PROGRAM

ABSTRACTS

and

PARTICIPANT DIRECTORY

Xanthomonas Genomics Conference 2009

Pingree Park, Colorado State University, USA
July 13-15, 2009

<u>**Organizing committee:**</u>

Adam J. Bogdanove, Iowa State University, Chair
Jeffrey B. Jones, University of Florida
Jan Leach, Colorado State University, co-Chair
Mary Beth Mudgett, Stanford University
Frank F. White, Kansas State University

Program

Talks are 25 minutes including questions unless otherwise noted

Monday, July 13

14:00-19:30 Check in and Registration

19:00-21:00 Reception

19:30 Welcome and Introduction to Pingree Park, Adam Bogdanove, Conference Chair, and Deborah Cowan, Pingree Park Coordinator

Tuesday, July 14

7:00-8:00 Breakfast

8:00-10:00 Session I: Genome sequencing, global genomic diversity of *Xanthomonas spp*. and molecular diagnostic tools. Moderator, Tika Adhikari

Adriana Bernal, Universidad de los Andes. "What it takes to infect cassava: genomics of *Xanthomonas axonopodis* pv. manihotis"

Marie-Anne Van Sluys, IBUSP, Sao Paulo and Colorado State University. "IS elements produce natural genome variation and shape *Xanthomonas* genomes"

Lionel Gagnevin, Université de la Réunion. "New powerful tools for epidemiological analyses of *Xanthomonas citri* pv. citri with a strong evolutionary aftertaste"

Richard Thwaites, Food and Environment Research Agency, York, U.K. "Biology, phylogeny and genomics of banana *Xanthomonas* wilt, an emerging disease in East Africa"

(15 min.) Casiana Vera-Cruz, International Rice Research Institute. "Genomics-based diagnostic marker development for *Xanthomonas oryzae* pv. oryzae and *X. oryzae* pv. oryzicola"

10:00-10:15 Break

10:15-12:15 Session II: Comparative and functional *Xanthomonas* genomics. Moderator, Jan Leach

Philippe Rott, CIRAD, Montpellier, France. "Genomics and functional studies of pathogenicity factors of *Xanthomonas albilineans*, an unusual xanthomonad causing sugarcane leaf scald"

Charles Manceau, INRA, Beaucouzé, France. "A multilocus sequence analysis of *Xanthomonas campestris* reveals a complex structure within crucifer-attacking pathovars of this species"

Prabhu Patil, University of Nebraska. "Function and evolution of lipopolysaccharide biosynthetic gene clusters in xanthomonads"

Valérie Verdier, IRD-CNRS, Université de Perpignan. "New insights into the genome of African *Xanthomonas oryzae* pv. oryzae strains"

(15 min.) Cornelius Schubert, Martin Luther University, Halle, Germany. "Identification and characterization of novel genes involved in the virulence of *Xanthomonas campestris* pv. vesicatoria"

12:15-13:15 Lunch

13:45-15:45 Session III: Gene function, A. Type III effector diversity and function. Moderator, Boris Szurek

Seiji Tsuge, Kyoto Prefectural University. “Genome-wide screening for T3S effectors in *Xanthomonas oryzae* pv. oryzae”

Bing Yang, Iowa State University. “Type III effector diversity and function in *Xanthomonas oryzae* pv. oryzae”

Mary-Beth Mudgett, Stanford University. “Xanthomonas T3S effector XopN suppresses PAMP-triggered immunity and interacts with a tomato atypical receptor-like kinase and TFT1”

Ulla Bonas, Martin Luther University, Halle, Germany. “New insights into type III effectors from *Xanthomonas campestris* pv. vesicatoria ”

(15 min.) Ahmed Hajri, INRA, Université d'Angers. “The ‘repertoire for repertoire’ hypothesis: repertoires of type III effectors may explain host specificity in *Xanthomonas*”

15:45-16:00 Break

16:00-18:00 Session IV: Gene function, B. Gene regulation and signaling in host-pathogen interactions. Moderator, Ulla Bonas

Sang-Won Lee, University of California Davis. “A Type I secreted, sulfated peptide triggers rice XA21-mediated innate immunity”

Robert Ryan, National University of Ireland, Cork. “Cyclic di-GMP signaling and virulence in the plant pathogen *Xanthomonas campestris* pv. campestris”

Max Dow, University College Cork. “Physical interactions between HD-GYP and GGDEF domain proteins mediate virulence-related signaling in *Xanthomonas campestris*”

Matthieu Arlat, Université de Toulouse. “*Xanthomonas campestris* pv. campestris exploits N-acetylglucosamine during infection”

(15 min.) Jung-Gun Kim, Stanford University. “XopD suppresses ethylene responses induced during the late stages of *Xanthomonas campestris* pv. vesicatoria infection in tomato”

18:00-19:00 Dinner

19:00-21:00 Poster Session (West Classroom)

Wednesday, July 15

7:00-8:00 Breakfast

8:00-10:00 *Session V: Gene function, C. Extracellular polysaccharides.* Moderator, Max Dow

Mari-Anne Newman, University of Copenhagen. “*Xanthomonas* microbe associated molecular patterns (MAMPs): elicitors of plant innate immunity”

Richard Cooper, University of Bath. “*Xanthomonas* oligomers and polymers: the complexity of elicitation and suppression of host innate immunity”

Anke Becker, University of Bielefeld and Freiburg, Germany. “Regulation of carbohydrate metabolism in *Xanthomonas campestris* pv. campestris”

Marie-Agnes Jacques, INRA, Beaucouzé, France. “Role of type III secretion system and adhesins in the fitness of *Xanthomonas fuscans* subsp. fuscans in bean phyllosphere and in transmission to seeds”

(15 min.) Gongyou Chen, Shanghai Jiao Tong University. “Extracellular protease deficient mutants of *Xanthomonas oryzae* pv. *oryzicola* are virulence deficient and unable to synthesize or to secrete extracellular protease”

10:00-10:15 Break

10:15-12:15 Session VI: High-throughput, "next generation" sequencing technology, data management, international online databases and standards, new-genome based resources for the international community. Moderator, Frank White

Jeff Jones, University of Florida. "Genomic comparisons of xanthomonads infecting tomato and pepper"

Ralf Koebnik, IRD-CNRS, Université de Perpignan. "Next-generation genomics of *Xanthomonas*"

Frank-Jörg Vorhölter, University of Bielefeld. "Bioinformatics for polyOmics analyses of xanthomonads"

(15 min.) Ya-Wen He, Institute of Molecular and Cell Biology, Singapore. "Gene discovery by genome re-annotation, similarity searching and microarray analysis in *Xanthomonas campestris* pv. campestris"

12:15-13:15 Lunch

13:15-14:05 Session VI, continued

Magdalen Lindeberg, Cornell University. "Genome analysis and information management for *Pseudomonas syringae*"

John Hamilton, Michigan State University. "The Comprehensive Phytopathogen Genomics Resource"

14:05-15:15 Session VII: Roundtable Discussions, topics to be announced

15:15-18:30 Free

18:30 Dinner and Closing

19:30 Party, music by *Finders and Youngberg* (Fireside Lounge)

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Acknowledgements

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Speaker Abstracts

(Ordered as in program)

Session I

Bernal

What it takes to infect cassava: genomics of *Xanthomonas axonopodis* pv. manihotis

Adriana Bernal[1], Luis M. Rodriguez-R[1], Mario Arrieta[1], Alejandro Grajales[1], Ralf Koebnik[2], Valerie Verdier[2], Boris Szurek[2], Silvia Restrepo[1]

[1]Departamento de Ciencias Biológicas. Universidad de los Andes, Bogotá, Colombia; [2]Laboratoire Génome et Développement des Plantes. Institute de Rechercher pour le Développement. Montpellier, France.

Xanthomonas axonopodis pv. manihotis is the causal agent of Cassava Bacterial Blight. This disease causes severe losses in all warm and humid regions where cassava is grown. In order to have a holistic understanding of the biology of this pathogen and its interaction with the plant, we have undertaken a genomic approach. The genome of *Xam* strain CIO151 was sequenced using Illumina technology and an initial annotation of a partial list of genes present in *Xam* was performed. Subsequently, 454 technology was used to sequence the same genome given the insufficient length of contigs obtained with the previous approach. A considerable proportion of the genome was assembled using de novo and similarity-based assembly programs, and the best assembly was selected on the basis of expected genome coverage, similarity with other *Xanthomonades* and identifiable coding sequences. An approximate of 4500 gene-coding sequences were identified and annotated using BLASTp and algorithms that search for conserved domains. A manual annotation was initiated for genomic regions coding for proteins that could be involved in pathogenesis, including candidate effector genes, which were predicted using a combination of statistical approaches. Finally, a phylogenomic analysis was performed of the Xanthomonadaceae family, using a variety of coding regions selected by established and experimental methods.

Van Sluys

IS elements produce natural genome variation and shape *Xanthomonas* genomes

Van Sluys MA[1, 2]; de Souza RF[1]; Gonçalves M[1]; Bogdanove A[3]; Leach J.[2]

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Bacterial genome evolution relies on mobile genetic element activity, as well as other phenomena, to impact genetic diversity. Small transposon units known as *Insertion Sequence* (IS) elements are major actors in disseminating antibiotic resistance within a microbial community as a result of composite mobile elements jumping into plasmids and then back to chromosomes. Growing evidence from sequencing projects is also suggestive that these elements may play a role in establishing the boundaries between bacterial species. A thorough search for IS elements was made on nine Xanthomonads.genomes and 45 distinct transposon units were defined. These belong to 10 of the 19 major IS families, including the IS5 and IS3 families that are found in all *Xanthomonas* sequenced species. IS256, IS3 and IS4 families are found in plasmids of *Xanthomonas axonopodis* pv. citri as well as *Xanthomonas axonopodis* pv. vesicatoria while the elements of these families have amplified to high copy numbers in the chromosome of the four sequenced *Xanthomonas oryzae* genomes. Several of the unique elements found for each genome lay by the side of a particular feature, not necessarily related to pathogenicity, but a distinguishing genetic characteristic. A global view of the IS element distribution along the chromosome backbone also provide evidence of their association with genomic breaks and gene islands. Also, none of the sequenced genomes have the same global pattern of IS element insertion and amplification. This observation supports independent amplification profiles among the Xanthomonads and may correlate with speciation/pathovar bottlenecks. Evidence for a particular environmental condition common to a plant host is lacking because *X. oryzae* pv. oryzicola and Xoo inhabit the same host but have distinct elements with unique amplification profiles. The results presented follow a common trend of the impact of these mobile elements in creating a unique evolutionary pathway for each bacterial lineage.

