



2013 APS-MSA Joint Meeting Abstracts of Special Session Presentations

Biology of Pathogens

Filling in the Gaps: How Do Xanthomonads Adapt to Diverse Hosts, Tissues, and Environments?

The xylan utilization system of *Xanthomonas campestris* controls epiphytic life and reveals common features with animal gut symbionts

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TonB-dependent receptors (TBDTs) are outer membrane proteins mainly known for the active transport of iron siderophore complexes in Gram-negative bacteria. Analysis of the genome of *Xanthomonas* species revealed an overrepresentation of these transporters. This overrepresentation is associated with the ability to exploit plant carbohydrate and we proposed the existence of specific carbohydrate utilisation systems with TBDTs (named CUT systems). Recently, we identified a CUT system involved in the utilisation of xylan, a major component of plant cell wall and the second most abundant plant polysaccharide in nature. This CUT system encompasses genes required for the degradation of xylan as well as genes for the transport and metabolism of xylo-oligosaccharides, xylose and glucuronate. Interestingly, these genes which expression is induced by xylo-oligosaccharides, are required for optimal growth on plant leaves. Part of the xylanolytic machinery of *Xanthomonas*, including TBDT genes, displays a high degree of conservation with the xylose-regulon of the oligotrophic aquatic bacterium *Caulobacter crescentus*. Moreover, it shares common features, including the presence of TBDTs, with the xylan utilisation systems of *Bacteroides ovatus* and *Prevotella bryantii*, two gut symbionts. These similarities and our results support an important role for TBDTs and xylan utilisation systems for bacterial adaptation in the phyllosphere, oligotrophic environments and animal guts.

Contribution of type III/TAL effectors to pathogenicity

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Most plant-pathogenic bacteria use a sophisticated protein delivery device to inject type III effectors (T3Es) into the host's cells for the benefit of the pathogen. The *French Network on Xanthomonads* studies several species of *Xanthomonas* and examines the role of T3Es during the infection process. Here, we will focus on two rice pathogens, *X. oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*). First, the diversity within T3E repertoires of 45 strains clearly showed that both pathogens belong to closely related, but distinct, phylogenetic groups. Both pathogens comprise core and variable T3E suites that probably have distinct roles in pathogenicity and different evolutionary histories. We examined whether or not one of the differential T3Es between *Xoo* and *Xoc*, XopO, acts as a determinant of tissue specificity. Second, we monitored 41 candidate *Xoo* T3Es for their ability to translocate an AvrBs1 reporter into plant cells. Nine proteins were identified as *bona fide* T3Es. Mutations in their genes revealed that one of them, *xopR*, contributes to virulence in hybrid rice. Third, we isolated a new TAL effector that induces a rice *SWEET* gene to promote colonization of the leaf blades. Artificial *TAL* genes were then used to systematically evaluate the potential of *SWEET* genes to support the growth of *Xoo* in the xylem vessel. Only five phylogenetically close *SWEET* proteins which presumably act as sucrose transporters were found to provoke virulence.

Genome and transcriptome analysis to reveal adaptation to new environments and hosts

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Pathovars of *Xanthomonas* are interesting working models for the analysis of mechanisms of adaptation and specialization to hosts, plant tissues, niches and environments. The understanding of such mechanisms is important on practical and academic points of view since they can help explain how emergence of new diseases or of diseases in new situations may occur. Four research groups belonging to the *French Network on Xanthomonas* (FNX) have recently focused on genome and transcriptome sequencing of several pathovars of *Xanthomonas* chosen for their adaptation particularities, in a project called "Xanthomix". *Xanthomonas* strains attacking Anacardiaceae,

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citrus, crucifers, legumes or rice were selected to help understand adaptation processes: genetic structure revealing either convergence or divergence for causing a specific disease on a specific host ; host tissue specialization (xylem vs. mesophyll) ; virulence variation (broad vs. narrow host range); contrasted epidemic situation (endemic vs. epidemic). RNAseq experiments were performed on a large set of strains to determine the extent and the diversity of the hrp regulon. In addition, the RNAseq dataset is also being used to improve *Xanthomonas* genome annotation and gene prediction. Analysis of transcripts allowed us to scan for unknown specific and non-specific effectors as well as small non-coding RNAs and hypothesize new mechanisms that could be involved in adaptation to host and environment.

Differences in patterns of host transcriptome modulation as a measure of diversity and adaptation of TAL effector-wielding *Xanthomonas* populations

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Many *Xanthomonas* deploy TAL effectors to modulate host gene expression for susceptibility. TAL effectors recognize their host genomic targets via a structurally modular mechanism that allows for rapid evolution of new specificities. Degeneracy in the mechanism and the presence of duplicated, variant TAL effectors in most strains result in collateral activation of host genes inconsequential to disease. Thus, the pattern of host gene expression changes following inoculation is a potentially useful phenotype to assess pathogen strain diversity. And, changes induced in common by multiple isolates may identify key susceptibility factors. We sought to test these hypotheses by comparing by RNAseq host transcriptional responses to 11 geographically diverse isolates of *X. oryzae* pv. *oryzicola*, which causes

bacterial leaf streak of rice. The number of genes induced by a strain ranged from 1177 to 3360 and those repressed from 800 to 4300. 570 genes were induced and 370 repressed by all 11 strains. Each strain induced some genes that were not induced by any other strain. A tree based on the gene expression changes grouped the strains in agreement with their geographical grouping. Thus host gene expression changes are a valid phenotype for assessing strain diversity. Ongoing work aims to relate the gene expression changes to TAL effector content to identify adaptations important in disease.

