one of the most devastating diseases in this crop. This disease can cause complete losses under the appropriate conditions. Previous studies have shown the presence of 19 type three effectors (T3E) in the genome of the model strain *Xam* CIO151 and these proteins, together, have a key role in the virulence of this strain. In this work we performed a systematic mutational analysis to determine the role of T3E on the virulence of this pathovar. Using the double crossing-over technique, we generated single and double mutants for a subset of genes mutants (Δ) for the T3E *avrBs2*, *xopN*, *xopZ*, and *hpaF* in the T3E repertoire of *Xam*. The mutants were inoculated in cassava susceptible cultivar MCOL2215. A reduction in disease symptoms was observed with single and double mutants for *avrBs2*, *xopZ* and *hpaF* when they were compared against the wild type strains. Therefore, these effector genes have a role in the virulence of this bacterium. The mutation effects were tested on additional strains CIO303, CIO536, CIO556, CIO560, CIO650, which are representative of the diversity present in Colombia, and similar results were obtained. This underscores the importance of these proteins in the virulence of *Xam* on Cassava. Results obtained in this study could be useful for the development of durable and sustainable resistance to CBB through the implementation of genes that recognize the effectors that are important for virulence.
Determination of the genetic and functional diversity of TAL effectors from *Xanthomonas axonopodis* pv. manihotis

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The TAL (Transcription Activator-Like) effector family comprises a group of type III-secreted proteins exclusive of the genus *Xanthomonas* and *Ralstonia*. During pathogenicity, they are imported into the host cell nucleus where they bind to upstream DNA sequences of target genes which they trans-activate, for the benefit of pathogen survival and/or multiplication. DNA-recognition is operated through Repeat Variable Di-residues (RVDs) of TAL effectors that match to nucleotides in the target DNA box. Although the N and C terminal regions of these effectors are highly conserved, the RVDs in the central repeat region can have a high degree of variability, thereby generating diversity and possible functional specialization. In this study, we aimed at identifying the molecular diversity of TAL effectors in *Xanthomonas axonopodis* pv. manihotis (*Xam*), the causal agent of cassava bacterial blight, in two groups of strains: some isolated from different regions of the world in the seventies, eighties and nineties, and some collected from the Colombian Caribbean coast and oriental plains from 2008 to 2012. Using Southern-blot analyses, we determined the presence of a variety of TAL effectors among different *Xam* strains. Performing bioinformatic analyses of RVD sequences, we predicted probable target genes with importance in the infectious process for a subset of them. We also compared the resolutive power of TAL effectors with that of AFLPs for determining the genetic distances between the strains under study.
TAL effectors enhance virulence on diverse rice varieties when introduced individually into a TAL effector-deficient strain of \textit{Xanthomonas oryzae}

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Most strains of \textit{Xanthomonas oryzae}, the causal agents of bacterial leaf blight and bacterial leaf streak of rice, encode large numbers of TAL (Transcriptional Activator-Like) effectors. TAL effectors activate transcription of specific host genes, but the roles of individual TAL effectors in virulence in different host plant genetic backgrounds are poorly understood. We transformed the weakly virulent, TAL effector-free strain X11-5A of \textit{X. oryzae} with individual TAL effector genes from more virulent strains to assess the virulence contributions of those TAL effectors in 21 diverse rice varieties. TAL effector genes that activate SWEET family sugar transporters increased X11-5A virulence on 13 of 21 varieties, to varying extents depending on the variety and the TAL effector gene. The previously identified transcriptional target for each effector was strongly activated by that TAL effector in all cultivars tested. These results confirm that SWEET-targeting TAL effectors are major contributors to pathogen virulence, yet reveals that their relative importance in virulence depends on host genetic background.
Effect of the TAL effector protein $\text{PthB}_{Xam}$ from $Xanthomonas axonopodis$ pv. manihotis on the transcriptome of susceptible cassava host plants