Gagnevin

New powerful tools for epidemiological analyses of *Xanthomonas citri* pv. *citri* with a strong evolutionary aftertaste

Lionel Gagnevin, Lan Bui Thi Ngoc, Christian Vernière, and Olivier Pruvost
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The aggravation of epidemic situations for bacterial diseases is often correlated with the emergence of new groups of strains, either because changes in the environment have favored such groups or because the strains themselves have evolved to become more fit to their environment (including the host). To understand the parameters of these emergences plant bacteriologists must improve their knowledge of the populations dynamics (ie epidemiology) as well as adaptation mechanisms (ie evolution). They also need to be able to efficiently monitor changes while they happen. For pathogens such as *Xanthomonas citri* pv. *citri* (*Xcc*), the causal agent of Asiatic citrus canker, common genotyping tools are often not discriminant enough to address questions at rather small time or spatial scales. The sequence of the complete genome of *Xcc* proved a goldmine of new appropriate markers. In order to delve further into the structure of the populations of *Xcc* at a large (Asia) or smaller (Vietnam) scale, genotyping schemes involving insertion sequences (IS-LM-PCR) and variable number of tandem repeats (MLVA) were developed and used in addition to a reference technique, AFLP. The data were analyzed by classical population genetics methods, as well as by Bayesian and phylogeographical approaches. The three types of markers were highly congruent in describing the genetic diversity and population structure. Besides a clear identification of the *Xcc* pathotypes, these genotyping techniques allowed to make assumptions on the relationships between them, to describe the distribution of *Xcc* in Asia and to understand the population dynamics and genetics of *Xcc* at a smaller regional scale. The higher genetic diversity of pathotype A* suggests that it may have a longer evolutionary history than pathotype A. In Vietnam, two differentiated pathotype A populations were identified with characteristics suggesting a recent and massive dissemination of a new population, likely through propagative material.

Thwaites

Biology, phylogeny and genomics of banana *Xanthomonas* wilt, an emerging disease in East Africa

Richard Thwaites[1], David Studholme[2], Eric Kemen[2], Daniel MacLean[2], Sebastian Schornack[2], Valentine Aritua[3], Wilberforce Tushemereirwe[4], Murray Grant[5], Jonathan Jones[2] and Julian Smith[1]
[1]Food and Environment Research Agency, Sand Hutton, York, U.K.; [2]The Sainsbury Laboratory, Colney Lane, Norwich, U.K., [3]Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas, U.S.A. [4]School of Biosciences, University of Exeter, Devon, U.K., [5]National Agricultural Biotechnology Centre, Kawanda Agricultural Research Institute, Kampala, Uganda.

Banana *Xanthomonas* wilt (BXW) was first recognized in Ethiopia in the 1960s as a pathogen Enset, a relative of cultivated banana. For many years the disease was not reported again, but it has recently reemerged and is spreading throughout banana growing regions of East Africa. So far the disease has caused serious loss of yield in Uganda, Democratic Republic of Congo, Rwanda, Tanzania, Kenya and Burundi. Fingerprinting using rep-PCR and sequencing of the gyrase B gene has identified the causal organism as a strain of *X. vasicola*, closely related to other strains from this species known to cause disease on sugarcane, sorghum and maize in East Africa. East African strains of *X. vasicola* do not share identical host ranges, and we are investigating the genetic basis for the differing host specificities amongst these phylogenetically closely-related taxa. We have generated draft genome sequences from two East African Xanthomonads; an isolate of BXW and a strain of *X. vasicola* pv. *vasculorum*, a pathogen of sugarcane. Comparisons of predicted proteins made between these strains and with other Xanthomonads have revealed intriguing insights into the evolution of pathogenicity and host range in these organisms. For example, several genomic regions indicative of introduction by horizontal gene transfer have been identified in the two East African strains, with likely origins both in other plant pathogens and also bacteria existing as plant endosymbionts. The evolutionary significance of these findings, as well as their impact on efforts to further understand and control BXW will be discussed.

Vera Cruz

Genomics-based diagnostic marker development for *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*

Jillian Lang[1], John Hamilton[2], Marie Anne Van Sluys[3], Casiana Vera Cruz[4], Ned Tisserat[1], C. Robin Buell[2], Jan E. Leach[1]

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A key hurdle in developing highly specific, easily used diagnostic tools for plant pathogens has been the difficulty in finding unique features that can be targeted by serological or DNA based applications, and that have been validated against an extensive collection representative of the pathogen population. We used a comparative genomics pipeline to rapidly identify unique regions in the genomes of the rice bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*) and bacterial blight (*X. oryzae* pv. *oryzae*), respectively. Using the unique regions, a suite of diagnostic primers were developed that monitor diverse loci and distinguish the two pathogens. A subset of these primers was combined into a multiplex polymerase chain reaction (PCR) set that accurately distinguished the two rice pathogens in a survey of geographically diverse strains of *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, other xanthomonads, other bacteria isolated from rice leaves and seed, and several different genera of plant pathogenic and plant-associated bacteria. This comparative genomic approach is a powerful tool that can be applied to other plant pathogens to expedite development of diagnostic primers.

Session II

Rott

Genomics and functional studies of pathogenicity factors of *Xanthomonas albilineans*, an unusual xanthomonad causing sugarcane leaf scald

Philippe Rott

UMR Cirad/Inra/Montpellier SupAgro, Biologie et génétique des interactions plante-parasite (BGPI), Campus international de Baillarguet, TA A-54K, Montpellier Cedex 5, F-34398.

Xanthomonas albilineans (*Xa*) is a systemic, xylem-invading pathogen that causes sugarcane leaf scald. Symptoms vary from a single, white, narrow, sharply defined stripe to complete wilting and necrosis of infected leaves, leading to plant death. *Xa* produces the toxin albicidin that blocks chloroplast differentiation, resulting in white foliar stripe symptoms. Albicidin is the only previously known pathogenicity factor in *Xa*, yet albicidin-deficient mutant strains are still able to efficiently colonize sugarcane. The complete genome of *Xa* strain GPE PC73 from Guadeloupe was recently sequenced and annotated (project by Genoscope and four French teams working on plant pathogenic xanthomonads). The genome size (3.769 Mb) is about 25% smaller than that of other already sequenced xanthomonads. Interestingly, *Xa* lacks both the xanthan gum gene cluster and a type III secretion system (T3SS) of the Hrp1 and Hrp2 injectisome families. Surprisingly, *Xa* possesses a T3SS SPI-1 gene cluster that is predicted to interact with animal cells. Four percent of the *Xa* genome encodes 12 large non-homologous non-ribosomal peptide synthetases (NRPSs) in six regions. Three of these NRPSs, encoded in the same gene cluster, were previously described as involved in albicidin biosynthesis. In attempts to identify additional pathogenicity genes, we used site-directed deletions of candidate genes and also random Tn*5* mutagenesis, both followed by inoculation of sugarcane plants with newly introduced mutations. Mutation of quorum sensing genes *rpfF* and *xanB2* did not appear to affect albicidin production nor sugarcane colonization by *Xa* strain FL07-1 from Florida. However, four independent Tn*5* mutations of an OmpA family gene strongly affected both disease symptom development and sugarcane stalk colonization without affecting *in vitro* albicidin production in *Xa* strain FL07-1. Other candidate pathogenicity genes are currently being investigated.

Manceau

A multilocus sequence analysis of *Xanthomonas campestris* reveals a complex structure within crucifer-attacking pathovars of this species

Emilie Fargier[1, 2], Marion Fischer-Le Saux[1] and Charles Manceau[1]
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Previous classification of *Xanthomonas campestris* has defined six pathovars (*aberrans, armoraciae, barbareae, campestris, incanae, raphani*) that cause diseases on cruciferous plants. However pathogenicity assays with a range of strains and different hosts identifies only three types of symptom: black rot, leaf spot and bacterial blight. These findings raise the question of the genetic relatedness between strains assigned to different pathovar or symptom sub-divisions. Here we have addressed this issue by multilocus sequence analysis of 42 strains. The *X. campestris* species was polymorphic at the 8 loci analyzed and had a high genetic diversity; 23 sequence types were identified of which 16 were unique. All strains that induce black rot (pathovars *aberrans* and *campestris*) were genetically close but split in two groups. Only three clonal complexes were found, all within pathovar *campestris*. The synonymy of pathovars *armoraciae* and *raphani* suggested from disease symptoms was confirmed, although this group of strains was particularly polymorphic. Strains belonging to pathovars *barbareae* and *incanae* were closely related, but distinct from pathovars *campestris* and *armoraciae*. There is evidence of genetic exchanges of housekeeping genes within this species as deduced from a clear incongruence between individual gene phylogenies and from network structures from SplitsTree analysis. Overall our study showed that the high genetic diversity was brought with equal importance by recombination events and accumulation of point mutations. However, *X. campestris* remains a species with a clonal evolution driven by a differential adaptation to cruciferous hosts.

Patil

Function and evolution of lipopolysaccharide biosynthetic gene clusters in xanthomonads

Prabhu B. Patil,[1] M. G. Anil,[2] Adam J. Bogdanove,[3] and Ramesh V. Sonti[2]
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The genus Xanthomonas displays extensive variation at a lipopolysaccharide (LPS) biosynthesis locus present between housekeeping genes, cystathionine gama lyase (*metB*) and electron transport flavoprotein (*etfA*). *Xanthomonas oryzae* pv. *oryzae* (Xoo), the bacterial blight pathogen of rice, encodes a 12.2 kb LPS gene cluster that is present in most Xoo strains from India and Asia, but absent in two strains, BXO8 and Nepal 624, from the Indian subcontinent. The LPS locus of BXO8 is 24 kb and the genes are organized as convergent transcriptional units with 5 ORFs being in one orientation and the rest in the other. Mutations in all five ORFs from the *metB* side of the BXO8 LPS gene cluster cause loss of surface polysaccharide production and virulence, while only 2/10 ORF's transcribed from the *etfA* side have an apparent role in surface polysaccharide production and virulence. A comparative analysis with LPS gene clusters in other xanthomonads suggests that the BXO8 LPS gene cluster might be ancestral type that was replaced by a totally novel LPS gene cluster during the course of evolution and spread of Xoo. Interestingly, the related Stenotrophomonas (Sma) strains also harbor a highly variable LPS locus between *metB* and *etfA*. Various Sma strains are found as endophytes in plants, in rhizosphere, soil, water and as opportunistic human pathogens. The Sma LPS gene clusters show high relationship with the LPS gene clusters of xanthomonads. However, most startling is that the LPS gene cluster of *Xanthomonas oryzae* pv. *oryzicola* (Xoc), the leaf streak pathogen of rice, is highly similar to that of Sma strain K279a, a clinical isolate. Fourteen out of sixteen genes that are present in the Xoc LPS locus have orthologs in Sma K279a. We speculate that owing to it's ability to occupy varied ecological niches, Sma can serve as a conduit for transfer of diverse LPS gene clusters into the xanthomonads.

Verdier

New insights into the genome of African *Xanthomonas oryzae* pv. *oryzae* strains

Valérie Verdier[1], Boris Szurek[1], Yanhua Yu[1], Mauricio Soto[1], Guillaume Robin[1], Thierry Mathieu[1], Jiaxun Feng[2], Ralf Koebnik[1]
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Xanthomonas oryzae pv*. oryzae* is the causal agent of rice bacterial blight, a disease occurring in most rice growing areas including Africa, where it appeared in the 80's. During the last decade the culture of rice has increased drastically in West-Africa. In the absence of appropriate resistant varieties the disease has expanded to almost all rice-cultivated areas. Developing control measures for *Xoo* as well as tools for detection and tracking is therefore of crucial importance. The success of these efforts will be accelerated by genomic analyses. In a first attempt to evaluate the importance, population structure and diversity of *Xoo* in West-Africa, we performed pathogenicity assays as well as molecular analyses on a collection of *Xoo* strains. We described new races among African *Xoo* strains. Pathogenicity on different rice varieties also suggested that the African strains may have a different host range and may have evolved on wild graminaceous species native in Africa. Whatever the genetic tools used (RFLP, AFLP, rep-PCR, SSH, study of CRISPRs), the African *Xoo* strains appear genetically different from the Asiatic ones and are more related to Asian *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*). A specific and intriguing feature of African strains is a smaller number of transcription activator-like (*tal*) effector genes. A functional analysis has been initiated on *tal* genes. One of them, named *talc,* plays a crucial role in the production of leaf lesion symptoms on susceptible rice lines. Our objective is to delve further into the evolutionary history of *Xoo*. Currently, the genome of two African *Xoo* strain (BAI3 and MAI1, isolated in Burkina and Mali respectively) is being sequenced. It should help deciphering evolutionary mechanisms at the genome level in relation with differences in pathogenicity. All together, the data presented will give new insights into the intriguing evolutive history of African *Xoo* strains.

