Trends in Microbiology

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Review

How *Ralstonia solanacearum* Exploits and Thrives in the Flowing Plant Xylem Environment

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The plant wilt pathogen *Ralstonia solanacearum* thrives in the water-transporting xylem vessels of its host plants. Xylem is a relatively nutrient-poor, high-flow environment but *R. solanacearum* succeeds there by tuning its own metabolism and altering xylem sap biochemistry. Flow influences many traits that the bacterium requires for pathogenesis. Most notably, a quorum sensing system mediates the pathogen's major transition from a rapidly dividing early phase that voraciously consumes diverse food sources and avidly adheres to plant surfaces to a slower-growing late phase that can use fewer nutrients but produces virulence factors and disperses effectively. This review discusses recent findings about *R. solanacearum* pathogenesis in the context of its flowing *in planta* niche, with emphasis on *R. solanacearum* metabolism in plants.

Highlights

Plant wilt pathogens are adapted to the low-nutrient but high-flow environment of xylem vessels.

Success in this physically and biochemically dynamic niche requires metabolic specialization, adhesion to vessel walls, and virulence factor production. Quorum sensing (QS) enables endophytic bacteria to optimally express these traits.

Pathogen biofilms in vessels may facilitate QS signal accumulation, plant cell wall digestion, type III secretion, and nutrient absorption from flowing xylem sap, but biofilms also limit bacterial spread. Sap flow may help *R. solanacearum* disperse from biofilms to colonize new niches.

R. solanacearum uses QS to transition between an adhesive early phase and a dispersive later phase. Early-phase *R. solanacearum* uses diverse nutrients to grow quickly, but at quorum, it narrows metabolic options and slows growth, investing instead in making virulence factors.

R. solanacearum Infection and the Plant Xylem Environment

Many, if not most, bacteria have adapted to environments with intermittently or constantly flowing liquids. Flowing microbial habitats range from the lumen of animal guts to soils, bioreactors, and deep marine vents [1]. Moving fluids can hinder bacterial motility, dilute diffusible extracellular signal molecules, and alter attachment. However, flow can also benefit microbes by introducing nutrients, removing waste, and promoting other attachment mechanisms [2]. Plant xylem vessels, which transport water and minerals from soil to above-ground plant parts, are home to diverse endophytic microbes. These include benign commensals and beneficial symbionts as well as destructive wilt pathogens that kill plants by disrupting normal water uptake [3,4].

One such pathogen, the Beta-proteobacterium *R. solanacearum*, causes bacterial wilt disease on a strikingly broad range of hosts, likely owing to its genetic diversity (Box 1) [5]. Infected plants are often stunted and yellowed before they develop characteristic wilting symptoms, which usually lead to death of the entire plant. In the model host plant tomato, symptoms appear after *R. solanacearum* populations exceed 10^8 CFU/g stem [6]. As disease progresses, the pathogen grows to the extremely high density of 10^{10} CFU/ml xylem fluid. Interestingly, *R. solanacearum* can also live for extended periods in xylem vessels of tolerant plant cultivars at moderately high cell densities (10^4 to 10^7 CFU/g stem) without triggering symptoms [7–9].

As a soil-borne pathogen, *R. solanacearum* locates plant hosts by sensing and chemotaxing toward root exudates using flagellar motility [10–12]. Initial bacterial interactions with plant surfaces involve both reversible and irreversible attachment via polysaccharides, adhesin proteins, and cell-surface appendages such as pili [13]. *R. solanacearum* cells attach to the

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Box 1. The Heterogeneous *Ralstonia solanacearum* Species Complex

R. solanacearum is a species complex of genetically diverse but related strains that colonize plant xylem vessels and cause wilt diseases. Historically, strains of plant-pathogenic *Ralstonia* were subdivided into races based loosely on host range, or biovars based on ability to oxidize various carbohydrates [87,88], but these classification schemes were neither biologically predictive nor phylogenetically meaningful. A DNA-based classification system now groups strains into four *phylotypes* that are further subdivided into *sequevars*. Phylotypes can be easily distinguished with multiplex PCR [5]. Comparative genomics of more than 50 sequenced strains in the *R. solanacearum* species complex suggest that phylotypes I and III are a single clade sharing average nucleotide identity (ANI) values greater than 96%, while strains in phylotype II and phylotype IV are clearly separable, with only 90–95% ANI to strains in other phylotypes [82,89,90] (Figure I). Interestingly, each phylotype has a different geographic origin: phylotype I strains come from Asia, phylotype III strains from Africa, phylotype II strains from the Americas, and phylotype IV strains from Pacific islands (Japan, Philippines, Indonesia, and Australia). The distinct geographic origins of the phylotypes suggest that *R. solanacearum* strains, which are typically soil-borne, did not readily move around the globe before human travel and agriculture [88]. This led to the hypothesis that the *R. solanacearum* species complex diverged when Pangea broke up 150–200 million years ago [88,89], but molecular clock dating of prokaryotic lineages suggests that the *Ralstonia* genus emerged more recently (Timetree.org and [91]).

