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The Virulence Factors of the Bacterial Wilt Pathogen *Ralstonia solanacearum*

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Abstract

The bacterium *Ralstonia solanacearum* causes bacterial wilt on more than 200 plant species, including important crops such as potato, tomato, eggplant, pepper, tobacco and banana. Many factors contribute to the virulence of this pathogen. This review discusses the major virulence factors, including extracellular polysaccharide I, the type III secretion system and effectors, swimming motility and twitching motility, cell-wall-degrading enzymes and type II secretion system, and their contribution to the virulence and pathogenicity of *Ralstonia solanacearum*.

Keywords: *Ralstonia solanacearum*; Bacterial wilt; Virulence factor; Extracellular polysaccharide; T3SS; Motility

Ralstonia solanacearum, previously named *Pseudomonas solanacearum* and *Burkholderia solanacearum*, is a soil-borne gram-negative bacterium that causes bacterial wilt disease on more than 200 plant species from 50 botanical families, including important crops such as potato, tomato, eggplant, pepper, tobacco and banana [1,2]. *R. solanacearum* is considered a species complex-a heterogeneous group of related but genetically distinct strains [3]. This bacterium infects plants through root wounds or at sites of secondary root emergence, then colonizes the xylem vessels and spreads rapidly to aerial parts of the plant through the vascular system. In xylem vessels, the bacterial population can multiply extensively and rapidly reach very high levels ($>10^{10}$ cells/cm of stem in tomato) [4,5]. Typical disease symptoms include browning of the xylem, chlorosis, stunting, wilting, and the infected plants usually die rapidly. Bacterial wilt is considered one of the most destructive bacterial plant diseases because of its extreme aggressiveness, world-wide geographic distribution, and unusually broad host range [6]. In fact, *R. solanacearum* was ranked 2nd in a list of the top 10 most scientifically/economically important plant pathogenic bacterial pathogens in 2012 [7].

R. solanacearum has been widely accepted as a model organism for the study of bacterial virulence and pathogenicity in plants [1]. To date, many factors have been found to contribute to the virulence of *R. solanacearum*, however, due to the limited space, this paper will only discuss the major virulence factors in this pathogen.

Extracellular Polysaccharide I (EPS I)

One of the most important virulence factors is a heterogeneous polymer of N-acetylated extracellular polysaccharide I (EPS I) [8]. EPS I-deficient mutants are nearly avirulent and do not colonize plant xylem vessels as efficient as wild type [4,9]. It has been suggested that EPS I directly causes wilting by physically blocking the vascular system and thereby alters water movement [8]. It has also been hypothesized that EPS protects *R. solanacearum* from plant antimicrobial defenses by cloaking bacterial surface features that could be recognized by hosts [4,9]. Interestingly, it has been recently found that *R. solanacearum* EPS I plays different roles in resistant and susceptible hosts [10]. In susceptible tomato plants, the wild-type and EPS I-deficient mutant induced generally similar defense responses; but in resistant tomato plants, the wild-type induced significantly greater defense responses than the EPS I-deficient mutants, suggesting that the EPS I itself is a specific elicitor of plant defense responses [10].

The Type III Secretion System

The Type III Secretion System (T3SS) has a central role in pathogenesis of many bacterial pathogens of plants and animals [11]. In *R. solanacearum*, the T3SS is encoded by the *hrp* gene cluster, which spans a 23-kb region on the mega plasmid [12]. As in other major groups of Gram-negative bacteria, *R. solanacearum hrp* genes are key determinants for disease development on compatible hosts and for induction of the defensive hypersensitive response (HR) on resistant plants [13]. *R. solanacearum* is estimated to produce 70–80 type III effectors [14]. The completely nonpathogenic phenotype of *R. solanacearum* T3SS-defective mutants illustrates the collective importance of the effector proteins that are injected into plant cells by the system, although mutants lacking single effectors are usually fully virulent [11,15]. The T3SS of *R. solanacearum* contributes greatly to pathogenesis, but hrp mutants retain the ability to invade tomato roots and systemically colonize the vascular system, although the population size of T3SS mutants in infected tissues was reduced by 10 to 1000 fold compared to wild-type strains [16,17]. Recently, *in planta* transcriptome study and qRT-PCR tests by Jacobs *et al.* [18] and *in planta* expression study using green fluorescent protein reporter fusions by Monterio *et al.* [19] found that the T3SS is still active even after *R. solanacearum* has taken over the xylem, suggesting that the T3SS is functional throughout disease. These results changed the wide spread view from *in vitro* studies that T3SS is only active at the first stage of infection and is not needed when bacteria reach high cell densities [20,21].