Schubert

Identification and characterization of novel genes involved in the virulence of *Xanthomonas campestris* pv. *vesicatoria*

Cornelius Schubert[1], Sven Findeiss[2], Peter Stadler[2], Ulla Bonas[1]
[1]Institute of Biology/ Plant Genetics, Martin-Luther University Halle-Wittenberg, Germany; [2]Institute of Informatics/ Bioinformatics, University of Leipzig, Germany.

The Gram-negative plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease on pepper and tomato. *Xcv* enters the plant tissue via natural openings or wounds and multiplies in the intercellular spaces. In *Xcv*, essential pathogenicity and many virulence-associated genes are induced upon contact with the plant cells by two known regulatory proteins, HrpG and HrpX. Both proteins regulate the expression of the *Xcv* type III secretion system, an essential pathogenicity factor. Genome sequencing of *Xcv* strain 85-10 revealed 4,854 genes in the chromosome and four native plasmids. High-throughput sequencing of cDNAs from *Xcv* strain 85-10 and derivatives and bioinformatic approaches identified novel genes, some of which are HrpG/HrpX dependently expressed. Genetic studies resulted in the discovery of new *Xcv* virulence factors involved in post-transcriptional regulation of gene expression.

Session III

Tsuge

Genome-wide screening for T3S effectors in *Xanthomonas oryzae* pv. *oryzae*

Ayako Furutani,[1] Kazuhiro Ochiai,[1] Takashi Oku,[2] Kazunori Tsuno[3] and Seiji Tsuge[4]
[1]National Institute of Agrobiological Sciences, Tsukuba, 305-8602, Japan; [2]Laboratory of Molecular Plant Pathology, Shobara, 727-0023, Japan; [3]Laboratory of Plant Pathology, Miyazaki, 889-2192, Japan; [4]Laboratory of Plant Pathology, Kyoto Prefectural University, Kyoto, 606-8522, Japan.

Like other Gram-negative phytopathogenic bacteria, a type III secretion (T3S) system encoded by *hrp* genes is indispensable for the pathogenicity of *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight of rice. Through the system, bacteria directly inject functional proteins, so-called effectors, into host cells. Among effectors in *X. oryzae pv. oryzae*, AvrBs3/PthA family proteins (transcription activator-like <TAL> effectors) have been well studied. Here, we conducted genome-wide screening for non-TAL effectors using the genome database of *X. oryzae* pv. *oryzae* MAFF311018. Sixty candidates were selected with the criteria: i) homologs of known T3S effectors in plant-pathogenic bacteria, ii) genes with expression regulated by *hrp* regulatory protein HrpX, or iii) proteins with N-terminal amino acid patterns associated with T3S substrates of *Pseudomonas syringae*. The calmodulin-dependent adenylate cyclase reporter assay revealed that 16 proteins of the candidates are translocated into plant cells in a T3S system-dependent manner. Of these 16 proteins, nine were homologs of known effectors in other plant-pathogenic bacteria and seven were not. Most of the effectors were widely conserved in *Xanthomonas* spp.; however, some were specific to *X. oryzae*. All these effectors were expressed in an HrpX dependent manner, suggesting coregulation of the effectors and the T3S system. To investigate the involvement of the effectors in pathogenicity, mutants of each effector were generated and inoculated to the host plant. Although most mutants showed similar virulence to the wild-type strain, lesion lengths caused by XOO1669- or XOO4134-mutants were repressed, suggesting that both effectors are involved in virulence of *X. oryzae* pv. *oryzae*.

Yang

Type III effector diversity and function in *Xanthomonas oryzae* pv. oryzae

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Xanthomonas oryzae pv. oryzae (Xoo), the causal agent of bacterial blight of rice, depends on a type III secretion system (T3SS) for pathogenesis. Some T3SS substrates, the so called type III (T3) effectors are translocated into host cells and play an important role in either eliciting host resistance in cultivars containing corresponding resistance genes or in contributing virulence to bacteria in the otherwise susceptible plants. Over years efforts have been devoted to identifying such T3 effectors and elucidating their mode-of-action. A large group of T3 effectors in Xoo belong to a family of transcription activation-like (TAL) proteins, characteristic of nuclear localization and transcription activation, and some have been shown to be specifically associated with the host gene activation that is responsible for either disease resistance (avrXa27/*Xa27*) or disease susceptibility (pthXo1/*Os8N3*). Each Xoo strain harbors multiple, but non-identical, TAL effectors with individuals contributing major, moderate or undetectable virulence to the bacterial infection. In addition, progress has also been made in identifying Xoo T3 effectors other than TAL effectors, including those that are conserved among *Xanthomonas* spp. and others that are unique in Xoo. With increasingly available genomic data from both pathogen and host, coupled with multiple approaches of genetics, genomics and biochemistry, more Xoo T3 effectors and their cognate host targets will be identified, and the molecular basis underlying their interactions leading to bacterial blight will be better understood.

Mudgett

Xanthomonas T3S effector XopN suppresses PAMP-triggered immunity and interacts with a tomato atypical receptor-like kinase and TFT1

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XopN is a virulence factor from *Xanthomonas campestris* pathovar *vesicatoria* (Xcv) strain 85-10 that is translocated into tomato (Solanum lycopersicum) leaf cells by the pathogen's type III secretion system. Xcv ∆xopN mutants are impaired in growth and have reduced ability to elicit disease symptoms in susceptible tomato leaves. We show that XopN action *in planta* reduced pathogen-associated molecular pattern (PAMP)-induced gene expression and callose deposition in host tissue, indicating that XopN suppresses PAMP-triggered immune responses during Xcv infection. XopN is predicted to have irregular, a-helical repeats, suggesting multiple protein–protein interactions *in planta*. Consistent with this prediction, XopN interacted with the cytosolic domain of a Tomato Atypical Receptor-Like Kinase1 (TARK1) and four Tomato Fourteen-Three-Three isoforms (TFT1, TFT3, TFT5, and TFT6) in yeast. XopN/TARK1 and XopN/TFT1 interactions were confirmed in planta by bimolecular fluorescence complementation and pull-down analysis. Xcv ∆xopN virulence defects were partially suppressed in transgenic tomato leaves with reduced TARK1 mRNA levels, indicating that TARK1 plays an important role in the outcome of Xcv–tomato interactions. These data provide the basis for a model in which XopN binds to TARK1 to interfere with TARK1-dependent signaling events triggered in response to Xcv infection. Progress in studying XopN and TARK1 function will be presented.

Bonas

New insights into type III effectors from _Xanthomonas campestris_ pv. vesicatoria

Ulla Bonas.

Martin Luther University, Halle, Germany.

Hajri

The "Repertoire for Repertoire" Hypothesis: Repertoires of type three effectors may explain host specificity in *Xanthomonas*

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Host range of many plant pathogenic bacteria is restricted to one or a few host plant species reflecting a tight adaptation of bacteria to specific hosts. Two hypotheses could explain host specificity: either it can be explained by the phylogenetic position of the strains, or, alternatively, by the association of virulence genes enabling a pathological convergence of phylogenetically distant strains. In this latter hypothesis, host specificity would result from the interaction between repertoires of bacterial virulence genes and repertoires of genes involved in host surveillance systems and defences (a "repertoire for repertoire" hypothesis). To challenge these two hypotheses, we selected 132 *Xanthomonas axonopodis* strains representative of 18 different pathovars which display different host range. First, the phylogenetic position of each strain was determined by sequencing the housekeeping gene *rpoD.* This study showed that many pathovars of *Xanthomonas axonopodis* are polyphyletic. We thus investigated the distribution of 37 T3E genes in these strains by both PCR and hybridization methods. Our study revealed that T3E repertoires are highly variable between these strains, both in terms of T3E presence and of size of repertoires. T3E repertoires comprise both core and flexible gene suites that likely have distinct roles in pathogenicity and different evolutionary histories. Our results showed a correspondence between composition of T3E repertoires and pathovars of *Xanthomonas axonopodis*. For polyphyletic pathovars, this strongly suggests that T3E genes explain a pathological convergence of phylogenetically distant strains. Finally, our results support the "repertoire for repertoire" hypothesis as the molecular basis of host specificity for plant pathogenic bacteria.

Session IV

Lee

A Type I secreted, sulfated peptide triggers rice XA21-mediated innate immunity

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Pathogen recognition receptors (PRRs) activate innate immunity by detecting specific microbial components. The rice XA21 PRR recognizes a Type I-secreted molecule present in most strains of the Gram-negative bacterium, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). We used reverse phase-high pressure liquid chromatography analysis of *Xoo* supernatants to isolate fractions that can trigger XA21-mediated immunity. Liquid Chromatography-Mass spectrometry analysis of these biologically active fractions led to the identification of peptides corresponding to a single 194 amino acid (aa) protein encoded by a gene that we designate *ax21* (activation of Xa21-mediated immunity). *Xoo* mutants defective in Type I secretion that are unable to trigger XA21-mediated resistance, no longer secrete Ax21. An *Xoo* mutant strain lacking *ax21* is no longer recognized by XA21. The Ax21 protein carries two predicted tyrosine sulfation sites. A sulfated, 17 aa synthetic peptide derived from the N-terminal region of Ax21 is sufficient for Ax21 activity, whereas peptides lacking tyrosine sulfation are biologically inactive. Ax21 is present in all sequenced *Xanthomonas* species as well as *Xylella fastidiosa* and the human pathogen, *Stenotrophomonas maltophilia*. Ax21 activity is conserved in *X. campestris* pv. *vesicatoria,* a pathogen of tomato. Thus, our results demonstrate that Ax21 is a pathogen associated molecular pattern that does not fall into any of the previously characterized classes.

Ryan

Cyclic di-GMP signaling and virulence in the plant pathogen *Xanthomonas campestris pv. camprestris*

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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. Furthermore, some PilZ domain proteins have recently been shown to bind the cyclic di-GMP and to exert a regulatory function. Recent work from our own laboratory has implicated cyclic di-GMP in regulation of virulence factor synthesis and biofilm formation through the action of the HD-GYP domain regulator RpfG. Here we report a comprehensive study of cyclic di-GMP signalling in bacterial disease in which we examine the contribution of all proteins with GGDEF, EAL or HD-GYP and PilZ domains to virulence and virulence factor production in the phytopathogen *Xanthomonas campestris pathovar campestris* (*Xcc*). Genes with significant roles in virulence to plants included those encoding proteins whose probable function is in cyclic di-GMP synthesis as well as others (including the regulator RpfG) implicated in cyclic di-GMP degradation. Furthermore, RpfG controlled expression of a subset of these genes. A partially overlapping set of elements controlled the production of virulence factors in vitro. Other GGDEF-EAL domain proteins had no effect on virulence factor synthesis but did influence motility. We also show that PilZ domains are also involved in the control of biofilm formation, virulence-factor production and motility in *Xcc*. These findings indicate the existence of a regulatory network that may allow *Xcc* to integrate information from diverse environmental inputs to modulate virulence factor synthesis as well as of cyclic di-GMP signalling systems dedicated to other specific tasks. However the available evidence indicates the existence of further cyclic di-GMP “effector” or binding proteins beyond PilZ.