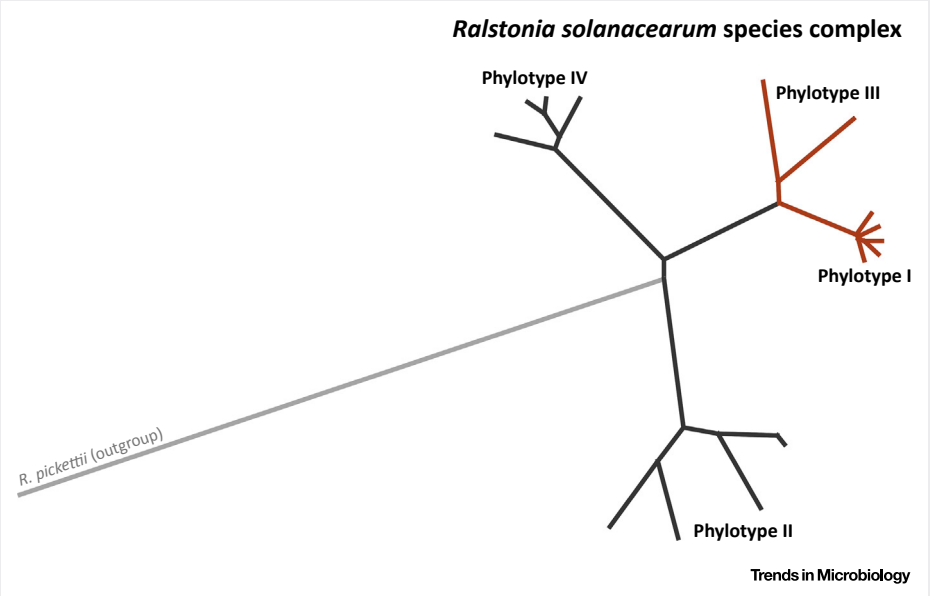

Figure I. Rooted Phylogenetic Tree of Representative Strains in the *Ralstonia solanacearum* Species Complex. A neighbor-joining tree was constructed using whole-genome average nucleotide identity (ANI). Phylotypes I and III, which form a single clade sharing >96% ANI, is colored brown.

root surface in a polar fashion via type IV pili and form microcolonies at the root elongation zone and sites of lateral root emergence [11,14–18]. *R. solanacearum* enters roots through wounds or natural openings and migrates to the developing vascular bundles, reaching the xylem vessels of susceptible tomato roots within 24 h [15,19]. After entering the xylem network, *R. solanacearum* spreads systemically through its host. Some *R. solanacearum* cells are planktonic in the xylem sap stream, while others use twitching motility to move along the vessel wall [20]. These solitary cells eventually grow into aggregates in a biofilm matrix that can fill entire vessels and potentially obstruct water flow [15,21] (Figure 1).

Bacterial wilt virulence depends on a large consortium of virulence factors, including secreted plant cell-wall-degrading enzymes, extracellular polymeric substances (EPS), and dozens of type III effectors [22]. Expression of these virulence factors is regulated by a complex regulatory cascade dominated by the Phc quorum sensing system, discussed in detail below [23].