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$Xanthomonas axonopodis$ pv. manihotis ($Xam$) is a gram negative bacteria causing the Cassava Bacterial Blight (CBB) in $Manihot esculenta$. The CBB disease represents an important limitation for cassava massive production. Bacterial pathogenicity often relies on the injection of effector proteins inside the eukaryotic host cells via a type III secretion system. Most of these type III effector proteins are involved in suppressing the basal defense responses to promote bacterial colonization and multiplication. TAL (Transcription Activator-Like) form a particular group of effector proteins characterized by tandem repeats of 34 amino acids in the central region, nuclear localization signals and an acidic activation domain at the C-terminus. TALs are translocated to the nucleus and bind directly to the promotor region of target host genes in order to modulate their expression. In $Xam$, the $\text{PthB}_{Xam}$ TAL effector is crucial for pathogenicity (Castiblanco et al., $\textit{in prep.}$). Based on the use of cassava microarrays and RNAseq, we aimed at evaluating the transcriptomic response of susceptible cassava plants challenged with $Xam$ strains carrying or not $\text{pthB}$. Combining both of these approaches, we were able to identify a set of $\text{pthB}$ target gene candidates. We will present our latest data on the nature and functional analysis of these genes induced and repressed by $\text{PthB}$.

Castiblanco L.F., Gil J., Rojas A., Osorio D., Gutiérrez S., Szurek B., López C., Restrepo S., Verdier V. 3, Bernal A.J Characterization of $\text{PthB}_{Xam}$, a TAL effector protein from $Xanthomonas axonopodis$ pv. manihotis. $\textit{In prep.}$
N-acetylcysteine (NAC) and copper as antimicrobial compounds by *Xanthomonas citri* subsp. *citri*.

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*Xanthomonas citri* subsp. *citri* (*Xcc*) is a pathogenic bacteria and the causal agent of citrus canker disease. This bacterium develops a characteristic biofilm on both biotic and abiotic surfaces. Biofilms are known to protect bacteria from environmental stresses, host defense mechanisms, and antimicrobial compounds. The ability of plant pathogenic bacteria to form and detach from biofilms may equally have considerable implications for survival on leaf surfaces and within host plants, spread throughout the plant and the completion of the disease cycle. However, the formation of biofilms by plant pathogens has received relatively little attention to date. NAC is a mucolytic agent that has anti-bacterial properties and also decreases biofilm formation by a variety of bacteria and copper-based products are routinely used as a standard control measure for citrus canker. We investigated if is possible to use the NAC as an antimicrobial compounds together copper. We found that minimum inhibitory concentrations (MICs) of NAC and of copper for *Xcc*. The treatment of first moment with NAC and after 24 hours put copper demonstrated interaction against the *X. citri*. Overall, our data indicate that NAC has anti-bacterial properties against *X. citri*. Use of NAC associated with copper may be a new strategy for the treatment of the control measure for citrus canker.

Financial Support: CNPq / INCT.
Biofilms are known to protect bacteria from environmental stresses, host defense mechanisms, and antimicrobial compounds. The ability of plant pathogenic bacteria to form and detach from biofilms may equally have considerable implications for survival on leaf surfaces and within host plants, spread throughout the plant and the completion of the disease cycle. However, the formation of biofilms by plant pathogens has received relatively little attention to date. Xanthomonas citri subsp. citri (Xcc) is a pathogenic biofilm-forming bacteria and the causal agent of citrus canker. In this study, we investigated biofilm formation by screening in Xcc mutants obtained by random mutagenesis of the transposon EZ::TN<KAN-2>. Bacterial clones were grown in Y minimal medium containing glucose (1% wt/vol) as the only carbon source. Large-scale screening, using colorimetric biomass assay with crystal violet staining, was performed to select clones with defective ability to form biofilm. The genomic DNA of the selected clones was isolated and the position of the transposon insertion was revealed by thermal asymmetric interlaced (TAIL-PCR) targeting unknown DNA sequences contiguous to known kan gene sequences. The sequences obtained were compared and aligned with sequences available at GenBank. In order to evaluate the capability of Xcc mutants to form biofilm in biological material, 2800 clones were analyzed and 160 were selected for their decreased ability to form biofilm. BLAST searches revealed that transposon inactivated open read frames with high identity with putative secreted protein, and an outer membrane receptor precursor, among others. These genes showed downregulate about biofilm formation. Extracellular polysaccharides (EPS) production by X. citri also been quantified. The mutants are being evaluated for pathogenicity in plants of sweet orange. Hypo- and hyper-virulent mutants will be challenged by differential tolerance to antimicrobial compounds. The identification of these genes as potential candidates involved in Xcc biofilm formation may help the understanding of epiphytic life of the bacteria on the leaf before infection and could allow the development of new strategies to control citrus canker.