Dow

Physical interactions between HD-GYP and GGDEF domain proteins mediate virulence-related signaling in *Xanthomonas campestris*

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The virulence of the plant pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) depends upon cell-cell signaling 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 a CheY-like receiver domain attached to an HD-GYP domain, which is a cyclic di-GMP phosphodiesterase. Mutation of *rpfF*, *rpfG*, or *rpfC* leads to a coordinate reduction in the synthesis of virulence factors such as extracellular enzymes, alterations in biofilm formation and reduced motility. We have used yeast two-hybrid analysis and fluorescence resonance energy transfer experiments in *Xcc* to address the role of RpfG in DSF signal transduction. We will demonstrate that physical interaction of RpfG with two GGDEF domain-containing proteins, which are diguanylate cyclases involved in cyclic di-GMP synthesis, acts to control a sub-set of RpfG-regulated virulence functions. RpfG interactions were abrogated by alanine substitutions of the three residues of the conserved GYP motif in the HD-GYP domain. Alterations in the GYP motif or loss by mutation of the two GGDEF domain proteins influenced *Xcc* motility but had no effect on the synthesis of extracellular enzymes or biofilm formation. RpfG-GGDEF interactions were dependent upon DSF signaling, being reduced in the *rpfF* mutant but restored by DSF addition. Mutagenesis of both of the genes encoding the GGDEF-domain targets lead to a substantial loss of virulence in Chinese radish. These observations expand our understanding of the role of the Rpf/DSF system in the virulence of *Xcc* and of the role in signal transduction of RpfG, which belongs to a widespread class of bacterial two-component regulator that have an HD-GYP domain.

Arlat

***Xanthomonas campestris* pv. campestris exploits *N*-acetylglucosamine during infection**

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Xanthomonas campestris pv. campestris (*Xcc*) possesses specific CUT systems (Carbohydrate Utilization systems containing TonB-dependent transporters), devoted to the scavenging of plant carbohydrates. We identified a new *Xcc* CUT system involved in the exploitation of *N*-acetylglucosamine (GlcNAc) from plant origin. This GlcNAc CUT system, which is under the control of NagR and NagQ regulatory genes, is divided into an upper pathway involved in the external generation and uptake through the outer membrane of GlcNAc-containing carbohydrates and a lower pathway corresponding to the uptake through the inner membrane and catabolism of GlcNAc. We show that this system is used during the infection of host plants. Indeed, a *nagA* mutant, affected in GlcNAc catabolism and sensitive to this compound, is unable to grow *in planta* and to induce disease symptoms. This phenotype is reversed when mutations in the upper pathway are introduced into *nagA* mutants, thus underlying the importance of this pathway in the generation of GlcNAc *in planta*. Based on glycoside hydrolase activities belonging to the upper pathway, we propose that glycans from glycoproteins might be the source of GlcNAc for *Xcc* during infection. Comparative studies show that the GlcNAc CUT system is specifically conserved in phytopathogenic *Xanthomonadaceae* suggesting that the degradation of glycans of plant origin is a common feature of this bacterial family. Although GlcNAc is an important nutrient source for chitinolytic bacteria, the exploitation of this molecule by phytopathogenic bacteria was never suspected. Therefore, this work broadens the source of GlcNAc metabolized by bacteria and sheds new light on metabolic capabilities of *Xanthomonas*.

Kim

XopD suppresses ethylene responses induced during the late stages of *Xanthomonas campestris pv. vesicatoria* infection in tomato

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Xanthomonas campestris pathovar *vesicatoria* (Xcv) strain 85-10 uses the type III secretion (T3S) system and a group of effector proteins to promote disease. We discovered that the XopD effector is required for maximal Xcv growth and suppression of disease symptoms in tomato. XopD contains two EAR motifs flanked by a DNA-binding domain and a SUMO protease domain. EAR motifs are conserved in plant transcription factors (TFs) that repress gene transcription induced during defense and stress. XopD EAR motifs and SUMO protease domain are required for suppression of defense- and senescence-associated gene transcription. In recent work, we found that XopD might directly affect ethylene signaling and/or biosynthesis. Ethylene levels and mRNA abundance of ethylene biosynthesis genes, LeACS2, LeACO1, and LeACO2 are significantly higher in Xcv ΔxopD-infected leaves compared to Xcv-infected leaves. Furthermore, XopD reduces the stability of ERF4, a TF required for the activation of ET-dependent transcription. Destabilization of ERF4 required XopD-dependent SUMO protease activity. Based on these findings, we propose that XopD modulates ethylene biosynthesis and/or signaling by altering the stability of ethylene-responsive TFs during pathogen attack. Progress in dissecting the impact of XopD on ethylene signaling and biosynthesis will be presented.

Session V

Newman

Xanthomonas Microbe Associated Molecular Patterns (MAMPs): elicitors of Plant Innate Immunity

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Plants perceive several general elicitors from both host and non-host microbial pathogens. These microbe-specific elicitors are essential structures for pathogen survival and are for that reason conserved. They are referred to as Microbe or Pathogen Associated Molecular Patterns (MAMPs or PAMPs) and are recognised by the plant innate immune system. We have chemically and biologically examined peptidoglucan (PGN) from two Gram negative plant pathogens, *Xanthomonas campestris* pv. *campestris* (Xcc) and *Agrobacterium tumefaciens* (At). We show that PGN functions as a MAMP in plants by triggering diverse innate immune responses, and that its perception is dose dependent. Clear differences in the structures of Xcc and At PGNs and their constituent muropeptides were found and may explain why Xcc PGN was a better elicitor of plant immune responses than At PGN; the lower efficacy of At PGN might relate with its subtle, biotrophic mode of invasion. However, for both bacteria the muropeptides were more effective in triggering immune responses in *Arabidopsis* than the native PGN. These findings demonstrate for the first time that PGN from true plant pathogenic bacteria functions as a MAMP. We have investigated the role of Xcc lipopolysaccharide (LPS) in plant innate immunity for a number of years. Here we present new data to show that *Arabidopsis* PEN1, a SNARE protein believed to be required for docking and fusion of intracellular transport vesicles, is involved in signal transduction leading to the induction of the innate immune responses by particular bacterial MAMPs. Specifically we show that PEN1 is required for induction of *PR1* gene induction, callose deposition and generation of reactive oxygen species by LPS but not by flagellin. These findings, which suggest multiple roles for PEN1 in determining plant resistance to pathogens, will be discussed in the light of previously published work that shows internalisation of LPS on application to suspension cultured cells.

Cooper

Xanthomonas oligomers and polymers: the complexity of elicitation and suppression of host innate immunity

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Xanthomonas spp. produce the complex extracellular polysaccharide (EPS) xanthan, which is required for virulence of several major pathovars. The biosynthetic *gum* genes are characterized and evident in all *Xanthomonas* genomes. Protective functions are known, but we reveal a more fundamental role, that of suppression of MAMP-induced innate immunity. Polyanionic xanthan chelates calcium ions and levels of *X. campestris* xanthan in the apoplast are well in excess of that required to deplete this key calcium pool. Xanthan prevented or reduced calcium influx to the cytosol and the consequent signalling cascade, which is a prerequisite for defence activation. This was shown by comparing induction of various defence components (Ca influx, oxidative burst, callose formation, defence genes) by wild type and xanthan-deficient mutants and by inoculating pure xanthan prior to mutants or bacterial MAMPs. Infiltrated pure xanthan mimicked ultrastructurally the biofilm formed during infection. We also examined effects of MAMP combinations and their interactions with the host cell wall. Early responses in *Arabidopsis*, elicited by non-saturating concentrations of flagellin peptide (flg22), elongation factor peptide (elf18), *X. campestris* peptidoglycan (PGN) and constituent muropeptides, lipo-oligosaccharide (LOS) and core oligosaccharides, revealed that some MAMPs have additive and even synergistic effects, while some mutually interfere. The peptide elicitors are potent at sub-nm levels, whereas PGN and LOS only at high µm levels induce low and late responses. This contrast may result from restricted access to receptors through the host wall of these macro-molecular MAMPs. Thus flg22 rapidly permeates a cell wall matrix, whereas LOS, which forms micelles, is severely constrained. Clearly, induction and suppression of innate immunity involves complex interactions between host and pathogen polymers.

Becker

Regulation of carbohydrate metabolism in *Xanthomonas campestris* pv. campestris

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Xanthomonas campestris pv. campestris is pathogenic for cruciferous plants like cabbage or the model plant *Arabidopsis thaliana*. Another feature of these bacteria is the production of the exopolysaccharide xanthan gum which is applied as viscosifier at large scale in industries related to food and pharmaceuticals production, and in the oil drilling industry. Recently, the genome of *X. campestris* pv. campestris B100 was completed, a strain which had been characterized particularly regarding xanthan production and carbohydrate metabolism (Vorhölter et al. 2008). Acquisition and utilization of carbohydrates are central for both, plant pathogenicity and xanthan production. Applying a transcriptomics approach, using oligonucleotide microarrays galactose scavenging genes were identified in *X. campestris* pv. campestris B100 (Serrania et al. 2008). This sugar is a constituent of various plant cell wall polysaccharides, making it likely to be recognized by the bacterium. Gene expression profiling was also applied to identify putative target genes of regulators that might be involved in the control of carbohydrate metabolism. The Biolog Phenotype Array was applied to characterize the pattern of carbon source utilization of the *X. campestris* pv. campestris B100 wild type and regulatory mutants. A *X. campestris* pv. campestris B100 mutant library was constructed based on a set of 412 signature-tagged transposons each barcoded by two tags. This library will be applied in competition assays to identify genes relevant for carbohydrate utilization and pathogenicity.

Serrania et al. (2008) J Biotechnol 135:309-317
Vorhölter et al. (2008) J Biotechnol 134:33-45

Jacques

Role of type III secretion system and adhesins in the fitness of *Xanthomonas fuscans* subsp. *fuscans* in bean phyllosphere and in transmission to seeds

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Deciphering the mechanisms enabling plant pathogenic bacteria to disperse, colonize and survive on their hosts provides the necessary basis to set up new control methods. We evaluated the role of the type III secretion system (T3SS) and adhesins in two steps of the asymptomatic host colonization process: phyllospheric colonization and transmission to seeds. *Xanthomonas fuscans* subsp. *fuscans* is responsible for the common bacterial blight of bean, a seedborne disease. T3SS cluster genes and adhesin genes were identified, characterized and mutated in *X. fuscans* subsp. *fuscans CFBP4834-R*. Unlike the wild-type *X. fuscans* subsp. *fuscans*, strains with mutations in T3SS regulatory genes were impaired in their phyllospheric growth as was *Escherichia coli* on bean. Strains with mutations in the *hrp* structural genes maintained the same constant epiphytic population densities as did *X. campestris* pv. *campestris* on bean in a non-host interaction. Among the five adhesins identified in *X. fuscans* subsp. *fuscans*, only the non-polar adhesin YapH was required for adhesion on leaves. Transmission to seeds by the vascular pathway was abolished for mutants in T3SS regulatory and structural genes, and remained possible but altered, for mutants in adhesin genes, except for mutant in *yapH* which behaved as the wild-type strain. Transmission to seeds by floral structures did not require any of the known adhesins and remained possible but with a low efficiency for *hrp* mutants and was repeatedly recorded for a non-host pathogen (*X. campestris* pv. *campestris*). *E. coli* did no transmit to bean seed. In conclusion, we showed that T3SS and bacterial adhesins are implicated in the various processes leading to host phyllosphere colonization and systemic transmission to seeds in the absence of symptoms in compatible interactions.