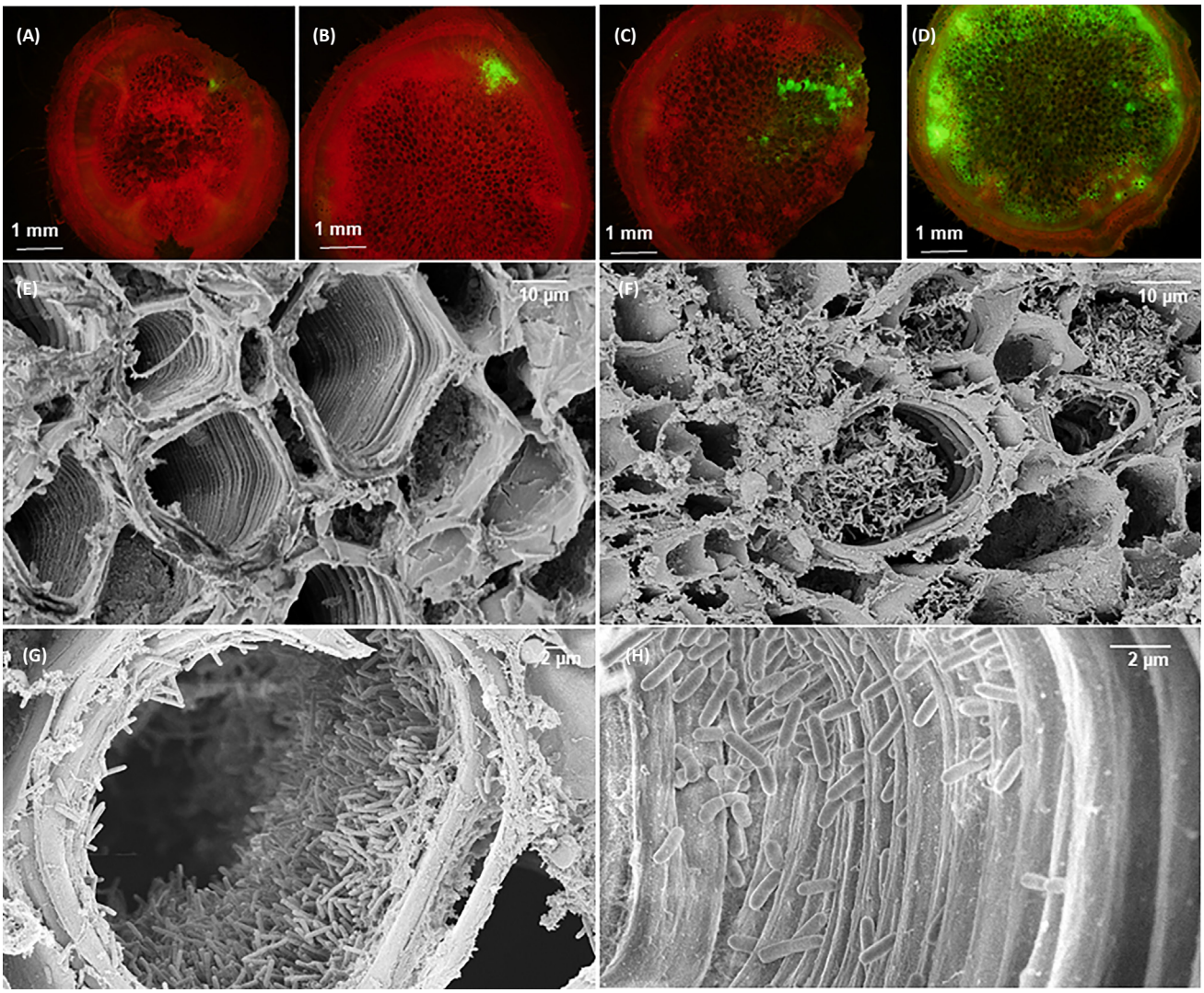

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Figure 1. Colonization of Tomato Xylem by *Ralstonia solanacearum*. (A–D) GFP-tagged *R. solanacearum* in tomato mid-stem cross-sections 48 h after soil-soak inoculation. Red is plant autofluorescence (image: Connor G. Hendrich). (E–H) Scanning electron microscopy of tomato xylem from (E) healthy tomato plants or (F–H) mid-stems of soil-inoculated plants showing first symptoms.

R. solanacearum behavior *in planta* is best understood in the context of its flowing environment. Xylem sap flows through the xylem vessels from roots to leaves, driven by tension (transpirational water evaporation from leaves that generates negative pressure due to water cohesion) and root pressure (soil water pulled into roots by accumulated ions in root xylem). Mature vessels are dead, and xylem sap has long been described as a nutrient-poor solution of water and minerals [24]. Although xylem vessels may appear to be simple plant plumbing, they are actually complex, dynamic environments with significant biochemical and biophysical variation (Box 2). Recent studies have shown that tomato sap contains concentrations of sugars, amino acids, and organic acids that can support bacterial growth *in vitro* [25–30].

Box 2. Plant Xylem Structure

Xylem tissue is composed of three components: water-transporting tracheids and vessel elements, fiber cells that provide structural support, and metabolically active parenchyma cells that regulate xylem homeostasis. Maturing vessels undergo programmed cell death in which they lose their cytoplasm, and the cell walls degenerate to create continuous open tubes. Vessel elements resemble corrugated pipes with interior walls that are structurally reinforced by rings of lignified secondary cell wall (Figure I). The vessels form an interconnected network with small openings, called pits, between adjacent vessels and between vessels and parenchyma cells. These pits provide semipermeable connections through pit membranes composed of a cellulose and pectin gel. Pores in pit membranes allow small molecules and water to pass through unimpeded, but restrict passive movement of microbial cells.

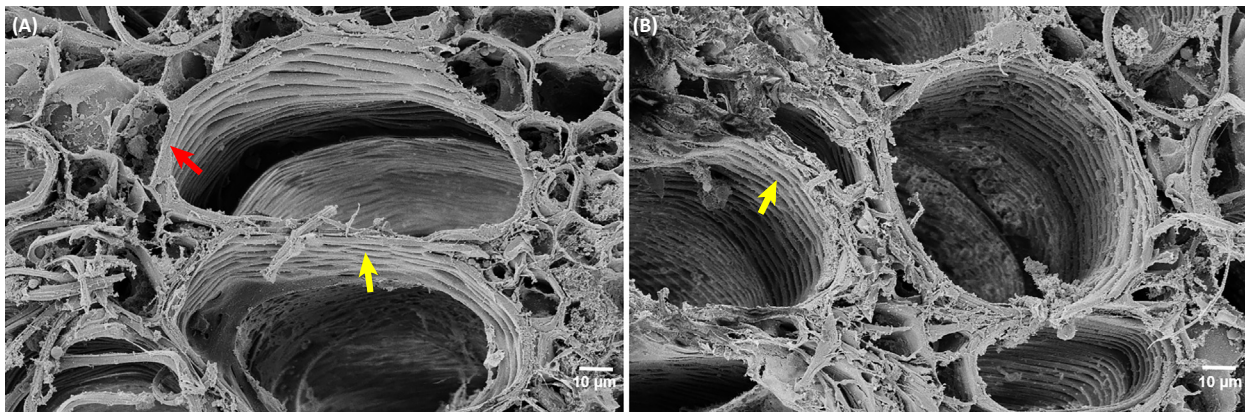

Figure I. Xylem Vessels Imaged Using Scanning Electron Microscopy of Healthy Tomato Plants. (A) Connections between xylem vessels and surrounding parenchyma cells (red arrow). (B) Bordered pits between two xylem vessels (yellow arrows).

Most previous analyses used sap from healthy plants, but we found that sap from diseased plants is enriched in many metabolites [31]. Indeed, the rapid growth of pathogens like *R. solanacearum* to high densities in xylem demonstrates that, for well-adapted microbes, xylem is a rich niche.

Xylem sap flow rates are influenced by environmental factors including ambient humidity, temperature, and the circadian cycle. During the day, tomato xylem sap flows at an average linear velocity of ~5 mm/sec but it slows to about a third of its maximum rate at night [32]. The effect of circadian rhythms on wilt disease remains to be investigated. Further, friction reduces the flow rate near surfaces like xylem vessel walls relative to in the open vessel lumen [1]. Finally, sap flow can be entirely halted by bacterial biomass, by plant defenses that seal xylem vessels, and by gas embolisms [3] (Box 3).