Financial Support: CNPq / INCT.
Molecular analysis of the role of the bacteriophytochrome protein in the virulence of the plant pathogen *Xanthomonas campestris* pv. *campestris*

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Light is an important source of energy information for a wide range of organisms. In plants and other photosynthetic organisms, photoreceptor proteins called phytochromes regulate functions related to growth and development. Surprisingly photoreceptors called bacteriophytochromes, homologues of the phytochromes, are also found in non-photosynthetic bacteria, including pathogens, although their role is poorly understood. *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot of crucifers is a phytopathogen of worldwide economic relevance. The *Xcc* genome encodes one putative bacteriophytochrome (*phy*). Recent results from our laboratory suggest that light modulates biofilm formation. Furthermore we have established that mutation of *phy* leads to altered level of xanthan polysaccharide, a virulence factor which is also directly involved in biofilm formation. Moreover the absence of the Phy protein in *Xcc* results in an increase of the production of secreted proteins, such as amylases and peptidases. Our results also show that *Xcc phy* is more virulent than the wild-type strain. Part of the *phy* mutant strain virulence may be attributed to its capacity to keep the plant stomata open. We have also studied callose synthesis during the infection and we have observed that it is significantly reduced in the *phy* strain compared to wild-type strain. Taken together, the results we are presenting in this work clearly indicate a direct link between virulence mechanisms and the Phy bacteriophytochrome in *Xcc*. It is likely that Phy senses light, probably Red and Far-Red ratio, modulating the *Xcc* infection cycle.
Analysis of the putative type VI secretion systems in *Xanthomonas campestris* pv. *vesicatoria*

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Recently, type VI secretion systems (T6SS) from several Gram-negative bacteria were characterized. T6SS fulfill different functions and contribute to virulence, antivirulence or interbacterial interactions (1). A set of conserved T6SS-core proteins has been identified and most of them are also encoded in most *Xanthomonas* spp. (2). *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is a plant-pathogenic bacterium that infects pepper and tomato plants (3). The sequenced *Xcv* strain 85-10 harbors two different T6SS-loci, each encoding 15 conserved T6SS-components. To analyse if the T6SS play a role in bacterial virulence we deleted several T6SS-components which were described to be essential for a functional T6SS in other bacteria. An example is the cytoplasmatic ATPase ClpV and the cell puncturing device protein VgrG (4). Here, we present our recent data on the genetic and functional characterization of the putative T6SS in *Xcv*.

Role of chemotaxis in host specificity of xanthomonads

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*Xanthomonas* spp. are plant pathogenic bacteria that are responsible for diseases with a high socio-economic impact worldwide. Events leading to host specificity occur as early as chemotactic attraction by host and adhesion to tissues. Indeed, Mhedbi-Hajri and colleagues have recently shown, using association genetic approach, that the repertoire of genes encoding chemotaxis sensors, other chemical environment sensors and adhesins correlate with the grouping of strains in pathovar and therefore with their pathology (host range and tissue specificity). They also found strong evidence of adaptive divergence acting on chemotaxis sensors and adhesins. The objective of this work is to demonstrate through a functional genetic approach by gene knockout, the involvement of chemotaxis in the host specificity of *X. campestris pv. campestris* (Xcc LMG568). Two mutants were constructed: one was deleted of *cheY*, a gene encoding a key protein in the chemotactic signal transmission and the other strain was deleted of a gene encoding a chemotactic sensor (MCP0324). The *cheY* mutant is not tactical and is altered, in its ability to colonize radish -a host plant- and tomato -a non-host plant- in comparison to the wild type. The strain deleted of the MCP0324 sensor gain the capacity to internalize into leaf tissues of non-host plants such as tomato, bean and stock, in contrast to the wild type which remains confined to the leaf surface of these plants. On the other hand, this mutant lost its ability to internalize into leaf tissues of a host plant such as *Arabidopsis*. Thus, this sensor should detect a repulsive molecule from the non-host plant and/or an attractive one from the host plant. All these data support the importance of chemotaxis in colonization of plants by Xcc leading or not to its internalization into the tissues.
Structure of the PilZ-FimX<sub>EAL</sub>-c-diGMP complex responsible for the regulation of bacterial Type IV pilus biogenesis

