"Listening In" on How a Bacterium Takes Over the Plant Vascular System

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ABSTRACT Bacteria that infect the plant vascular system are among the most destructive kind of plant pathogens because pathogen proliferation in the vascular system will sooner or later shut down the plant's water and nutrient supply and necessarily lead to wilting and, in the worst case, death of the entire plant. How bacterial plant pathogens adapted to life in the plant vascular system is still poorly understood. As described in a recent article, Caitilyn Allen and her group studied the archetypical vascular pathogen *Ralstonia solanacearum*, the causative agent of bacterial wilt disease in almost 200 crop and ornamental plant species, and they have described the results of a microarray analysis that allowed them to "listen in" on the pathogen's sabotaging activity inside the plant [J. M. Jacobs et al., *mBio* 3(4):e00114-12, 2012]. Besides gaining for the first time an almost complete picture of *R. solanacearum* gene expression during infection, this approach allowed revision of a wrong assumption about the activity of the pathogen's type III secretion system during infection and uncovered the importance of sucrose as an energy source for vascular pathogens like *R. solanacearum*.

The plant vascular system fulfills two essential functions: it carries water and minerals from the roots to the leaves and transports sugars from the leaves to nonphotosynthetic parts of the plant, like roots and tubers. The xylem fulfills the first function. This amazing structure is able to transport water all the way from the roots to the leaves at the tops of the tallest trees through long tubes of connected dead plant cells. The phloem fulfills the second function but is made of living interconnected plant cells.

Since xylem consists of relatively nutrient-poor dead plant cells that are mainly transporting water and minerals, pathogens that have adapted to life in xylem can usually also survive well outside the xylem and are not considered intracellular pathogens. Pathogens living in the phloem are instead highly adapted to life inside the living nutrient-rich sugar-transporting phloem cells (and to life inside the insect vectors that transmit them from plant to plant). They are thus often impossible to culture, having lost the ability to survive freely in the environment, and are considered intracellular pathogens.

BACTERIAL VASCULAR PATHOGENS ARE A SERIOUS THREAT TO WORLD AGRICULTURE

Ralstonia solanacearum, the pathogen studied by Caitilyn Allen and coworkers in recent work (1), represents without doubt the largest and most diverse species complex of vascular plant pathogens. It includes dozens of variants attacking almost 200 different plant species around the world, mainly in warm tropical climates (2). At the same time, it is also one of the most intensively studied pathogens of the vascular system (3).

Although all types of plants can suffer damage from vascular pathogens, these pathogens are a particularly serious problem in tree crops. Because it takes years to grow a tree until it starts producing fruits or until it is ready to be cut down for its wood, the investment lost due to infection of tree crops with vascular pathogens is economically much more serious than that with herbaceous annual crops. Within the *Ralstonia solanacearum* species complex, there is one tree pathogen, *Ralstonia syzygii*, the causative agent of Sumatra disease of clove trees (4). While this pathogen is limited to Indonesia, other vascular pathogens that have recently emerged and/or spread to many parts of the world are putting entire industries in jeopardy: phloem-limited "*Candidatus* Liberibacter asiaticus," the presumed causative agent of citrus greening, is threatening the pure survival of the Florida orange industry (5), *Pseudomonas syringae* pv. actinidiae is threatening the economic viability of kiwifruit production in Europe and New Zealand (6), and *P. syringae* pv. aesculi is destroying horse chestnut trees in the parks of the United Kingdom (7). Another group of vascular pathogens threatens the wine industry in California and the citrus industry in Brazil: xylem-adapted *Xylella fastidiosa* (8). Therefore, studies such as Caitilyn Allen's (1) of how bacterial pathogens take over the plant vascular system are essential to developing new control strategies for all these pathogens. Below, I want to highlight two of the most interesting results obtained in the study.

THE TYPE III SECRETION SYSTEM IS ACTIVE EVEN AFTER *R. SOLANACEARUM* HAS TAKEN OVER THE XYLEM

R. solanacearum has long been known to have a type III secretion system (T3SS) to inject effector proteins into plant cells that can subvert the plant immune system (9). However, while for foliar plant pathogens the T3SS is the most important secretion system during pathogenesis, *R. solanacearum* also uses a type II secretion system to secrete cell-wall-degrading enzymes when invading plant roots (9). Once *R. solanacearum* reaches the xylem, it lives within dead xylem cells, which obviously cannot be manipulated through injected effectors. Finally, *R. solanacearum* was found not to even express its T3SS at high density when grown in culture, suggesting that it also does not express it at high cell density *in planta* (10). This finding invites the question of when during plant infection *R. solanacearum* actually does use its T3SS. On the other hand, however, *R. solanacearum* genomes have been predicted to

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encode dozens of effector proteins (9), and it was shown that a nondiffusible cell wall component activates transcription of T3SS genes (11). C. Allen and coworkers have now shown that although the *R. solanacearum* T3SS may not be transcribed at high cell density in culture, it is in fact transcribed at high cell density *in planta* (1). Independently of the work of C. Allen, a similar result has been obtained by Monteiro et al. (12) using a green fluorescent protein (GFP) reporter construct. However, what we still do not know is into which plant cells *R. solanacearum* actually injects its effectors, since the majority of bacteria during infection appear to be localized to dead xylem cells.

Careful reading of the article by Jacobs et al. reveals another intriguing result: almost half of the genes known to be directly positively regulated by HrpB, a key regulator of transcription of the T3SS and other virulence factors, were not found to be significantly upregulated *in planta* (1). This shows again the limitations of studies in culture and illustrates the importance of global studies of gene expression *in planta* to further our understanding of plant pathogen biology.

SUCROSE IS ONE OF THE ENERGY SOURCES USED BY *R. SOLANACEARUM* DURING ITS LIFE IN XYLEM

How do bacteria adapt to life in nutrient-poor xylem? Recently it was reported that the vascular pathogens *P. syringae* pv. aesculi and *P. syringae* pv. actinidiae contain genes for sucrose uptake and utilization (7, 13). However, some foliar *P. syringae* pathogens, such as the bean pathogen *P. syringae* pv. phaseolicola, contain the same genes. C. Allen and her group found that the operon (*scrRABY*) and the gene *scrK*, which encode a putative sucrose-specific phosphoenolpyruvate-carbohydrate phosphotransferase system, are expressed at much higher levels *in planta* than in rich medium, with *scrA* expression induced 1,724-fold based on quantitative PCR (qPCR) analysis (1). Genetic analysis revealed that sucrose is indeed an important energy source for *R. solanacearum* in xylem, since the wild-type *R. solanacearum* strain consistently outcompeted a *scrA* mutant *in planta*. It will be interesting to see if the genes for sucrose uptake in *P. syringae* pathovars play a similarly important role.

Interestingly, despite its defect in competition assays, when inoculated solo, the *R. solanacearum scrA* mutant reached a high population density in xylem and wilted plants. Therefore, the question of what *R. solanacearum*'s main energy source during life in xylem is remains unanswered. In this regard, *R. solanacearum* is not alone—the main energy source is not known for any xylem-inhabiting pathogen. We can safely assume that C. Allen is asking this question, and we should be looking out for her answer and for the answers to many other questions regarding *R. solanacearum*-plant interactions in the future.

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