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Ralstonia Solanacearum Species Complex and Bacterial Wilt Disease

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Abstract

The bacterium *Ralstonia solanacearum* is the causal agent for bacterial wilt on more than 200 plant species from 50 botanical families, including important crops such as potato, tomato, eggplant, pepper, tobacco and banana. *R. solanacearum* is considered a species complex, and is also a widely accepted model organism for the study of bacterial pathogenicity in plants. This review discusses the disease caused by *R. solanacearum*, the classification of the pathogen, the major virulence and pathogenicity factors and their complex regulation in *R. solanacearum*.

Keywords: *Ralstonia solanacearum*; Bacterial wilt; Species complex; Classification; Virulence; Pathogenicity factors; Regulation.

Introduction

The bacterium *Ralstonia solanacearum* is a widely accepted model organism for the study of pathogenicity in plants [1]. This soil-borne bacterium, which belongs to the betaproteobacteria, is responsible for bacterial wilt on more than 200 plant species from 50 botanical families, including important crops such as potato, tomato, eggplant, pepper, tobacco and banana. In fact, bacterial wilt is considered the single most destructive bacterial plant disease because of its extreme aggressiveness, wide geographic distribution, and unusually broad host range [2].

R. solanacearum inhabits the vascular tissue of its hosts. The bacterium normally invades plant roots from the soil through wounds or natural openings where secondary roots emerge [3], colonizes the intercellular space of the root cortex and vascular parenchyma, and eventually enters the xylem vessels and spreads up into the stem and leaves, where the pathogen cell density commonly surpasses 10^9 CFU/g of host tissue [3,4]. After *R. solanacearum* has colonized the xylem, large numbers of bacterial cells are shed from roots, providing a pathway for bacteria to return to the soil and initiate new infections [1]. Affected plants suffer chlorosis, stunting, wilting, and usually die rapidly.

R. solanacearum species complex and classification

R. solanacearum is considered a species complex-a heterogeneous group of related but genetically distinct strains [5,6]. DNA-DNA hybridization studies have revealed that the identity between *R. solanacearum* genomes is often less than the 70% threshold level commonly expected within a bacterial species [7,8]. Gillings and Fahy first used the term "species complex" to describe the high genetic variation between isolates, and Taghavi et al. [9] expanded the concept of the *R. solanacearum* species complex by including two closely related species from Indonesia, *Ralstonia syzygii* (a pathogen of clove trees) and the agent of blood disease of banana, known as the BDB. *R. solanacearum* species complex strains are highly competent for genetic exchange *in planta* [10] and show substantial pathogenic variability in host range and aggressiveness [5]. However, the genetic basis for this variation is unknown. For the past four decades, two different systems, race and biovar, have been widely used to differentiate *R. solanacearum* strains [11-13]. Historically, *R. solanacearum* was subdivided into "races" based loosely on host range [13]. Race 1 strains attack tobacco, many other solanaceous crops and many hosts in other plant families; race 2 strains are limited to musaceous species including *Heliconia* spp. and triploid banana; race 3 strains primarily attack potato; race 4 strains are particularly virulent on ginger; race 5 strains infect and cause disease on mulberry tree, and are only found in China [14]. The race structure of *R. solanacearum* is poorly defined and not taxonomically useful.

Based on their different abilities to utilize and oxidize several disaccharides (cellobiose, lactose, and maltose) and hexose achohols (dulcitol, mannitol, and sorbitol), *R. solanacearum* strains were originally divided into five biovars [12]. Biovar 1 strains metabolize none of them; biovar 2 strains only metabolize disaccharides; biovar 3 strains metabolize all of them; biovar 4 strains metabolize only hexose alcohols; biovar 5 strains metabolize all of them except dulcitol and sorbitol [12,14]. Later, a new group of *R. solanacearum* isolates from the Amazon basin was differentiated from original biovar 2 using ribose and trehalose [15]. This group is named biovar 2-T or biovar N2 and the original biovar 2 strains are now referred to as 2-A. Except for biovar 2-A, which almost always corresponds to race 3, and biovar 5, which is identical to race 5, there is no correlation between biovars and races.

Recently, a new phylogenetic classification system consisting of four phylotypes was formed based on phylogenetic analysis of sequence data from the 16S-26S internal transcribed spacer region, the *egl* gene, the *hrpB* gene, and the *mutS* gene [5,6,16]. The phylotypes correlate with the geographical origin of the strains: phylotype I includes strains originating primarily from Asia, phylotype II from America, phylotype III from Africa and surrounding islands in the Indian Ocean, and phylotype IV from Indonesia [5,6]. Each phylotype is further divided into sequevars, which are defined as isolates with less than 1% nucleotide variation in the endoglucanase (*egl*) locus [6,16]. The *R. solanacearum* phylotype system is a stable and phylogentically meaningful classification scheme that represents the evolutionary lineages within the species complex [6].

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The Major Virulence and Pathogenicity Factors in *R. solanacearum* and Their Regulation

The major virulence and pathogenicity factors

Many factors contribute to virulence and pathogenicity of *R. solanacearum* strains, including a heterogenous polymer of N-acetylated extracellular polysaccharide I (EPS I) [17-19], the type III secretion system (T3SS) [20], flagella-driven swimming motility and type IV pili-driven twitching motility [21-24], Cell-Wall-Degrading Enzymes (CWDEs) and type II secretion sytem (T2SS) [25-27].