Flagellar motility and fitness in xanthomonads

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Xanthomonads are plant-associated bacteria that establish neutral, commensal or pathogenic relationships with plants. These γ -proteobacteria are motile by a single polar flagellum. Motility is an important feature involved in biofilm formation, plant colonization and hence considered as a pathogenicity factor. The French Network on *Xanthomonads* studies genomics of several species of *Xanthomonas*. *FNX* sequenced and annotated the genomic sequence of *X. fuscans* subsp. *fuscans* strain 4834-R (*Xff* 4834-R), the agent of common bacterial blight of bean. Sequencing revealed a deletion of 34 genes (33 kbp) involved in flagellar biosynthesis, which results in abolition of motility. This feature is not solely restricted to *Xff* 4834-R, since some flagellar biosynthetic genes are missing in approximately 5% of 300 *Xanthomonas* strains tested. As a consequence, these strains are also non-motile. Interestingly, half of the *Xff* strains isolated from the same epidemic than strain 4834-R are non-motile and this ratio is conserved among strains colonizing the next bean seed generations. Isolation of such variants in natural epidemics reveals that either flagellar motility is not a key function for fitness or that some compensation occurs within the bacterial population. Fine characterization of pathogenicity and ability to aggregate in biofilms give clues to interpret maintenance of non-motile strains in populations.

Insect-Transmitted Bacterial Diseases: Passing the Gift

Recent advances in understanding the biology of the insect-transmitted bacterium, *Xylella fastidiosa*

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Xylella fastidiosa is a Gram-negative bacterium that colonizes the xylem tissue of its plant hosts. This pathogen causes serious diseases such as Pierce's disease (PD) of grapevine, Almond Leaf Scorch and Citrus Variegated Chlorosis among many others. *X. fastidiosa* is transmitted by xylem-feeding insects such as sharpshooters (Hemiptera, Cicadellidae) and spittlebugs (Hemiptera, Cercopidae). The majority of the work has been performed in the *X. fastidiosa*-grape-sharpshooter pathosystem, and this presentation will cover what is known about the epidemiology of PD as well as information about the possible mechanisms used by the insect during the inoculation process. Thus far, bacterial cell surface proteins and carbohydrates have been identified as important mediators of bacterial acquisition and transmission by insect vectors, and special attention will be given to summarizing what is known about the molecular mechanisms governing this interaction between *X. fastidiosa* and its insect host.

Phytophagous insects, *Salmonella enterica*, and fresh produce: A tri-trophic interaction that can make you sick

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Salmonella enterica is the number one cause of bacterial food-borne illness in the United States. Despite fresh produce being considered the main vehicle of food-borne illness, to date, routes of *S. enterica* contamination and mechanisms of growth in plant environments remain unclear. This presentation will focus on the potential role of phytophagous insects in the growth, survival, and transmission of *S. enterica* on and to plants. Previously, we have found that thrips can acquire and harbor elevated *S. enterica* populations from contaminated produce. Following acquisition, contaminated thrips moved the human pathogen from inoculated produce to non-inoculated plants. Movement of *S. enterica* by thrips results in the spread of the human pathogen among plants. Taken together, these results suggest that an important insect pest of agricultural crops can transmit the human pathogen *S.*

enterica. Ongoing research complements these findings with further analysis of mechanisms of insect transmission and feeding behaviors that might have a direct influence on *S. enterica* growth in the phyllosphere.

Pantoea stewartii uses distinct type III secretion systems to alternate between host kingdoms

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Bacterial pathogens use phylogenetically distinct type III secretion systems (T3SS) that produce needle-like injectisomes or pili for the delivery of effector proteins into plant and animal host cells. The genome of *Pantoea stewartii* subsp. *stewartii* (*Pnss*), which causes Stewart's bacterial wilt and leaf blight of maize, encodes two phylogenetically distinct T3SSs. One is an Hrc-Hrp T3SS that is essential for bacterial pathogenesis in maize. In contrast, the second T3SS (*PSI-2*) belongs to the Inv-Mxi-Spa T3SS family that is typically found in animal pathogens. Mutations in the *PSI-2 psaN* gene, which encodes an ATPase essential for secretion of T3SS effectors by the injectisome, greatly reduced the persistence of *Pnss* in the gut of its flea beetle vector, *Chaetocnema pulicaria* (Melsh). The mutations also reduced transmission of *Pnss* to maize by beetles, but not by mechanical inoculation, indicating they did not affect pathogenesis in maize. Ectopic expression of the *psaN* gene in *Pnss* complemented the mutant phenotypes. In addition, the *PSI-2 psaN* gene was expressed at higher levels in insects compared to maize tissues. These data indicate an important role for *PSI-2* in vector transmission of the bacteria. Thus, the Hrp and *PSI-2* T3SSs play different roles in the life cycle of *Pnss* as it alternates between its insect vector and plant host.

Erwinia tracheiphila: Getting around with a little help from my friends

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Cucurbit bacterial wilt, caused by *Erwinia tracheiphila* (*Et*), is a major cucurbit disease in the Midwest and Northeastern United States. *Et* is a Gram-negative, xylem-inhabiting bacterium, that affects economically important crops such as cantaloupe, cucumber, squash, and pumpkin. The bacterial wilt