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Signal transduction pathways mediated by c-diGMP are ubiquitous in bacteria where they control many important and complex behaviors. C-diGMP is synthesized through the action of GGDEF domains that possess diguanylate cyclase activity and is degraded by EAL or HD-GYP domains with phosphodiesterase activity. Several families of c-diGMP receptors have been characterized, the largest of which are PilZ domains and degenerate (enzymatically inactive) EAL domains. Most Gram-negative bacterial genomes code for at least one and often dozens of different proteins with GGDEF, EAL or HD-GYP and PilZ domains. There is mounting evidence that some important c-diGMP-mediated pathways require protein-protein interactions between members of these domain families. For example, in Xanthomonas species, the control of motility via DSF-dependent signaling during quorum sensing is dependent on interactions between the HD-GYP domain from RpfG and at least two GGDEF domain-containing proteins. Interactions have also been observed between PilZ and the EAL domain from FimX. These latter proteins are involved in the regulation of type IV pilus biogenesis via interactions of PilZ with the hexameric PilB ATPase associated with the bacterial inner membrane. In spite of the importance of protein-protein interactions in c-diGMP-mediated pathways, no high resolution structures of these domains in protein-protein complexes are yet available. Here, we present the crystal structure of the ternary complex made up of PilZ, the FimX EAL domain and c-diGMP. PilZ interacts principally with the lobe region and the N-terminal linker helix of the FimX EAL domain. PilZ interactions with the FimX<sub>EAL</sub> involve both the stitching to together of beta sheets from both proteins as well as a hydrophobic surface made up of amino acids conserved in a non-canonical family of PilZ domains that lack intrinsic c-diGMP binding ability. Interestingly, the c-diGMP binds to isolated FimX<sub>EAL</sub> and to the PilZ-FimX<sub>EAL</sub> complex in a novel conformation never before encountered in c-diGMP-protein complexes in which one of the two glycosidic bonds is in a rare syn conformation while the other adopts the more common anti conformation. While direct PilZ-c-diGMP interactions in the complex are very tenuous, the structure points to a means by which both c-diGMP and PilZ binding could work together to modulate both the relative orientation of the EAL domain with respect to the neighboring GGDEF domain in the FimX protein and interactions between the conserved PilZ C-terminus and PilB.
Molecular characterization of the *Xanthomonas citri* subsp. *citri* Type IV pilus.

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Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (*Xac*), is one of the most serious diseases affecting citrus production worldwide. The pathogen enters host plant tissues through stomatal openings and wounds and then colonizes the apoplast causing the break of the epidermis due to cell hyperplasia. The infection is visualized as raised lesions on fruit, foliage and young stems. Severe disease can cause defoliation, dieback and fruit drop reducing production volume and causing high market losses. The genome of Xac has been completely sequenced and gives insights into the importance of genes and gene clusters governing strategic mechanisms of pathogenesis. Bacterial T4P are long, flexible surface filaments involved in a variety of important bacterial behaviors, including twitching motility, surface adhesion, pathogenicity, natural transformation, immune escape, biofilm formation, phage transduction, protein secretion and chemotaxis. The filament of the T4P is composed of a helical polymer of mostly pilin subunits. Cycles of polymerization, attachment and depolymerization mediate several pilus-dependent phenomena. The sequenced Xac genome codes for a large set of genes involved in T4P biogenesis and regulation, including several pilin homologs. We produced a Xac knockout strain in the gene coding for the major pilin subunit (*fimA*) and expressed and purified recombinant Xac pilin in *E. coli*. Bacterial suspensions of the *fimA* strain were sprayed onto leaves of host plant orange (*Citrus sinensis*). The *fimA* mutant strain produced a lower number of cankers as well as a different morphology compared with wild type *Xac*. In order to further characterize the function of the T4P, we analyzed the production of biofilm on polystyrene and glass surfaces and observed that the *fimA* mutant formed a more robust biofilm than wild type *Xac*. In addition, microscopy analyses were performed for compare patterns of bacterial migration between knockout and wild type *Xac* strains. The results of this study improve our understanding of how *Xac* T4P influence bacterial migration, biofilm and pathogenicity.
Identification of a large repertoire of TAL effectors in *Xanthomonas campestris* pathovars and their plant targets.