Because flowing environments are so biophysically and biochemically different from agar surfaces or culture broth, *R. solanacearum* pathogenesis and growth are best studied *in planta*. Bacterial biophysics studies have shown that flow affects many traits that *R. solanacearum* requires for pathogenesis. For example, flow near surfaces generates high shear forces that promote bacterial attachment via a process called shear-trapping [1]. Flow orients rod-shaped bacterial cells to set the direction of their twitching and swimming motility, changes the shape of biofilms, increases nutrient availability, and dilutes diffusible waste products, secondary metabolites, and bioactive molecules such as quorum sensing signals [1,2,33]. In this review, we highlight our analysis of the xylem metabolome and explore ways that the dynamic xylem habitat may affect *R. solanacearum's* quorum sensing-regulated traits, including attachment, motility, and central metabolism.

Box 3. When Sap Flow Stops: Xylem Dysfunction during Bacterial Wilt Disease

The exact cause of wilting during *Ralstonia solanacearum* infections is unknown, but several mechanisms likely contribute to plant vascular dysfunction (Figure I). In heavily colonized vessels, bacterial cells and extracellular polymeric substances (EPSs) can occlude xylem vessels [15,92,93]. Vessels can also be blocked by tyloses, a type of plant immunity. Tyloses are balloon-like out-growths of the living parenchyma cells adjacent to vessels that extend into and block vessels (reviewed in [3]). Gas embolisms are a less-discussed mechanism of plant vascular dysfunction during wilt disease. Embolisms could be formed by bacterial damage to xylem pit membranes or by dinitrogen gas, a byproduct of *R. solanacearum* denitrifying respiration. Evaporative transpiration from leaves generates negative pressure in xylem vessels that draws sap upward, as in a straw. The resulting negative pressure can suck gases into the vessels and displace the sap. In response, some plant structures reduce embolisms and limit their spread between vessels. The pit membrane, a modified primary cell wall that separates vessels from the xylem apoplast, has microscopic pores that deter embolism spread by bolstering the surface tension of sap and limiting gas entry into vessels [94,95]. Although embolisms have not been studied in *R. solanacearum*-infected plants, they are common in plants infected with another xylem pathogen, *Xylella fastidiosa* [96,97]. *X. fastidiosa* uses cell-wall-degrading enzymes to digest pit membranes in order to spread between vessels, and degraded pit membranes allow embolisms to more readily form in vessels and cascade throughout the xylem network [95]. Like *Xylella*, *R. solanacearum* has several cell-wall-modifying proteins that likely target primary plant cell walls: two cellulases that degrade cellulose (ChbA and Egl), one expansin that nonenzymatically loosens cellulose by disrupting interstrand hydrogen bonds (ExlX), and four enzymes that degrade pectin (Pme, PehA, PehB, and PehC) [23,98]. Cellulose degradation is important for *R. solanacearum* virulence, and pectin degradation makes a smaller contribution to virulence [23]. It remains to be determined whether *R. solanacearum* infections increase xylem embolisms by degrading the pit membranes that protect vessels.

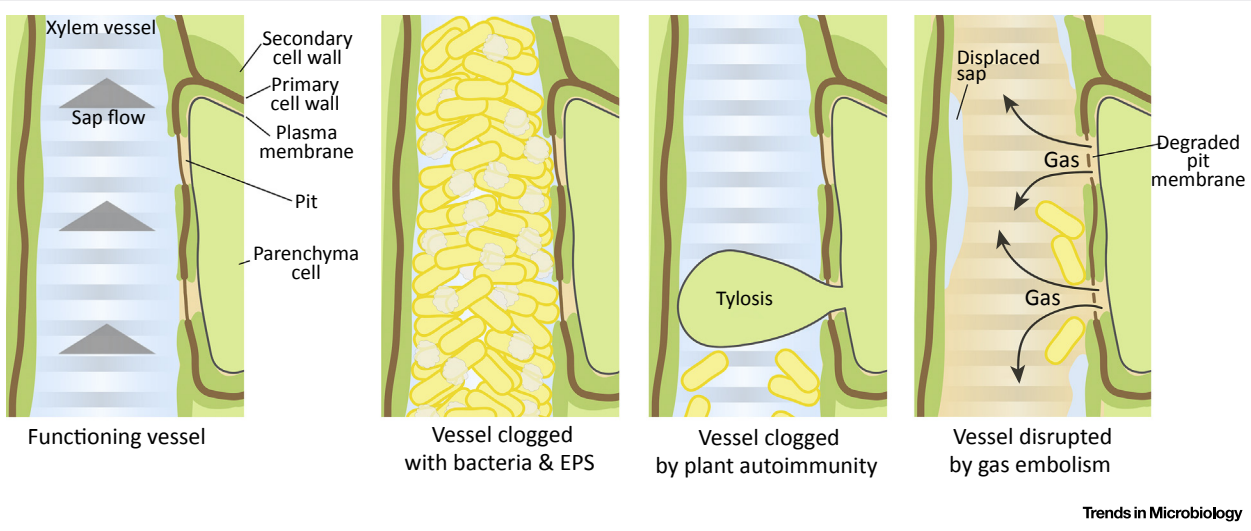

Figure I. Mechanisms That May Contribute to Plant Vascular Dysfunction during Bacterial Wilt Disease. EPS, extracellular polymeric substance.