Motility

R. solanacearum possesses flagella-driven swimming motility and type IV pili-driven twitching motility that are important to its ecological fitness and virulence [22-25]. Both nonmotile and nontactic mutants are significantly reduced in virulence on soil-drench inoculated tomato plants but exhibit normal virulence when directly inoculated into plant xylem, indicating that *R. solanacearum* needs directed motility and that

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swimming motility contributes to virulence in the early stage of host colonization and invasion [22,23]. However, when *R. solanacearum* grows in plant xylem, virtually all the bacterial cells are nonmotile [22,23]. Interestingly, recently it is reported that a hypermotile *motN* mutant of *R. solanacearum* is also reduced in virulence [26], indicating the importance of precise regulation of motility in this bacterium. *R. solanacearum* strains with mutations in *pilQ, pilT* or *pliA* lost twitching soil-drench and cut-petiole inoculation [24,25]. Furthermore, the *pilA* mutant was also affected in biofilm formation, adherence to multiple surfaces and natural transformation [24]. Together, these results demonstrate that type IV pili and twitching motility are important for several stages of wilt disease development.

Cell-Wall-Degrading Enzymes (CWDEs) and Type II Secretion System (T2SS)

R. solanacearum secretes several CWDEs, including three polygalacturonases (PehA , PehB and PehC) [27,28], an endoglucanase (Egl) [29], a pectin methylesterase (Pme) [30], and a cellobiohydrolase (CbhA) [31]. Gene disruption analysis revealed that Egl, PehA, PehB and CbhA, each contribute to the pathogen's ability to cause wilt [31-33]. An *R. solanacearum* strain GMI1000 pyramid mutant lacking all known CWDE genes, although significantly less virulent than parent strain GMI1000, was more virulent than a T2SS mutant. This suggests that additional extracellular proteins secreted by the T2SS contribute to the virulence of *R. solanacearum* [31].

Other Virulence Factors

Besides the above mentioned virulence factors, *R. solanacearum* also has other factors that contribute to its virulence. For example, *R. solanacearum* encounters reactive oxygen species (ROS) during bacterial wilt pathogenesis and expresses diverse oxidative stress response genes to detoxify ROS or otherwise tolerate this oxidative environment [34-36]. Inactivation of *oxyR*, which encodes the only identified regulator of oxidative stress gene in *R. solanacearum* significantly reduced virulence [37]. Two genes, *acrA* and *dinF*, encoding multidrug efflux pumps, were also found to contribute to bacterial wilt virulence [38]. Some metabolic pathways also appear to be required during pathogenesis of *R. solanacearum*, and deletion of *metER* methionine biosynthesis genes produced significantly reduced disease symptoms without causing auxotrophy or affecting growth inside the plant [39,40]. *R. solanacearum* was also found to need Flp pili for virulence on potato [41].

The virulence factors of *R. solanacearum* are controlled by a complex regulatory signal transduction pathway that responds to both environmental signals and quorum sensing molecule 3-hydroxypalmitic acid methyl ester (3-OH PAME) [20,42]. For details, the readers are referred to a very comprehensive review on regulation of virulence and pathogenicity genes written by Schell [43]. Over the years, substantial progress has been made in studying *R. solanacearum* and bacterial wilt disease [43]. With genomes of more than 10 strains from *R. solanacearum* species complex being available now [44-50], our knowledge about this pathogen will be broadened further. Based on comparative genomic analysis of eight sequenced strains, it has been proposed that the *R. solanacearum* core genome comprises ~2,850 conserved genes, whereas the variable genome contains ~3,100 genes and the numbers of strain-specific genes vary from strain to strain [46,47]. This great genetic variation may account for the broad host range of *R. solanacearum* species complex and makes it more challenging to determine which genes are responsible for host-range speciation. A better understanding of the *R. solanacearum* virulence factors and their complex regulation will lead to novel avenues for research and effective disease control strategies.

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