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Transcription Activator-Like (TAL) genes, also known as *Hax* (homologues of *avrBs3* in *Xanthomonas*), encode Xanthomonads-specific transcription factors which are injected inside plant cells through the type 3 secretion system to activate expression of specific host genes. *Xanthomonas campestris* (Xc) infects Brassicaceae and are organized into 3 pathovars: *campestris* (Xcc), *incanae* (Xci), and *raphani* (Xcr). Here, we used AFLP to phylogenetically organize a collection of 62 Xc strains covering all the pathovars isolated on various host plants worldwide. Interestingly, though TAL genes are absent in the 3 Xcc reference strains for which genomic sequence is available, we have identified TALs in 32 out of 51 Xcc strains studied. So far, we have cloned and sequenced 46 TALs including 41 from pv. *campestris*. Corresponding proteins are organised in 22 haplotypes and 11 families (namely Hax2 to Hax12) based on their RVD sequences and inferred specificities. Moreover, while most RVD were already identified in known TALs, we also identified one yet undescribed RVD sequence in Hax7 family, which specificity is being determined. Finally, using *in silico* prediction tools, we searched for TALs target promoters in the genome of *B. rapa*, a natural host of Xc. Our progress in the experimental validation of these targets by qRT-PCR experiments will be presented.
Dynamic interplay between abscisic acid, cytokinin and salicylic acid molds innate immunity of rice against the leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*.

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Although recent findings have uncovered the plant hormone abscisic acid (ABA) as a key determinant of plant-pathogen interactions, little is known about the underlying mechanisms. Aiming to further decipher the molecular logic of ABA-modulated immunity, we have analyzed the impact, dynamics and interrelationship of ABA with other hormones during progression of rice infection by the leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (XOO). Interestingly, exogenous application of ABA compromises basal immunity to XOO, whereas chemical disruption of endogenous ABA levels enhanced resistance. Moreover, successful XOO infection stimulated endogenous ABA synthesis, indicating that in planta ABA levels are positively correlated with XOO growth in rice plants and, hence, that ABA is a virulence factor for XOO. Several lines of evidence demonstrate that this immune-suppressive effect of ABA is due at least in part to suppression of effectual defences controlled by the classic immune hormone salicylic acid (SA). In addition, ABA restricts an independent disease susceptibility pathway regulated by the plant hormone cytokinin (CK). Exogenous application of kinetin, a natural adenine-derived CK, rendered rice more susceptibility to XOO. However, gene expression analyses revealed that CK and ABA interact in a mutually antagonistic manner. Moreover, SA negatively interferes with both ABA and CK pathways. Together, these results underscore the importance of multidirectional ABA-CK-SA crosstalk in shaping the outcome of rice-XOO interactions and favor a scenario whereby virulent XOO hijacks the rice ABA machinery to inflict disease.
The effector HpaF from Xanthomonas axonopodis pv. manihotis is a virulence determinant that suppresses plant defense responses

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Bacterial Type Three effectors (TTE) are proteins that play crucial roles in the pathogenesis process in plants and animals. These proteins have been studied in diverse plant-pathogen interactions. However, roles for the TTEs of Xanthomonas axonopodis pv. manihotis (Xam), a cassava bacterial pathogen, have not been extensively studied. To determine the importance of TTEs for Xam pathogenicity, we undertook a mutagenesis approach. The individual disruption of several effector genes did not affect the virulence of Xam. However, the disruption of hpaF reduced the ability of Xam to cause symptoms on cassava plants. Additionally, ectopic expression of hpaF in Arabidopsis thaliana partially suppressed PAMP-dependent callose deposition and the production of reactive oxygen species, based on assays performed with the heterologous species Pseudomonas fluorescens. HpaF was also able to suppress the hypersensitive response (HR) elicited by HopA1 in tobacco plants. Furthermore, three potential HpaF target proteins were identified in cassava using a yeast two-hybrid assay, and co-immunoprecipitation assays were performed to test these interactions. The data provide insights into the role HpaF plays during the interaction between cassava and Xam.
Molecular action of *Xanthomonas* effector proteins in the plant cell