Chen

Extracellular Protease deficient mutants of *Xanthomonas oryzae* pv. *oryzicola* are virulence deficient and unable to synthesize or to secret extracellular protease

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Xanthomonas oryzae pv. *oryzicola* (Xooc) is the causal agent of bacterial leaf streak, a serious disease of rice in South Asia. Previously, 12 extrocellular protease deficient mutants were screened from a Tn5-based transposon randomly insertional mutant library. Here we identified Tn5 inserted positions in each of these mutants and found that any mutation in components of type II secretion machine, cAMP regulatory protein, integral membrane protease subunit, S-adenosylmethionine decarboxylase proenzyme, or extracellular protease (*ecpA*) completely or partially led to the loss of extracellular protease activities and deduced virulence in rice. Ectopic expression of *ecpA* in *Escherichia coli* demonstrated its extracellular protease activity. Reverse transcriptional polymerase chain reaction (RT-PCR) showed that the *ecpA* expression was induced *in planta* in all the mutants except the mutant *ΔecpA*. Genetic complementation of the mutant with the *ecpA* gene restored not only extracellular protease activity, but virulence and *in planta* growth. Intriguingly, the heterologous expression of the ecpA gene conferred the extracellular protease activity to the vascular bacterium X. oryzae pv. oryzae. In addition, the purified extracellular protease mimicked water-soaking symptoms as the same caused by the pathogen when it was injected into rice leaves. Based on our results, our hypothesis is that the *ecpA* gene product is a tissue-specific effector for *Xooc* pathogenesis in rice which is absent in *Xoo*.

Session VI

Jones

Genomic comparisons of xanthomonads infecting tomato and pepper

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Xanthomonas-tomato/pepper appears to be a suitable system to study host-pathogen co-evolution due to diversity among xanthomonads. *X. vesicatoria* (*Xv*1111), *X. perforans* (*Xp*91-118), and *X. gardneri* (*Xg*101) were sequenced for comparison with the previously sequenced *X. euvesicatoria* (*Xcv*85-10). Genome-wide screening for homology with effectors from plant and animal pathogens revealed diversity in effectors possessed by each strain. Most of the unique genes possessed by each strain within this group have low GC content and are flanked by IS elements which explain their acquisition from other xanthomonads or other genera. All four xanthomonads have at least 7 common effectors. *Xg*101 seems to have acquired several effectors from *Pseudomonas syringae*. Comparison of hrp cluster showed *Xp*91-118 having conserved hrp cluster with that of *Xcv*85-10, whereas core hrp cluster genes of *Xv*1111 and *Xg*101 showed more homology to hrp cluster of *X. campestris* pv. *campestris* ATCC33913 (*Xcc*) and contained a *hrpW* like *Xcc*. Phylogenetic relationships using MLST correlated with phylogeny based on hrp/ gum cluster and the common effector, avrBs2. Whole genome alignments of the three strains against reference genomes using MUMmer (nucmer program) also showed similar trends in relatedness. *Xp*91-118 has at least 7 chromosomal rearrangements, while *Xv*1111 and *Xg*101 show at least 10 rearrangements compared to *Xcv*85-10. Thus, *Xp*91-118 is closely related to *Xcv*85-10 and both group with *X. citri pv. citri* 306. *Xg*101 is more closely related to *Xcc* group than *Xcv*85-10. *Xv*1111 forms a distinct clade showing relatedness to both groups *Xcv*85-10 and *Xcc*, with *Xcv*85-10 to a greater extent. *Xv*1111 and *Xg*101 could be said to be mosaic genomes having originated from *Xcv*85-10 and *Xcc*.

Koebnik

Next-generation genomics of *Xanthomonas*

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One of the main questions in phytopathology is: what are the specific features that make a pathogen out of a microbe? Based on experiments and intuition, several factors have been put forward as candidate factors, such as the repertoire of type III effectors, adhesive molecules, sensory circuits, and hydrolytic enzymes. Suppression of defense response and/or escape from detection by the plant's surveillance system are amongst the hottest topics in the field of plant-microbe interactions. Recently, comparative genomics approaches have been applied in order to tackle such questions. For instance, it was concluded that differentiation of xanthomonads with respect to host- and tissue-specificity does not involve major modifications or wholesale exchange of gene clusters. Instead, subtle changes in a small number of genes, incl. non-coding sequences, are likely to account for such traits. Therefore, more genome sequences are required to fully understand the evolution of host- and tissue-specificity. At present, complete genome sequences of eleven *Xanthomonas* strains are publicly available. Four new genome sequences of *Xanthomonas* have been solved in France, using next-generation sequencing technologies, and projects on 40 more strains have been launched. Here, we will give an overview about current projects, discuss first analyses of selected loci, and present a new website for the *Xanthomonas* community.

Vorhölter

Bioinformatics fo PolyOmics Analyses of Xanthomonads

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The increasing amount of genome and post-genome data from *Xanthomonas* species opens new opportunities for their deeper understanding. The size and complexity of the data however make them difficult to be interpreted appropriately. In many cases suitable bioinformatics software can facilitate data analysis. An important aspect related to data analysis is visualizing the results from high-throughput experiments in a way that eases understanding. Examples are given that illustrate the bioinformatics-based analysis of *Xanthomonas campestris* pv. campestris at the genome, transcriptome, proteome and metabolome levels.

He

Gene discovery by genome re-annotation, similarity searching and microarray analysis in *Xanthomonas campestris* pv. *campestris*

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A thorough search for new CDS in *Xanthomonas campestris* pv. campestris (*Xcc*) genomes was conducted by using bioinformatic, experimental postgenomic and genetic approaches. Our bioinformatic approach relied on a combination of advanced intrinsic and extrinsic methods. In first step, intrinsic sequence information (nucleotide composition and presence of RBS) was served to identify 2850 putative new CDS. The second step consisted of screening for true CDS by extrinsic evidence involving similarity searching. A total of 278 new CDS were found to have one or more homologs in other bacterial species. Most of these new CDS encode hypothetical proteins or conserved hypothetical proteins. Eleven CDS encode putative secreted proteins or exported proteins and 3 CDS encode regulatory proteins or transcription factors. Furthermore, transcriptional analysis of 1724 putative new CDS under different conditions was conducted using the constructed 50mer oligonucleotide microarray. A total of 147 new CDS were identified with detectable transcript. Among them, 75 CDS were constitutively expressed; 72 CDS were only transcribed at high cell density (OD_{600}=2.0). Further analysis showed that transcription of 15 putative new CDS is induced by DSF signal and the expression of one CDS was induced only in poor NYG medium. The transcriptional expression of 11 DSF-regulated CDS was further confirmed by RT-PCR analysis. Finally, CDS deletion and subsequent phenotype analysis confirmed that Xcc_CDS002 encoding a conserved SIR2-like domain protein is associated with bacterial virulence and Xcc_CDS1553 encoding an ArsR family transcriptional repressor is involved in arsenate resistance in *Xcc*.

Lindeberg

Genome analysis and information management for *Pseudomonas syringae*

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Pseudomonas syringae pathovars and strains differ significantly in their host range, degree of virulence, preferred environment, and nature of symptoms elicited on host plants. To better understand the factors underlying this variability, multiple genomics-enabled approaches are being employed. Conserved regulatory and translocation motifs have been used to identify predicted type III effectors, and genome comparisons conducted to identify regions of variability likely to account for strain-to-strain differences in the bacterial-host interaction. Verification of predicted but uncharacterized genes in the variable regions has been initiated using proteome and transcriptome sequencing. With four strains of *P. syringae* currently sequenced and over 10 more currently in process, systematic management of the emerging wealth of data is critical to its utility to the *P. syringae* research community. To achieve this goal, ongoing genome annotation is being conducted with particular emphasis on: (i) linking functional descriptions to supporting evidence so users can assess annotation accuracy, (ii) updating annotations to reflect the most current research, and (iii) minimizing misleading transitive annotation. While every effort is made to accurately describe experimentally characterized genes in the various sequenced *P. syringae* strains, genome annotation records for *P. syringae* pv *tomato* DC3000 are receiving the most attention for the purpose of creating a highly annotated reference genome on which annotation of subsequent related genomes can be based. Strategies in use for Pto DC3000 include incorporation of new feature types and evidence qualifiers into the annotation records at NCBI, adoption of a systematic nomenclature for the type III effector proteins, and Gene Ontology (GO) annotation of the type III effectors for the purpose of capturing the complex body of emerging experimental data on the molecular functions, cellular locations, and biological processes of individual effectors. A database of Hop effector proteins, updates made to the NCBI records, a list of GO annotations, and other resources for genome analysis can be found at the Pseudomonas Genome Resources website (http://www.pseudomonas-syringae.org/).

Hamilton

The Comprehensive Phytopathogen Genomics Resource

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The Comprehensive Phytopathogen Genomics Resource (CPGR) provides a web-based portal for plant pathologists and diagnosticians to access genomic data for all bacterial, fungal, stramenopile, nematode, viral, and viroid plant pathogens and to provide tools to rapidly create candidate diagnostic markers. A central feature of the CPGR is the Genome Warehouse, a frequently updated database of finished, draft, and in progress genome projects and Expressed Sequence Tag (EST) projects. The finished genome projects in the Genome Warehouse are linked to the Genome Tools and the Genome Browser through a database using the chado schema of the Genomic Model Organism Database project. The Genome Tools include Gene Lists, Pfam Domains, Interpro Matches, Putative Simple Sequence Repeats (SSRs), Intergenic regions, Unique loci, and a SSR Candidate Marker Search Tool. The plant pathogen Transcript Assemblies (TA) Database provides a resource for plant pathogen EST projects. The database is populated with unique transcripts or TA from an EST clustering and assembly pipeline. A comprehensive report page is available for each TA including predicted SSRs and primers flanking the SSRs picked by Primer3 that can be used as candidate diagnostic markers. We have also developed a plant pathogen ribosomal DNA (rDNA) database. This database contains all of the rDNA sequences from GenBank for the organisms we have identified as plant pathogens and the species in the same genus of each pathogen. The web interface for the rDNA database offers multiple search and download options. The CPGR can be accessed at http://cpgr.plantbiology.msu.edu. Funding for the work is provided by a grant to C.R.B. by the USDA National Research Initiative Cooperative State Research Extension Education Service (2006-55605-16645 and 2008-03779).

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1

Bacterial leaf streak: an old disease but a new threat to wheat growers

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The genus *Xanthomonas* consists of many different species and pathovars that cause plant disease. Among them, *Xanthomonas translucens* pv. *undulosa* is one of several closely related pathovars in the *X. translucens* group. *X. t.* pv. *undulosa* causes bacterial leaf streak (BLS), also known as black chaff, is an economically important pathogen of small grains worldwide. Yield losses up to 40% have been attributed to this pathogen in the United States. Although the bacterium was reported as early as 1917, severe disease epidemics in the northern Great Plains of the United States have only recently occurred. Despite the economic importance of this disease in U. S. agriculture, the establishment of *X. t.* pv. *undulosa* in susceptible hosts and how it causes disease are poorly understood. A long-term goal of our research is to identify the molecular mechanisms operative in the interaction of *X. t.* pv. *undulosa* and wheat. In our recent studies, over 600 wheat landraces and advanced breeding lines around the world were evaluated for resistance to *X. t*. pv. *undulosa*. The majority of wheat genotypes were highly susceptible to *X. t.* pv. *undulosa*. Strains of *X. t*. pv. *undulosa* collected from North Dakota were analyzed by repetitive sequence-based polymerase chain reaction (rep-PCR) and insertion sequence-based PCR (IS-PCR). The strains varied significantly in virulence and were genetically diverse. Currently, the genome sequencing of two strains of *X. t.* pv. *undulosa* from wheat and barley is in progress. The completed sequence of *X. t.* pv. *undulosa* should help to identify genes involved in pathogenesis and provide a foundation for intervention strategies to control this devastating disease in wheat.