Quorum Sensing during *R. solanacearum* Infection

Bacterial quorum sensing (QS) is a powerful communication tool that coordinates behaviors based on cell density (reviewed in [34]). Bacteria produce and secrete QS signals, which are small diffusible molecules. As the bacterial population increases, QS signal accumulates until it reaches a threshold that activates a signal transduction cascade that transcriptionally and phenotypically reprograms the cells. QS ensures that public goods such as virulence factors are produced only when beneficial to the community, and it also enables subpopulations in the community to quickly adjust to a different lifestyle.

Many QS systems use LuxI/LuxR-type regulators that respond to *N*-acylhomoserine lactone (AHL) signal molecules [34]. *R. solanacearum* has LuxI/LuxR homologs (SolI/SolR) that produce and respond to AHLs, but the SolI/SolR system is dispensable for virulence [23,35]. The lead QS system in *R. solanacearum* involves the LysR-family transcriptional regulator PhcA, which is activated by either 3-hydroxymyristatic methyl ester or 3-hydroxypalmitatic methyl ester [36,37]. When sufficient signal accumulates, PhcA is activated, triggering expression of some virulence traits, such as EPS and cellulases, and repression of others, such as siderophores and swimming motility [23]. Transcriptomic analysis of a *phcA* mutant growing in tomato xylem

revealed that PhcA-mediated QS affects expression of over 12% of *R. solanacearum's* genes [38] (see Figure 2 for summary). Along with the expected virulence factors, PhcA negatively regulates *R. solanacearum* metabolic versatility and growth *in planta* and *in vitro* (described further below) [38,39].

Importantly, *in vitro* findings do not always apply *in planta* because the plant environment overrides PhcA repression of some traits. An *R. solanacearum phcA* mutant growing in culture has a very different transcriptional profile from the same mutant during tomato infection. Many genes are differentially expressed in culture but not *in planta*, and 53 genes are differentially expressed in divergent directions in the two conditions [40,41].

For example, PhcA negatively regulates twitching motility and type III secretion at high cell densities in culture, but in tomato xylem, twitching is expressed independently of cell density and T3SS is expressed at high cell density [20,38,42,43].

Flow can wash away signals and repress QS, but bacterial behaviors such as EPS production and the formation of dense biofilms provide resilience to flow-mediated quorum quenching [33,44–46]. The physical complexity of the xylem environment creates pockets of reduced flow, for example, in crevices or near the bottom of thicker biofilms [33]. The spatial distribution of QS signal in xylem is unknown, but there are some indirect data. Transcriptional fusions to the highly expressed *eps* promoter, which is activated by PhcA, were used to study the distribution of cells at quorum *in planta*. Kang *et al*. found that P_{eps} expression varied minimally in individual *R. solanacearum* cells isolated from tomato stem [47]. However, the homogenous population of planktonic cells that diffused out of stem slices may be phenotypically different from the adherent cells that remained in the plant tissue. Additionally, Monteiro *et al*. imaged whole plants inoculated with a bioluminescent P_{eps}*::lux* reporter strain, and showed that *R. solanacearum* expresses P_{eps} in host roots as early as 1 day postinoculation, with increased expression over time [43]. It is likely that *R. solanacearum* populations inside infected plants are a heterogenous mixture of quorum-active and -inactive cells, at least in newly colonized vessels. It may thus be more accurate to reframe our view of 'high cell density' traits (like slower growth and cell wall degradation) as behaviors *R. solanacearum* preferentially uses along the xylem wall where the QS signal is more concentrated.

Interestingly, *Xylella fastidiosa,* a xylem-dwelling plant pathogen with a life history strategy very different from *R. solanacearum*, uses QS to mediate a phenotypic switch between a form adapted for spread in the plant and a form that is efficiently disseminated by its insect vector [48].

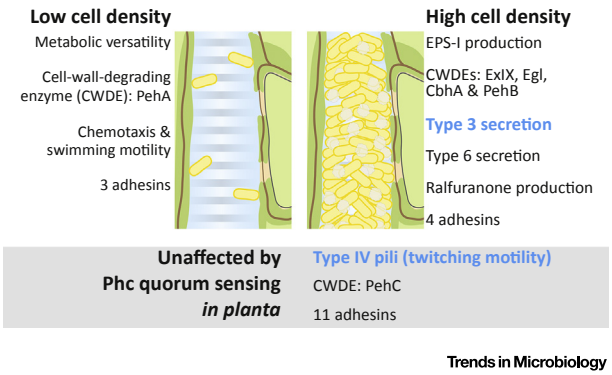

Figure 2. Effects of Phc Quorum Sensing on *R. solanacearum* Traits in Tomato Xylem. Blue text indicates traits that are regulated differently *in planta* and in culture [38]. In culture, PhcA represses both type IV pili and type III secretion genes at high cell density [20,99].

Attachment and Biofilm Formation

Inside tomato xylem vessels, *R. solanacearum* forms biofilm-like aggregates that range from a few dozen cells to thick mats that fill vessels and may obstruct water flow [15,21] (Figure 1). Likewise, xylem sap flow likely influences biofilm architecture, as it does for *X. fastidiosa* [49]. High-resolution SEM images show *R. solanacearum* cells embedded in a fibrillar matrix within colonized vessels, although the fibrils visible in desiccated SEM samples are probably a hydrated gel *in vivo* [15,21,38]. These fibers likely contain EPS-I, the *N*-acetylated acidic extracellular polysaccharide that gives *R. solanacearum* its distinctive mucoid colony morphology; when plates are inverted, *R. solanacearum* cells and EPS-I drip onto the plate lids [23]. Most (~80%) EPS produced in culture is not covalently attached to *R. solanacearum* cells, but some capsular EPS-I is bound to cells; this is true of both cells grown in culture and cells isolated from the xylem [50,51]. Additionally, the extracellular matrix contains extracellular DNA because (i) exogenous DNAse dissolves *R. solanacearum* biofilms *in vitro*, and (ii) fibrils are thicker and more abundant in xylem biofilms of a Δ*nucAB* mutant that lacks two extracellular DNases [21]. The source of the extracellular DNA is interesting and unexplored: does *R. solanacearum* actively secrete DNA, like *Neisseria gonorrhoeae,* or does a subset of the population release DNA by lysis, like *Streptococcus pneumoniae* [52,53]?