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The plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* delivers more than 25 effector proteins into the plant cell cytoplasm via the type III-secretion (T3S) system. In susceptible pepper and tomato plants, type III effectors interfere with host cell processes to the pathogen’s benefit. One example is AvrBs3, which functions as transcription factor and induces hypertrophy of mesophyll cells in susceptible pepper and tomato plants. We are currently assaying for the molecular function of other type III effectors, termed Xop (*Xanthomonas* outer protein), in the plant cell. In contrast to AvrBs3, the effector proteins XopG and XopI probably are involved in plant protein stability. The effector protein XopG has homology to zinc-dependent metalloproteases, e.g. butulinum and tetanus neurotoxins, including the zinc-coordinating motif. Whether XopG has protease activity in the plant cell is not known yet. Transient expression of XopG induces cell death reaction in leaves of pepper plants and *Nicotiana tabacum*. Mutant studies revealed the importance of a zinc-binding sequence element for these phenotypes. We will present data on the biochemical characterization and cell death induction of XopG in *planta*. Homology searches for the effector protein XopI revealed an F-box, which is known to be part of the SCF-complex which mediates protein degradation. Therefore XopI potentially mimics plant F-box-proteins to engage protein turnover *in planta*. We will present interaction studies of XopI with plant proteins.
Evaluation of resistance to *Xanthomonas citri* subsp. *citri* in transgenic citrus plants overexpressing *AtNPR1* gene

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Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (Xcc), is a main problem in Brazil, where a well-established eradication program is employed to control the disease. However, despite its local efficacy, new disease foci are frequently detected. Because most citrus varieties are susceptible to the disease, the development of resistant genotypes through genetic engineering is considered to be a potential control strategy. The *NPR1* gene is a key regulator of the salicylic acid (SA)-mediated activation of pathogenesis related genes (PR) at the onset of systemic acquired resistance (SAR). Its overexpression in transformed citrus plants increased disease resistance and induced the expression of PR genes. The main objective of this work was to evaluate the response of transgenic plants of Hamlin sweet orange carrying the *AtNPR1* gene to inoculations with Xcc. Leaves were inoculated by spreading a bacterial suspension (10⁶ UFC/ml) over the lower leaf surface with a cotton swab dipped in inoculum. Plants were kept in the greenhouse and the lesion area was evaluated 30 days after inoculation. The expression of the transgene and of the PR2 gene was quantified by RT-qPCR. Differences in transgene expression of up to 15 fold were detected among the six transgenic plants and the two plants that expressed the higher levels were more resistant to Xcc as their lesion areas were reduced by 50 to 80% compared to non-transgenic plants. Interestingly, the expression of PR2 in these two transformants was also high. These results indicated that the heterologous expression of *AtNPR1* increased the resistance to *Xanthomonas* in transgenic Hamlin and induced the expression of PR2, which is considered a marker of systemic acquired resistance.
**In vitro screening to evaluate *Xanthomonas citri* subsp. *citri* in transgenic citrus plants**

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*Xanthomonas citri* subsp. *citri* is the causal agent of citrus canker, an important disease of citrus crop. In Brazil, eradication of infected plants is mandatory in the State of Sao Paulo and citrus growers spend more than US$ 30 million on eradication practices. Most of the citrus varieties are susceptible to canker, but under field conditions tolerance is observed in some genotypes. The development of fast and reliable methods of screening for identification of resistant genotypes is of great importance in citrus breeding. An *in vitro* screening method was used to evaluate the resistance of transgenic plants expressing the *AtNPR1*. This gene is a key regulator of salicylic acid (SA)-mediated activation of the plant immune system. Leaves of transformed sweet orange (*Citrus sinensis* cv ‘Hamlin’) plants, 60-70% expanded were collected from greenhouse grown plants. Leaves were disinfested and injected with a suspension \((10^6 \, \text{CFU/ml})\) of *X. citri* subsp. *citri* strain 306, cultured on NA medium at 28ºC and resuspended in PBS buffer. The injection was performed with a syringe with or without a needle in six leaves at three inoculation points on each leaf. Mock inoculations were performed in the same leaves but at the opposite side in relation to the midrib. After inoculations, leaves were placed in Petri plates containing 0.3% agar/water and maintained under a 16-h photoperiod at 27 ± 1 °C. The experiment was repeated four times. The lesion area was measured 14 days after inoculation and the results were compared with the performance of the transgenic plants when inoculated in the greenhouse in a previous experiment. The responses of the leaves of the transgenic plants *in vitro* correlated well with those observed in the corresponding plants in the greenhouse, indicating that the method was adequate, faster and easy, especially when the inoculation was done using a needle. The proposed method facilitates the identification of resistance genotypes to citrus canker.
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