2

***Os11N3* is a host susceptibility gene induced by *Xoo* effector AvrXa7**

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Xanthomonas oryzae pv. oryzae (*Xoo*) uses type III Transcription Activator like (TAL) effectors for effective host invasion and pathogenicity. *Xoo* TAL effectors PthXo1, PthXo6 and PthXo7 induce rice genes *Os8N3*, *OsTFX1* and *OsTFIIA*γ*,* respectively. An alternate TAL effector AvrXa7 reprogrammes the transcription of another host gene *Os11N3,* which belongs to the same family as *Os8N3*. RNAi mediated knockdown of *Os11N3* resulted in plants resistant to strains with AvrXa7 but not PthXo1. *Os11N3* T-DNA insertional mutants also showed a similar response. Plants homozygous for insertion were resistant to strains with *avrXa7* as their major TAL effector but remained susceptible to strains containing *PthXo1*. Plants heterozygous for insertion were susceptible to strains with either effector. The results indicate that the pathogen may overcome *xa13* mediated resistance by targeting alternate susceptibility genes.

3

Silencing the 14-3-3 gene family member GF14e results in a lesion mimic phenotype and enhanced resistance to the bacterial blight pathogen

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The 14-3-3 protein family is found in all eukaryotes and is implicated in diverse biological functions including cell cycle regulation and signal transduction. In rice, the 14-3-3 gene family has at least eight members, named GF14a-h. *GF14e* has been associated with quantitative disease resistance to the rice blast pathogen in multiple mapping populations. *GF14e* expression, as measured by reverse transcriptase PCR (RT-PCR) in susceptible and resistant rice lines, did not show differences correlated with host-pathogen interactions. Silencing of *GF14e* by RNAi in a stable transgenic rice line resulted in a lesion mimic phenotype that appears at approximately 25 days after sowing. The silenced lines exhibit enhanced resistance (reduced bacterial numbers and shorter lesions) to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae;* the enhanced resistance is correlated with mimic lesion formation. However, the silenced line did not show enhanced resistance to the fungal pathogens *Rhizoctonia solani* (sheath blight) or *Magnaporthe oryzae* (rice blast). Current experiments are focused on understanding the role of *GF14e* in cell death by exploring potential interactions between the GF14e protein and bacterial effectors.

4

Identification of an avirulence gene, *avrxa5,* from a rice pathogen *Xanthomonas oryzae* pv. *oryzae*

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Xanthomonas oryzae pv. *oryzae*, the causal agent of bacterial blight in rice, interacts with rice plants in a gene-for-gene principle. *avr*-gene specificity is dictated by both avirulence (*avr*) genes in the pathogen and host resistance (*R*) genes. However, there is no evidence so far to show that an *avr* gene in the pathogen matches a recessive *R* gene in rice. Here we isolated an *avrBs3/PthA* family gene, *avrxa5*, from our previous clone p58, which was originally from the strain JXOIII. The gene specifically converted PXO99[A] strain from compatibility into incompatibility in rice IRBB5, but not in rice IR24. IRBB5 and IR24 are resistant and susceptible alleles of *xa5*, respectively. Sequencing indicated that *avrxa5*, highly similar to members of the *avrBs3/PthA* family, encodes a protein of 1,238 amino-acid residues with 19.5 thirty-four amino-acid direct repeats and its conserved carboxy-terminal region contains three nuclear localization signals and a transcription activation domain. Intriguingly, the thirteenth amino acids in the fifth and ninth repetitive units are missed. Importantly, the replacement of the repetitive region of *avrxa5* or *avrXa7* with the corresponding fragments of *avrXa7* or *avrxa5* changed their avirulence specificity in *xa5* or *Xa7* rice line. The results above indicated that *avrxa5* is distinct from previous characterized *avrBs3/PthA* members, suggesting that *avrxa5* specificity towards rice recessive *xa5* gene could facilitate our understanding on molecular mechanisms of plant-pathogen interactions.

5

Genome-wide analysis of a transposon inserted library of a model rice pathogenic bacterium *Xanthomonas oryzae* pv. oryzicola

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Bacterial leaf streak, caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xooc*), is an important disease in rice. To genome-widely mine pathogenesis-related genes of the pathogen, a Tn5 transposon-mediated mutation library of the pathogenic strain RS105 was produced. Twenty five thousand transformants were generated, appropriately corresponding to 5 X ORF coverage of the genome, and inoculated into rice and tobacco individually and respectively. Southern blot and thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR) analyses of Tn5 insertion sites of randomly selected mutants suggested a random mode of transposition. *in planta* pathogenicity assay revealed 321 mutants reducing virulence in rice, 19 mutants being deficient in extracellular polysaccharide production, 12 mutants losing extracellular protease activities completely or partially, 38 mutants not triggering hypersensitive response in tobacco and pathogenicity in rice, and 3 mutants maintaining hypersensitive response (HR) in tobacco but no pathogenicity in rice. Sequencing of inserted sites revealed novel genes which have not been reported so far. The above suggests that this high-quality library will facilitate identification of pathogenicity-related genes as well as functional genomics in *Xooc*-rice pathosystem.

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See **Chen** under **Speaker Abstracts**

7

Regulation of a new utilization pathway of xylan degradation products by the LacI repressor XyxR in *Xanthomonas campestris* pv. campestris

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The microbial degradation of plant cell wall is not only an important biological process but is of increasing scientific interest for biotechnological applications. *Xanthomonas campestris* pv. campestris (*Xcc*), the causal agent of black rot disease of Brassicaceae, is known for its ability to exploit plant compounds. This characteristic seems to be linked to the over-representation of TonB-dependent transporters (TBDTs) and the presence of numerous CUT systems (Carbohydrate Utilization systems containing TBDTs) required for the scavenging of plant carbohydrates. In this study, we describe a new *Xcc* CUT system involved in xylan utilization which is controlled by the LacI-type repressor XyxR (encoded by *XCC4101*). We show that xylo-oligosaccharides are better inducers than the monomer, xylose. This repressor regulates the expression of genes present in three loci. These loci encompass two TBDTs, two inner membrane transporters, two xylanase, a xylosidase and other degradative enzymes involved in the utilization of xylan and its degradation product. Based on xylanase activities, we show that these loci contain one gene encoding for the major *Xcc* xylanase. Based on indirect transport assays, we show that the two TBDTs of the xylan CUT system, XCC4120 and XCC2828, are required for the uptake of xylo-oligosaccharides, confirming their role in carbohydrate scavenging. Moreover, xylo-oligosaccharides and xylose are transported exclusively across the two inner membrane transporters of the xylan CUT system. Xylan is the major component of hemicellulose in plant cell walls. Xylo-oligosaccharides uptake through TBDTs represents a new aspect in the exploitation of these molecules by bacteria. It suggests the existence of active and specific mechanisms for nutrient uptake through the outer membrane.

8

Harpin domain in *hrpW* gene from *Xanthomonas citri* subsp. *citri* is involved in pathogenicity

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Harpin proteins are hypersensitive response (HR) elicitors secreted through type III secretion system (T3SS) and present in most *Xanthomonas* spp. HrpW protein in *Xanthomonas citri* subsp. *citri* (Xcc), cause citrus canker, is a T3-secreted protein which contains two domains, harpin and pectate lyase. Additionally, *hrpW* is located outside of the hypersensitive response and pathogenicity (*hrp*) cluster, which is a unique feature of *hrpW* in Xcc. To evaluate the role of HrpW in pathogenicity of Xcc we constructed two different in-frame deletion mutants in $hrpW_{Xcc}$, where 87 bp was excised from harpin domain creating a harpin domain mutant and leaving the pectate lyase domain intact, and 885bp was deleted in $hrpW_{Xcc}$ creating $hrpW_{Xcc}$ null mutant with both domains deleted. Mutation in the harpin domain partially impaired disease development in young grapefruit leaves. In contrast, the $hrpW_{Xcc}$ null mutant inoculation showed typical citrus canker symptoms. In addition, bacterial population assays revealed that both mutants had no differences in bacterial growth when compared with the wild type strain. Further experiments are ongoing to explain these results.

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See **Hajri** under **Speaker Abstracts**

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The prokaryotic sulfotransferase, RaxST, catalyzes sulfation of a tyrosine residue in the N-terminal domain of the Ax21 PAMP

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The XA21 pathogen recognition receptor confers immunity against strains of *Xanthomonas oryzae* pv. *oryzae* carrying the pathogen associated molecular pattern, Ax21. In our previous studies, we identified eleven genes that are required for Ax21 activity (*rax*) in *Xoo.* One of these genes encodes the 194 aa pathogen associated molecular pattern (PAMP), Ax21. Three of these genes, *raxP*, *raxQ,* and *raxST,* encode proteins involved in *Xoo* sulfation metabolism. Here, we show that Ax21 activity in the *raxQ* knockout strain is complemented by exogenous 3'-phosphoadenosine-5'-phosphosulfate (PAPS), clearly demonstrating that RaxQ is critical for PAPS synthesis and that a PAPS-dependent molecule is essential for Ax21 activity. In order to monitor the enzymatic activity of the *raxST* product, an enzyme coupling assay system was developed for sulfotransferase activity. Using this assay system, we demonstrate that *Xoo* RaxST possesses tyrosine sulfotransferase activity and utilizes the phosphoadenosine phosphosulfate produced by RaxP and RaxQ to transfer a sulfuryl group to the Ax21 peptide. This report both demonstrates that tyrosine sulfotransferase activity in a prokaryotic species and shows that its substrate is the Ax21 PAMP.

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See **He** under **Speaker Abstracts**

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Occurrence of citrus canker caused by *Xanthomonas axonopodis* pv. *citri* on commercial fruits of navel orange and mandarin in Saudi Arabia

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Citrus fresh fruits with canker-like symptoms exported to Saudi Arabia from Pakistan and China countries. Fruits of Mandarin (*Citrus rediculate* cv. Kinnow) and navel orange (*Citrus sinensis*) from Pakistan and China respectively, were taken from several markets. The samples consisted in several fruits (from 1–20, with one to several canker-like spots on each), and the analyses were only performed on the lesions observed on the fruits. Each lesion, and 2 mm of the peel around it, was cut in pieces with a sterilized scalpel or razor blade, comminuted in PBS buffer and left for 10–20 min at room temperature. 100 µl of the PBS extract were streaked onto plates of YPGA medium and incubated at 25°C. After 3–7 days, Xac-like colonies were selected and purified for further analyses. Based on physiological, biochemical and genetic characterizations including NaCl tolerance, hydrolysis of gelatin and polymerase chain reactions with primer pair 4/7 to *X. axonopodis* pv. *citri* (*Xac*) and BOX- PCR, all isolates were identified as *Xac*. Pathogenicity of purified Xac colonies was evaluated on detached grapefruit leaves (*Citrus grandis*). Surface of young leaves was disinfected with ethanol 70%, washed with sterile water and placed on 1% agar plates. The leaf was cut with a scalpel, the edge of the wound inoculated with 10µl of a suspension of 10^9 CFU ml), and incubated at 25–28°C until symptoms appearance (1–2 weeks). Water-soaked lesions, 2-3 mm in diameter, developed at the inoculation sites after 10 days and the bacteria were consistently re-isolated from the affected tissues. Negative controls with sterile water were performed. The laboratory practices throughout the processes involved with the management of suspected contaminated fruits, extraction procedures, microbiological techniques (isolation, purification of suspected colonies, pathogenicity tests, etc), were performed following strict safety measures to avoid any possibility of pathogen escape.