Phc QS changes *R. solanacearum* attachment behavior and biofilm morphology. Low-cell-density mode-mimicking Δ*phcA* mutant cells display several aberrant attachment behaviors [38]. While they attach to tomato roots much better than the wild-type, and form abnormally thick mats inside tomato xylem vessels, Δ*phcA* cells do not attach to glass slides or polyvinylchloride surfaces as well as wild-type cells. These results suggest that, in order to adapt to diverse environmental substrates, *R. solanacearum* must separately regulate attachment to the host (adhesion) and to fellow bacterial cells (cohesion). Bacterial attachment to root surfaces early in infection and to xylem vessel walls later in disease development may also be mechanistically distinct, both because these two surfaces are so physically different and because the bacterium uses attachment for different purposes in these two disease stages. Tissue-specific binding assays with individual adhesin mutants could test this hypothesis.

Bacterial attachment is facilitated by pili, lectins (proteins that bind sugars), and adhesins [16,35,54]. Adhesion processes are also influenced by pH, temperature, and the physiochemical properties of host surfaces such as root surfaces and xylem vessel walls [55]. The *R. solanacearum* genome encodes several large (>2500 amino acid) surface proteins that share homology with nonfimbrial adhesins in other bacterial pathogens. These nonfimbrial adhesins contribute to the virulence of several plant pathogens [56–59], but have not been characterized in *R. solanacearum*. Given their abundance, understanding temporal and spatial expression of these putative attachment proteins may reveal their specific roles in virulence. For example, bacteria must attach to deploy Type 3 secretion; during this process a bacterial pilus traverses the plant cell wall. We now know that *R. solanacearum* cells living in the dead xylem vessels express genes encoding the T3SS and effectors, probably to inject virulence effectors into the cytoplasm of adjacent xylem parenchyma cells [42,43].

Motility of *R. solanacearum* in the Xylem

What traits allow *R. solanacearum* to systemically colonize the xylem network? Xylem sap flow likely passively disperses planktonic cells upwards. Additionally, *R. solanacearum* has two forms of active motility: swimming mediated by flagella and twitching mediated by type IV pili.

R. solanacearum uses flagellar motility and chemotaxis to locate host roots, but swimming motility is largely repressed in xylem. Although up to 60% of the population is motile in culture,

bacteria in xylem are essentially nonmotile until populations reach 10^9 CFU/ml sap, at which point <5% of cells exhibit swimming motility [10,60]. Interestingly, the point when *R. solanacearum* xylem populations reach 10^9 CFU/ml is also when wilt symptoms appear, likely due to reduced xylem sap flow. Swimming motility propels bacteria at velocities of 20–40 μm/sec, but xylem sap flows much faster, at 1–5 mm/sec, so swimming in functional xylem vessels may be energetically expensive and futile [32,61]. So why does *R. solanacearum* swim in the plant at all? In liquid, swimming is 10- to 100-fold faster than twitching, which propels *X. fastidiosa* at ~1 μm/min [62,63], so *R. solanacearum* may use swimming motility to escape the stagnant environment of mature biofilms and to exit roots in endstage disease.

Bacteria move along surfaces with type IV pili-mediated twitching motility. Flow orients polarly attached bacterial cells such that the pilus points upstream; this allows bacteria to move down plant xylem vessels, against the sap flow [62]. The biology of twitching in the xylem has been studied in depth in *X. fastidiosa,* where twitching is specifically upregulated by 4-mm Ca^{2+} present in xylem sap [64]. *R. solanacearum pilA* and *pilQ* mutants, which lack type IV pili, are reduced in virulence following both root and stem inoculation assays, suggesting that pili contribute to multiple stages of pathogenesis [16,20]. However, the specific mechanism(s) underlying these virulence defects are unclear because type IV pili are required for both twitching motility and surface attachment. An *in vitro* study suggested that twitching behavior is repressed by the Phc QS system at high cell density, but transcriptomic data indicate that PhcA does not regulate type IV pilus gene expression *in planta* [38] (Figure 2).

Metabolism and Growth of *R. solanacearum* in Plants

Targeted genetic studies have identified individual metabolic pathways that help *R. solanacearum* succeed in plants [42,65–72]. However, a global view of *R. solanacearum* metabolism in the host is emerging, thanks to transcriptomic, metabolomic, and metabolic modeling studies [27,31,38,39,42,73–75]. These broader analyses reveal that, although xylem sap is dilute relative to other *in planta* niches [26], it is chemically complex, containing >110 known compounds [27,31]. *R. solanacearum* has the capacity to exploit many of the metabolites found in xylem sap: it can use 42 of 76 tested xylem metabolites as sole carbon or nitrogen sources *in vitro* [27,31,38]. Further, 22 metabolites are enriched in xylem sap from diseased plants, including eight verified nutrients [31]. The mechanisms that enrich nutrients in sap from wilting plants remain to be determined. Does *R. solanacearum* use type III effectors that function like *Xanthomonas* TALs to induce plant sucrose transporters [76]? Alternatively, if bacterial wilt disease induces vessel embolisms (Box 3), the intriguing but yet-unproven phloem-unloading hypothesis could enrich nutrients [77,78]. This hypothesis proposes that plants repair embolisms by moving phloem sugars into xylem vessels in order to draw water back into the vessel to collapse the embolism.