R. solanacearum EPS I-deficient mutants are nearly avirulent and had reduced stem colonization compared to wild type [3,28-30]. Two functions have been hypothesized for EPS I: one is physically blocking the vascular system and thereby altering water movement [19,28,29], the other is protecting *R. solanacearum* from plant antimicrobial defenses by cloaking bacterial surface features that could be recognized by hosts [3,30]. In *R. solanacearum*, the T3SS is encoded by a 23-kb *hrp* gene cluster on the megaplasmid [20] and is essential for pathogenicity, since *R. solanacearum hrp* mutants are unable to cause disease in many susceptible plants [31-33]. Both flagella-driven swimming motility and type IV pili-driven twitching motility contribute to the ecological fitness and virulence of *R. solanacearum* [21-24]. While swimming motility contributes to virulence only in the early stage of host colonization and invasion [21,22,34], the type IV pili and twitching motility are important for several stages of wilt disease development [23,24]. *R. solanacearum* secretes several plant CWDEs via the T2SS that contribute to the pathogen's ability to cause wilt, although none is essential for disease [25-27]. A T2SS mutant was much less virulent than the mutant lacking all known CWDEs, suggesting that additional extracellular proteins secreted by the T2SS contribute to the virulence of *R.solanacearum* [27].

Complex regulation of virulence and pathogenicity factors

The key virulence and pathogenicity factors of *R. solanacearum* are controlled by a complex regulatory network that responds to multiple signals [35]. The central player of this regulatory network is the transcriptional regulator PhcA, involved in the Phc cell density sensing system [36]. PhcA positively regulates the production of EPS I, Pme and Egl exoproteins, and an acyl-homoserine lactone (AHL) quorum sensing system called SolI/R. PhcA also represses the expression of genes involved in motility, T3SS, polygalacturonase and siderophore production [24,37-42]. The levels of active PhcA protein are controlled by a diffusible endogenous signal molecule, 3-hydroxypalmitic acid methyl ester (3-OH PAME). When extracellular 3-OH PAME accumulates above threshold concentrations (i.e., at high cell density in a confined space, such as the plant vascular system), PhcA is expressed, resulting in activation or repression of its various target genes [35,43].

In addition to the Phc cell confinement sensing system mediated by 3-OH-PAME, *R. solanacearum* also produces acylated homoserine lactones (acyl-HSLs) and has a functional acyl-HSL-dependent autoinduction system mediated by the above-mentioned SolI-SolR regulators, which are LuxI/LuxR homologs [40]. At present, only one gene of unknown function (*aidA*) is known to be regulated by SolI-SolR in phylotype II seq 7 strain AW1. *aidA* is not present in strain GMI1000 [40]. While inactivation of *solIR* does not affect any virulence factor production, the role of SolIR in the physiology of *R. solanacearum* requires more investigation. The acyl-HSL-dependent autoinduction system in *R. solanacearum* is part of a more complex autoregulatory hierarchy, since expression of *solR* and *solI* requires PhcA, which is itself controlled by another autoregulatory system that responds to 3-OH-PAME [40]. Expression of SolIR is also dependent on the alternate sigma factor RpoS [44].

R. solanacearum regulates deployment and use of its T3SS with a complex environmentally responsive signal transduction cascade via the Prh system [35,45]. *R. solanacearum* T3SS genes are induced by bacterial contact with plant cells or cell wall fragments [46]. This contact-dependent induction requires PrhA [47], an outer membrane-bound protein that may sense and transfer signal to activate T3SS expression through a linear pathway including the PrhR, PrhI, PrhJ, HrpG and HrpB proteins [48,49]. HrpG, an OmpR family two-component response regulator, is involved in *hrpB* gene activation in response to both nutrient and metabolic signals. HrpG plays a central role in regulating T3SS gene expression [49]. In addition, HrpG also independently regulates bacterial production of plant cell wall-degrading enzymes, exopolysaccharide, and the phytohormones ethylene and auxin [50]. Moreover, in culture the activity of HrpG is modulated by the Phc confinement sensing system [41]. HrpB is an AraC-like transcriptional activator required for the transcription of other *hrp* genes involved in T3SS and the effector protein genes [51-53]. HrpB was also found to regulate genes involving in chemotaxis, biosynthesis or catabolism of various low-molecular-weight chemical compounds, and siderophore production and uptake [51].

Conclusion

The present review has focused on the bacterial wilt disease caused by *R. solanacearum*, the classification of the pathogen, the major virulence and pathogenicity factors in *R. solanacearum* and their complex regulation. *R. solanacearum*, as a model organism for the study of bacterial pathogenicity in plants, survives in diverse environments and cause destructive disease on a wide range of plants by precisely controlling its gene expression through an elaborate regulatory network. Over the years, significant progress has been made in studying *R. solanacearum* species complex and bacterial wilt disease. However, further research is needed to better understand questions such as how does *R. solanacearum* regulate its gene expression in different niches, especially inside host plants? How do different plants respond to *R. solanacearum*? A better understanding of these questions will help us to design innovative tools and strategies to combat bacterial wilt disease more effectively.

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