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Diverse bacterial plant pathogens contain homologs of the *Xanthomonas oryzae* pv. *oryzicola avrRxo1* effector gene

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The maize NB-LRR gene *Rxo1* confers a hypersensitive response (HR) and induction of defense response genes after challenge with the rice bacterial streak pathogen *Xanthomonas oryzae* pv. *oryzicola. X. o.* pv. *oryzicola*, which is not a pathogen of maize, encodes a type III secretion-dependent effector *avrRxo1.* Four additional homologs of *avrRxo1* were cloned from *Burkholderia andropogonis, Acidovorax avenae* subsp. *avenae, A. a.* subsp. *citrulli,* and *X. axonopodis* pv. *vesicatoria.* While the amino termini of the homologs are diverged, the carboxy termini are similar, with the *Xanthomonads* grouping into one cluster, and the *Burkholderia* and *Acidovorax* grouping into a second cluster. The amino acid similarities between AvrRxo1 homologs ranged from 49% to 86%. The five homologs are adjacent to an ORF with similarity to chaperones; the two ORFs have GC contents distinct from the genomes of each source bacterium, suggesting that they were horizontally acquired. All five AvrRxo1 homologs contain a putative ATP/GTP binding site; mutation of this site in the *X. o.* pv. *oryzicola avrRxo1* eliminates HR function. While all *X. o.* pv. *oryzicola* strains from Asia contain *avrRxo1* and exhibit a strong HR in maize and rice with *Rxo1,* only some strains of *B. andropogonis* contain the homolog and elicit an HR on *Rxo1*-containing plants. When *avrRxo1* from *X. oryzae* pv. *oryzicola* is introduced into *X. oryzae* pv. *oryzae* strain PXO86, the strain shows enhanced virulence on rice. Thus the presence of *avrRxo1* homologs in diverse bacterial pathogens indicates that the gene might play a role in pathogenic fitness/aggressiveness.

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See **Kim** under **Speaker Abstracts**

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A novel molecular typing system for pathogenic xanthomonads

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated sequences (*cas*) form hypervariable loci which are widely distributed in prokaryotes. The repetitive region is characterized by short interspersed unique spacer sequences which often match to bacteriophage-derived DNA, thus providing immunity against foreign genetic elements, in particular bacteriophages. Bacteria of the genus *Xanthomonas* cause diseases on over 400 different host plants, including many economically important crops, such as rice, wheat, citrus, and banana plants. We screened more than 300 strains of *Xanthomonas*, representing ten different species (44 pathovars), for the presence of CRISPR loci. Only a few pathovars were found to possess a CRISPR locus, among them *X. axonopodis* pv. *vasculorum*, *X. axonopodis* pv. *cassavae*, *X. campestris* pv. *raphani, X. citri* pv. *citri*, *X. oryzae* pv. *oryzae* (*Xoo*), *X. translucens*, and *X. vasicola* pv. *musacearum*. Presence/absence of CRISPR loci appeared to be conserved at the pathovar level, except for *Xoo*. The apparent absence of CRISPR loci from African *Xoo* isolates confirms previous results showing that African *Xoo* isolates form a phylogenetic group that is distant from the Asian *Xoo* group. Comparative genomics suggested that the common ancestor of all xanthomonads had two CRISPR loci which in most species/pathovars got lost during evolution. Based on DNA sequence information about the terminal spacers of 32 Asian *Xoo* CRISPR loci we postulate that the common ancestor of these strains had all the spacers which are nowadays still found in a few strains and that some spacers got lost during evolution in some *Xoo* lineages. This work represents the first proof of concept of CRISPR analysis as a molecular tool for high-resolution strain typing, phylogenetic studies, and global surveillance of a phytopathogen.

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A conserved 22-amino-acid peptide of *Xanthomonas oryzae* pv. *oryzae* – a candidate for AvrXa21?

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The Gram-negative bacterium *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*) causes bacterial leaf blight (BLB) of rice, one of the most devastating diseases of rice worldwide. Until now, about 30 resistance genes against BLB have been isolated from domesticated or wild rice species, among them *Xa21*. *Xa21* was the first rice disease resistance gene to be cloned. It encodes a receptor-like kinase which is thought to detect a small molecule, called AvrXa21, which is produced from *Xoo*. Eight *rax* genes (required for AvrXa21 activity), organized in four operons, have been identified in *Xoo*. Yet, the molecular nature of AvrXa21 is still unknown. Since *Xa21* is one of the most important resistance genes that has been used in several breeding programmes, characterization of the AvrXa21 molecule and understanding of the ligand-receptor interaction would be extremely useful for future breeding programmes and for studies of the defense response in rice. By *in silico* analysis, we have identified a candidate *avrXa21* gene within the *raxSTAB* operon. This candidate gene shares overlapping stop/start codons with its upstream and downstream genes, *raxST* and *raxA*, respectively. It encodes a 22-amino acid peptide with a central double-glycine motif which is characteristic for small peptides secreted by a subfamily of ABC transporters, including RaxA. DNA sequence analysis shows that this small gene is strictly conserved within *X. oryzae* and that a similar peptide might be produced by other *Xanthomonas* species, such as *X. axonopodis* and *X. translucens*. We will show our latest results on the role of the candidate *avrXa21* gene in the pathogen-plant interaction.

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Are virulence factors involved in initial host colonization processes responsible for host specificity?

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Bacteria belonging to the genus *Xanthomonas* are grouped in pathovars defined on the basis of their host range and type of symptoms. Within a pathovar strains are highly specialized on a restricted host range. Currently, the genetic basis of host specificity for pathogenic bacteria remains poorly understood. We hypothesize that virulence factors which are involved in the initial stages of host colonization play a role in host specificity. Candidate host specificity factors selected are methyl accepting chemotaxis proteins (MCPs) and adhesins. Actually, nothing is known about the repertoires of MCPs and adhesins in strains of the genus *Xanthomonas*. We have determined the distribution of 30 MCPs and 13 adhesins among 180 strains belonging to 18 pathovars of *X. axonopodis* and 3 pathovars of *X. campestris* , as well as phylogenetic relationships of the strains based on polymorphism analyses of housekeeping genes. The selected strains were isolated from different host plants and various geographical origins. Some pathovars were polyphyletic while others were monophyletic. Repertoires of MCPs and adhesins were polymorphic among strains and displayed both ubiquitous and variable genes. Among the pathovars and genetic lineages tested, thirteen were distinguished by their distinct suites of MCPs and adhesins. The other pathovars and genetic lineages remained difficult to differentiate: five groups with unique repertoires were constituted. Polyphyletic pathovars displayed essentially homogeneous repertoires. These results show that events leading to host specificity occur as early as initial steps of chemotactic attraction and adhesion on host tissues.

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Dissection of *Xanthomonas oryzae pv. oryzae* regulatory circuit for Ax21

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Bacterial blight disease has been reported to reduce the annual rice production by as much as 50 percent in rice-growing countries. Thus, it is imperative that we develop methods to control this disease because it is an important crop for a large part of the world's human population. Pathogens have developed integrated regulatory circuits that control the coordinated expression of one set of genes in one environment and a different set of genes in another. In pathogenic bacteria, these regulatory circuits are generally controlled by two-component systems (TCSs), composed of histidine kinases and response regulators. In response to environmental stimuli, the histidine kinase phosphorylates the cognate response regulator, which then regulates gene expression. We have recently shown that the Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae (Xoo)*, a causal agent of bacterial blight in rice, requires two TCSs for Ax21 activity, a small peptide that triggers rice XA21-mediated resistance. One of them, the RaxR/H system, regulates *rax*-gene expression by Ax21 depending on cell population density, and the other, PhoP/Q, governs *Xoo* virulence through regulation of *hrp*-genes as well as control of Ax21 activity. Furthermore, PhoP expression is induced in RaxR knockout mutant strain. We hypothesize that the two TCSs coordinate production of Ax21. Ax21 appears to be a key regulator of the transition from a quiescent or epiphytic state to an invasive or pathogenic state. To investigate the PhoP regulon and relationship between the two TCSs, the binding motif that are directly regulated by PhoP protein is identified by performing promoter analysis *in vivo* and *in vitro* assays and using regulatory sequence prediction software analysis approaches. Secondly, To assess the interaction between the PhoP/Q and RaxR/H two component regulatory systems, the phosphorylation process that is occurring between PhoQ, PhoP and RaxR is investigated by performing trans-phosphorylation assays. These studies provide a comprehensive understanding of the regulation circuits in the global scale that regulate the expression of multiple virulence factors and an answer why *Xoo* secretes the Ax21 molecule.

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Mapping and identification of the *Xv4* resistance gene in *Solanum pennellii*

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Xanthomonas perforans is the prevalent species causing bacterial spot disease of tomato in Florida. Sequence data from twenty Florida field isolates of *X. perforans* showed that the effector gene *avrXv4* is highly conserved, suggesting that it plays an important role in pathogen fitness. With the eventual goal of molecular breeding for durable resistance, we set out to identify a resistance gene whose product recognizes the AvrXv4 effector protein. AvrXv4 was previously found to induce a hypersensitive response (HR) in the wild species *Solanum pennellii*, but the *R* gene responsible for this recognition, designated *Xv4*, has not been identified. We have made progress toward identifying the chromosomal location of *Xv4* using map-based cloning with molecular markers. Screening a collection of fifty introgression lines (ILs) of *S. pennellii* in *S. lycopersicum* (cultivated tomato) for an AvrXv4-dependent HR revealed that the *Xv4* resistance locus is within a 12.9-cM region of chromosome 6, in ILs 6-2 and 6-2-2. To more precisely map *Xv4*, we are genotypically and phenotypically characterizing a segregating F_2 population, using molecular markers available from the SOL Genomics Network that have been converted to cleaved amplified polymorphic sequence (CAPS) markers. F_2 recombinant plants identified to date suggest that *Xv4* resides within a 1.5-cM region between the markers TG352 and SSR128. We are continuing to identify molecular markers that co-segregate with *Xv4* resistance, which will be employed to probe a *S. pennellii* bacterial artificial chromosome (BAC) library. A BAC contig that spans the *Xv4* locus will be isolated and the DNA sequence will be determined,. After computational identification of candidate resistance genes, we will employ a combination of *Agrobacterium tumefaciens*-mediated transient expression, virus-induced gene silencing, and stable transgenic complementation to isolate *Xv4* and demonstrate its functionality.