R. solanacearum uses several nitrogen sources over its life cycle. Nitrate assimilation, wherein nitrate is converted to ammonia, contributes to plant root infection, but this trait is dispensable for growth in the xylem, suggesting that *R. solanacearum* acquires sufficient organic nitrogen from xylem sap [65]. Most proteinogenic amino acids are present at micromolar concentrations in xylem sap [25,27–30], but *R. solanacearum* must synthesize tryptophan [74], methionine [67], and likely cysteine because they are limited or not present in xylem sap. Leucine biosynthesis was reported as essential for *R. solanacearum* growth in tomato xylem based on chemical mutagenesis, but targeted mutants are needed to validate this [28]. Genomic analysis and *in silico* metabolic modeling suggest that *R. solanacearum* makes tryptophan, proline, lysine, cysteine, and aspartate using redundant, horizontally acquired pathways in order to synchronize virulence functions with primary metabolism [79]. Amino acids, organic acids, and sugars are all potential carbon sources for *R. solanacearum* in xylem sap. Reported

sugar concentrations in xylem sap are highly variable [25,27,80], but suggest that tomato xylem sap may contain millimolar levels of simple sugars like glucose, fructose, and sucrose.

During growth in xylem, *R. solanacearum* rapidly consumes the scant available oxygen, creating a hypoxic environment that demands an alternative respiratory strategy [66,81]. Denitrifying respiration is a key source of energy for phylotype I and III *R. solanacearum* strains in tomato xylem, but is less important for phylotype II and IV strains, which lack *nosZ*, encoding the final step in denitrifying respiration [66,82]. Interestingly, transcriptome analysis suggests that banana xylem may contain more oxygen, so *R. solanacearum* may not require nitrate to respire in musaceous host plants [75].

In addition to limitations imposed by its genomic capacity, *R. solanacearum* metabolite preferences are shaped by at least three global regulators: EfpR, RpoN1, and the Phc QS system [38,39,71–73]. EfpR was identified in an elegant experimental evolution study involving serial passage of *R. solanaceaurm* through plants for more than 300 bacterial generations [83]. Although *efpR* mutants can catabolize a broader range of organic acids and amino acids, they also have increased motility and reduced EPS production [73]. The increased motility of the *efpR* mutants may have given them a fitness advantage in this experiment, because bacteria were reisolated from each serial passage plant host by briefly incubating cut stems in water [83]. The alternative sigma factor RpoN1 (σ^{54}) is required for *R. solanacearum* growth on dicarboxylates and a few amino acids [71,72]. Although *rpoN1* mutants have reduced virulence on tomato, further work is needed to determine if this is due to the mutant's metabolic defects, its impaired twitching motility, or both [72].

The *in silico* metabolic modeling and *in planta* transcriptomic analysis described above both indicate that the Phc quorum sensing system mediates a strategic switch between growth and virulence over *R. solanacearum*'s life cycle [38,39]. At low cell density, *R. solanacearum* has a generalist metabolic strategy to take advantage of diverse nutrients, including pectin-derived galacturonate, phenolic hydroxycinnamic acids, and at least 23 additional nutrients [38,39,68,84] (Table 1). This broad metabolic capacity likely confers a fitness advantage for survival in soil and water, where nutrients are unpredictably available and rapidly depleted by competing microbes, and it also facilitates rapid growth early in plant colonization.

The high-cell-density version of *R. solanacearum* can use a much narrower range of nutrients. Specifically, the pathways for sucrose, galactose, and trehalose are upregulated by PhcA at high bacterial densities, suggesting that once *R. solanacearum* has successfully colonized the xylem its metabolism is tailored to exploit these three plant sugars [38]. Several other lines of evidence support this idea: (i) although bacterial wilt disease generally enriches xylem sap in metabolites, galactose was the only metabolite depleted in sap from infected plants [31]; (ii) an *scrA* sucrose catabolism mutant had reduced virulence and competitive fitness in tomato xylem [31,42]; (iii) trehalose is 19-fold enriched in tomato xylem sap during bacterial wilt disease [31], and (iv) some *R. solanacearum* strains deploy the RipTPS type III effector, which has trehalose-6-phosphate synthase activity [85].

How does xylem flow affect *R. solanacearum* metabolism? As described above, xylem sap is relatively dilute, and yet *R. solanacearum* grows as fast in xylem as it does in rich medium, with an *in planta* doubling time of ~2.1 h vs ~2.2 h in rich medium. However, *R. solanacearum* growth quickly plateaus when it is cultured on harvested tomato xylem sap [31]. Ongoing spread to new vessels may account for part of the increased growth *in planta* relative to growth in *ex vivo* sap, but *in planta* growth must also depend on the continually replenished nutrients provided by xylem flow [33]. In this sense,

Table 1. Xylem Sap Metabolites That Support *Ralstonia solanacearum* Growth[a]

Substrate	Relative growth
Glucose	Good
Sucrose	*Good*
Glucarate	Good
Galactarate	Good
Succinate	Good
Citrate	Good
Methionine	Good
Trehalose	*Good*
Ammonia	Good
Taurine	Good
Nitrate	Good
Glucose-1-phosphate	Good
Galactose	*Good*
Quinate	Good
Histidine	Fair
Urea	Fair
Malate	Fair
Beta-alanine	Fair
Aspartate	Fair
Gluconate	Fair
4-Aminobutyrate	Fair
Glucuronate	Fair
Serine	Fair
Fumarate	Fair
Glutamate	Fair
Proline	Fair
Galactitol	Fair
Alanine	Fair
Citrulline	Poor
Threonine	Poor
Pyruvate	Poor
3-Hydroxybutyrate	Poor
M-inositol	Poor
Glutamine	Poor
Glycine	Poor
O-acetylserine	Poor
Asparagine	Poor
Mannitol	Poor

[a]The low-cell-density form of *R. solanacearum* grows faster on many metabolites found in tomato xylem sap (in roman type). At high cell density, the Phc quorum sensing system tailors the *R. solanacearum* transcriptome for growth on galactose, sucrose, and trehalose (in italics). Good, fair, and poor indicate the relative *in vitro* growth of wild-type *R. solanacearum* GMI1000 cells on each metabolite (adapted from [31,38]).

R. solanacearum growth in xylem resembles microbial growth in a chemostat. Chemostats are bioreactors where microbes grow in a physiological steady state as fresh media is continuously supplied and an equal volume of spent media is removed. Early investigations into *R. solanacearum* nutrient consumption in *ex vivo* sap overlooked the impact of the continuous nutrient supply delivered by flowing xylem sap. Zuluaga *et al*. analyzed changes in amino acids and sugars in sap harvested from healthy plants after *R. solanacearum* grew to stationary phase [27]. Unsurprisingly, *R. solanacearum* depleted most (65%) detected sugars and amino acids. In contrast, we found *R. solanacearum* depleted only 4% of metabolites in the first 3 h of *ex vivo* growth on sap from infected plants: glucose, gluconate, proline, pipecolate, and 3-hydroxybutyrate. These nutrients may fuel *R. solanacearum* growth in the xylem chemostat. Glucose and gluconate consumption is consistent with the observed high expression of glycolysis, pentose phosphate pathway, and tricarboxylic acid pathway genes when *R. solanacearum* grows in tomato xylem [38,42,75]. 3-Hydroxybutyrate is a precursor of the carbon-storage molecule polyhydroxybutyrate, which *R. solanacearum* cells accumulate in plant xylem [86]. Recently, a genome-scale metabolic model was constructed for *R. solanacearum* GMI1000 [39]. It would be exciting to update this *in silico* model with finer-scale time-series analysis of the *R. solanacearum* consumption of xylem sap metabolites. Flux analysis may be possible if *R. solanacearum* were grown in ^{14}C- and ^{15}N-labeled xylem sap harvested from tomato plants grown with ^{15}N nitrate fertilizer in a ^{14}C carbon dioxide atmosphere. Although much has been uncovered about *R. solanacearum* metabolism in plants, we can learn much more by incorporating flow into models and experiments.

Concluding Remarks

In summary, like blood vessels, rivers, or pipes, plant vasculature presents colonizing bacteria with significant biophysical opportunities and constraints. This experimentally challenging niche warrants exploration because of the high socioeconomic impact of plant vascular pathogens and endophytes. Recent studies using mutant analysis, microscopy, modeling, metabolomics, and transcriptomics suggest that the moving stream of nutrients in xylem sap shapes microbial behavior by forcing bacteria to attach to xylem walls and form biofilms that shelter subpopulations from the effects of flow. We hypothesize that physiologically heterogeneous bacterial subpopulations develop when bacteria occupy different microenvironments in xylem. For *R. solanacearum*, this differentiation is mediated by a QS system that generates two biologically distinct versions of the organism: a more sessile form adapted to consume a wide diversity of nutrients, and a more planktonic form adapted to specialize on a few abundant nutrients, produce virulence factors, and disseminate throughout and beyond the plant. This model, based on analysis of whole tissue, can be more rigorously tested with emerging tissue-specific and single-cell 'omics techniques (see Outstanding Questions). More broadly, considering physical and biochemical aspects of a wilt pathogen's natural environment will enrich our understanding of this important group of microbes.

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Outstanding Questions

How does xylem sap flow affect *R. solanacearum*? Does sap flow influence the architecture of *R. solanacearum* biofilms? How does sap flow affect the distribution *R. solanacearum* quorum signal molecules? How does flow rate influence motility and systemic spread of *R. solanacearum*? Does flow increase the metabolites available to *R. solanacearum*? Can *R. solanacearum* mechanically sense and respond to variation in sap flow rates?

Why does *R. solanacearum* make biofilms? Fine-scale *in situ* studies are needed to determine if biofilms help *R. solanacearum* extract carbon from xylem vessel walls, facilitate injection of T3 effectors, protect *R. solanacearum* from flow so quorum sensing signals can accumulate, hold *R. solanacearum* cells in place so they can optimally extract nutrients from the flowing sap, or a combination of these activities?

What triggers *R. solanacearum* attachment? How are the different types of attachment (adhesion versus cohesion, or root surface versus vessel wall) deployed over the pathogen's life cycle? How do root and xylem surface chemistries influence pathogen attachment? Does nutrient availability influence attachment behavior?

How does *R. solanacearum* adapt to the strikingly variable microenvironments that the bacterium encounters inside its plant hosts? The temporal, biophysical, and biochemical heterogeneity of xylem vessels must demand correspondingly heterogeneous strategies. Emerging single-cell technologies will help to reveal them. For example, the transcriptomes of root-surface-attached, xylem wall-attached, and planktonic bacteria could be compared to determine if *R. solanacearum* regulates gene expression in response to different types of attachment.

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