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XopZ is a type III effector and virulence factor in *Xanthomonas oryzae* pv. oryzae

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Xanthomonas oryzae pv. oryzae (Xoo) depends on a type III secretion system (T3SS) to translocate the effectors into host cells for its ability to cause bacterial blight of rice. The majority of type III (T3) effectors with known virulence function in Xoo belong to a family of transcription activator-like (TAL) effectors, and some have been found to specifically induce host gene expression, which is partially responsible for disease susceptibility. Additionally, other, non-TAL related effector genes are present in the genome. However, their role for virulence and their mode of action have yet to be elucidated. Here we report the identification of one T3 effector gene that contributes to virulence in blight disease from Xoo strain $PXO99^A$. *XopZ* (PXO01041) encodes a predicted 1414 amino acid protein with unknown function. $PXO99^A$ contains two identical copies of the gene due to a large ~200kb duplication in the genome. Strains with Knock-out mutations of one copy of *XopZ* did not exhibit any visible virulence defect as measured by leaf clipping inoculation. However, strains with mutations in both copies of *XopZ* displayed reduced virulence in terms of lesion length and bacterial multiplication when compared to the wide type strain $PXO99^A$. The introduction of one genomic copy of *XopZ* or its open reading frame (ORF) under the control of the promoter from the neomycin phosphotransferase II gene in plasmid pHM1 restores the mutant to full virulence. Transient and ectopic expression of *XopZ* in *Nicotiana benthamiana* suppresses host basal defense responses that are induced by a T3SS mutant of $PXO99^A$, suggesting a role for XopZ in suppressing host innate immunity during Xoo infection. *XopZ* is widely distributed among Xoo strains, and homologs are found in all the sequenced *Xanthomonas* spp., suggesting a conserved role for this type of effector genes in *Xanthomonas* plant pathogens. Our results indicate that XopZ is a novel type III effector and contributes virulence to Xoo strains for bacterial blight of rice.

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Transcription profiling analysis of two response regulators, PhoP and RaxR, propose mechanism beneath the regulation of Ax21 expression in Xanthomonas oryzae pv. oryzae

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Xanthomonas oryzae pv. *oryzae* (*Xoo*), a causal agent of bacterial blight disease in rice, like other bacteria, uses two-component regulatory systems (TCSs) for modulation of gene expression in response to environmental cues. Previously, we observed the partially loss of Ax21 activity of Xoo knockout strains on rice line containing Xa21 in Xoo that are two TCSs, composed of RaxR/H and PhoP/Q indicating that these TCSs mediate the activation of Ax21 activity by perceiving extracellular signals, phosphorelating the certain response regulators, and ultimately regulating gene expression. The evaluation of gene expression by quantitative real time PCR of rax genes suggested that Ax21 molecule is a novel quorum sensing molecule, we, recently, have promising progress to identify and characterize Ax21 gene. Based on previous proteomic analysis in our lab, we observed that the PhoP proteins is increased in *raxR* knockout strains suggesting the negative regulatory effect between these two response regulators (RRs), though little is known about the mechanism whether this effect is from direct or indirect signal transduction. By using transcriptional profiling analysis and hierarchical clustering analysis of PhoP and RaxR regulon, we discovered that Ax21 gene has unique expression pattern and is regulated by PhoP and RaxR. To study the mechanism how these RRs control expression of Ax21 gene and do signaling crosstalk in transcription level. The *in vitro* gel shift mobility assay was used to assess the direct interaction between the response regulator protein and promoter of raxR, phoP, and Ax21 gene, as well as, GFP-promoter fusion was used as *in vivo* assay indicating the direct regulation of RaxR on Ax21 gene expression, however, the interaction between RaxR and PhoP is still under investigation process. Here, the proposed regulatory loop of three components is demonstrated based on preliminary integration of transcriptional profiling analysis, quantitative realtime PCR, and promoter study results.

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The *Xanthomonas* effector AvrBsT suppresses the AvrBs1 HR in pepper

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The Gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) causes bacterial spot disease in pepper and tomato plants. Pathogenicity depends on a functional type III secretion system (T3SS) and translocation of more than 20 effector proteins into the plant cell. In case of recognition of a given effector protein by a plant that carries the corresponding resistance (R) gene bacterial growth is arrested and often a hypersensitive response (HR), a rapid local plant cell death, is induced. The effector AvrBs1, e.g. in *Xcv* strain 85-10, elicits a rapid HR in *Capsicum annuum* (pepper) carrying the Bs1 resistance gene. However, the presence of AvrBsT in the plant cell leads to a strong reduction of the AvrBs1-specific HR. AvrBsT is a member of the YopJ/AvrRxv family that is highly conserved in plant and animal pathogenic bacteria and has predicted acetyl transferase activity. A pepper serine/threonine kinase was identified as interaction partner of AvrBsT. Virus-induced gene silencing showed that this kinase is involved in the specific induction of the HR by AvrBs1.

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***talc* is a new major virulence determinant of the *avrBs3/pthA* gene family essential for African *Xanthomonas oryzae* pv. oryzae strain BAI3 pathogenicity**

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The pathogenicity of many Gram-negative pathogenic bacteria relies on a type III secretion system mediating the secretion of effector proteins into the extra-cellular milieu and/or their translocation into the host cells directly. Loss of individual effector genes usually results in no or poor effect on virulence because of functional redundancy or inappropriate testing conditions. Here, we report on the identification of *talc*, a new *Xanthomonas oryzae* pv. oryzae (*Xoo*) major virulence effector of the *avrBs3/pthA* gene family. *Xoo* is the causal agent of bacterial blight of rice, a disease occurring in most rice growing areas including Africa, where it has become emergent in the 80's. Recently, new races originating from West Africa were characterized, highlighting substantial differences between Asian and African *Xoo* genomes. Among other specific features, African strains were shown to harbor a reduced set of *avrBs3/pthA* genes. A systematic mutagenesis approach has been initiated, aiming at deciphering the contribution of each of the 8 paralogs of African *Xoo* strain BAI3 to its lifestyle and pathogenicity. Several mutants were characterized, one of which mutated in *talc*, appeared severely affected in the production of leaf lesion symptoms on susceptible rice lines. *In planta* growth curves analysis show that *talc* mutant multiplies at almost Wild-type levels upon leaf-clip inoculation, but remains restricted to the site of inoculation at the apex of the leaf, suggesting a requirement of *talc* for vascular tissues colonization. As expected, complementation of the mutant with a plasmid-born copy of *talc* under the control of its native promoter complemented for growth and virulence, while other *Xoo* major virulence TAL effectors such as *avrXa7* failed. In addition, *talc* is able to delay nonhost HR in tobacco. Finally, a phylogenetic analysis of the *tal* family in *X. oryzae* was performed and revealed *talc* to be closer to *tal* members of *X. oryzae* pv. oryzicola, the causal agent of bacterial leaf streak, rather than to those of other *Xoo* strains of Asiatic origins. Altogether, our data give insight into the intriguing evolutive history of TAL effectors in African *Xoo* strains.

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Increased temperature favored effectiveness of a rice bacterial blight disease resistance gene

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High temperatures promote development of many plant diseases and reduce effectiveness of disease resistance (*R*) genes. Thus, increasing environmental temperatures associated with climate change will complicate *R* gene mediated control of plant disease. In many rice producing countries, two crops of rice are produced, with more disease occurring in the season with higher day/night temperatures. While studying the factors that influence durability of rice bacterial blight *R* genes, we identified *Xa7*, which restricts disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) at high day/night temperature regimes (31°C /29°C and 35°C /31°C) more effectively than at low temperature regimes (29°C /21°C). In contrast, other *R* genes (*Xa3*, *Xa4*, *xa5*, and *Xa10*) are less effective at high temperatures, and allow more disease development than at low temperatures. We monitored the performance of a bacterial blight (BB) resistance gene *Xa7* in field studies, and addressed the influence of high temperature on its effectiveness. Disease severity was monitored in field plots over 11 yr (22 cropping seasons). The virulence of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) field populations to *Xa7* was assessed. While exposure to *Xa7* over 11 yr is selecting for virulence in the *Xoo* population, we found that the resistance conferred by *Xa7* is still effective. *Xa7* restricts disease more effectively at high than at low temperatures. In contrast, other *R* genes are less effective at high temperatures. We propose that greater effectiveness of *Xa7* at high temperatures may contribute to the durability of *Xa7*.

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Plant cell wall degrading enzymes: How many sources in *Xanthomonas*, *Xylella* and *Stenotrophomonas*?

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As a result of the genome sequencing projects of the genera *Xanthomonas*, *Xylella* and *Stenotrophomonas*, several genes were identified as possibly coding for plant cell wall degrading enzymes (PCWDE). Some of these gene clusters have been previously associated to pathogen virulence. We propose a phylogenetic relationship and genome repertoire enumeration for whole ORFs and putative individual domains of these genes, using bioinformatics toolbox. The pipeline was written in order to establish single linkage BLAST clusters of selected sequences along with annotation, COG and KO information retrieval, PFam HMM search, multiple alignment and reliability score generation and bootstrapped maximum likelihood phylogenetic analysis for the whole gene and individual PFam domains. Another algorithm was also written that generates a table comparing the PFam domain architectures on an inter-genome maximum repertoire basis for each genome, with a placement criterion obeying clusterization. Through this analysis we aim to trace a profile of the quantitative and qualitative characteristics for the PCW degrading apparatus of each genome to be correlated with the infection phenotype of each pathogen. This and other analysis will serve as basis for candidate gene selection for future molecular characterization work and elaboration of degradation cocktails with cell wall composition specificity, an important aspect for the biomass manipulation industry.

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Molecular cloning of avrXa23, a type-III effector gene from Xanthomonas oryzae pv. oryzae

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Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the causal agent of bacterial blight of rice. *Xa23* is a bacterial blight resistance gene originally identified in wild rice, *Oryza rufipogon*, and transferred into the *indica* rice cultivar JG30 by cross and back crosses, resulting in the near-isogenic line CBB23. *Xa23* is dominant and resistant to all *Xoo* field isolates tested. The map-based cloning of *Xa23* in our laboratories is in progress. To investigate the interaction between *Xa23* and *Xoo,* we seek to isolate the corresponding avirulence gene *avrXa23*. Since no natural *Xoo* stain that overcomes CBB23 has been identified, we generated a random insertion mutant library of *Xoo* strain PXO99 using a Tn5-derived transposon tagging system, and isolated the mutant strains that are virulent on CBB23. A total of 24,192 Tn5-insertion clones was screened on CBB23 by leaf-clipping inoculation and at least eight of them caused lesions on CBB23 comparable to those on JG30, the susceptible recurrent parent of CBB23. Polymerase chain reaction and Southern blot analysis showed that all the eight mutants, designated as P99M1, P99M2, P99M3, P99M4, P99M5, P99M6, P99M7 and P99M8, have a single Tn5-insertion in their genomes. The flanking DNA sequences of the Tn5-insertion sites were isolated by PCR-walking and sequenced. Bioinfomatic analysis of the flanking sequences, by aligning them with the whole genome sequences of Xoo strains PXO99, KACC10331 and MAFF311018 through NCBI, revealed that the Tn5-insertions disrupted genes that encode TAL effector AvrBs3/PthA, ISXo1 transposase, Type II secretion system protein like protein or outer membrane protein, glycogen synthase, cytochrome C5 and conserved hypothetical protein, respectively. We also screened a TAL effector gene mutant library of $PXO99^{A}$ for strains virulent on CBB23. Five out of thirty mutants are completely compatible with CBB23. Reintroduction of one $PXO99^{A}$ genomic cosmid into one mutant ($PXO99^{A}$ ME8) restores its avirulence on CBB23. The cosmid can also complement the avirulence defect of Tn5-insertion mutants P99M2, P99M4 and P99M5. One of three TAL effector genes in the cosmid clone is the functional avirulence gene on CBB23 and referred to as *avrXa23*. *avrXa23* encodes a novel TAL effector of 1238 amino acids, containing the C-terminal nuclear localization signals and transcription activation domain, and the central region of 26.5 direct repeats of 34 amino acids, characteristic of TAL effector family.

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