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Review

Global regulation of pathogenicity mechanism of *Ralstonia solanacearum*

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Abstract Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating plant diseases worldwide. *R. solanacearum* first invades intercellular spaces of roots where it multiplies before invading xylem vessels and producing exopolysaccharide (EPS), leading to wilt of the infected plant. In this review, we focus on regulation of *R. solanacearum* pathogenicity, which requires proliferation in intercellular spaces. *R. solanacearum* possesses *hrp* encoding the type III secretion system (T3SS), and its pathogenicity depends on interactions between the host plant and type III effectors. HrpB positively regulates expression of not only *hrp* but also genes encoding exoproteins secreted through the type II secretion system (T2SS). A consortium of T2SS-secreted exocellular proteins containing plant cell wall-degrading enzymes contributes to not only invasion of xylem vessels, leading to systemic infection, but also quantitative control of virulence. Moreover, T2SS functionally interacts with T3SS. PhcA activated by quorum sensing in response to the bacterial cell density induces expression of *xpsR*, leading to biosynthesis of EPS. Moreover, active PhcA also suppresses expression of *prhIR*, resulting in suppression of *hrp* expression. These results suggest that *R. solanacearum* pathogenicity is globally regulated by mutual regulation by pathogenicity factors through multiplication of the bacteria in intercellular spaces.

Key words: *Ralstonia solanacearum*, type II secretion system, type III secretion system.

Bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi et al. 1995) is one of the most devastating bacterial plant diseases in the tropics, subtropics, and warm temperature regions worldwide (Hayward 1991). *R. solanacearum* invades intercellular spaces of roots through openings such as wounds then accumulates around the stele before breaking into and filling the xylem vessels (Roberts et al. 1988). On invasion of the xylem vessels, the bacteria grow and travel rapidly to the upper parts of the plant. This results in extensive wilting because of reduced sap flow caused by the presence of a large number of bacteria cells and exopolysaccharide (EPS) slime produced by the bacteria in some xylem vessels. The main virulence factor of *R. solanacearum* is therefore thought to be EPS.

R. solanacearum GMI1000 (GMI1000), which is nonpathogenic to tobacco plants and pathogenic to tomato plants, elicits a hypersensitive response (HR) when infiltrated into tobacco leaves (Boucher et al. 1985). GMI1000 possesses *hrp* (hypersensitive response and pathogenicity), which confer the bacterium's ability to elicit the HR in tobacco leaves as well as its pathogenicity to tomato plants (Boucher et al. 1987; Arlat et al. 1992; Van Gijsegem et al. 1995). Several putative *hrp*-encoded proteins of this bacterial pathogen share homology with proteins from the animal pathogens *Yersinia*, *Salmonella* and *Shigella* (Galan and Collmer, 1999). These proteins are assumed to be structural constituents of the type III secretion system (T3SS), which translocates effector proteins out of the cell (Van Gijsegem et al. 1995; Wei et al. 1992; He et al. 1993; Gaudriault et al. 1997; Bogdanove et al. 1996; Mudgett and Staskawicz 1998). *hrp* genes are expressed in the presence of plant cells through the HrpB regulator. This activation, which requires physical interaction between the bacteria and plant cell, is sensed by the outer membrane receptor PrhA, which transduces plant cell contact-dependent signals through a complex regulatory

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Abbreviations: CbhA, β-1,4-cellobiohydrolase; CWDEs, plant cell wall-degrading enzymes; Egl, β-1,4-endoglucanase; EPS, exopolysaccharide; HR, hypersensitive response; *hrp*, hypersensitive response and pathogenicity genes; 3-OH PAME, 3-hydroxy palmitic acid ester; PC, phenotype conversion; PehA, endopolygalacturonase A; PehB, exopolygalacuturonase B, PehC, exopolygalacuturonase C; Pme, pectin methyl esterase; T2SS, type II secretion system; T3SS, type III secretion system.

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cascade integrated by PrhJ, HrpG, and HrpB regulators (Aldon et al. 2000; Brito et al. 1999 & 2002; Cunnac et al. 2004).

R. solanacearum also produces several known virulence factors including a consortium of plant cell wall-degrading enzymes (CWDEs), which are secreted via the type II secretion system (T2SS) (Denny et al. 1990; González and Allen 2003; Huang and Allen 1997 & 2000; Tans-Kersten et al. 1998). The bacteria reportedly produce one β-1,4-endoglucanase (Egl), one endopolygalacturonase (PehA), two exopolygalacuturonases (PehB and PehC), one β-1,4-cellobiohydrolase (CbhA) and a pectin methyl esterase (Pme). Genetic inactivation of single genes has shown that Egl, PehA and PehB each contribute to its virulence (Liu et al. 2005). Furthermore, Liu et al. showed that a mutant lacking the six genes encoding these six CWDEs wilted plants significantly more slowly that the wild-type. These CWDEs are thus thought to be involved in quantitatively controlling the virulence of this bacterium.

Expression of pathogenicity factors in *R. solanacearum* is controlled by a complex regulatory network that responds to environmental conditions, the presence of host cells, and bacterial density (Schell 2000). At the center of this network is PhcA, a LysR family transcriptional regulator (Brumbley et al. 1993), which, directly or through intermediary regulatory genes, coordinates the expression of several virulence factors such as EPS and various CWDEs (Huang et al. 1995). Active PhcA is regulated in response to cell density by a quorum-sensing mechanism that involves the specific autoinducer molecule 3-hydroxy palmitic acid methyl ester (3-OH PAME) (Flavier et al. 1997). At a low cell density, presumably corresponding to saprophytic life and early plant colonization, PhcA is not expressed in culture, leading to expression of early disease virulence factors, including several polygalacturonases and both twitching and swimming motility (Liu et al. 2001; Kang et al. 2002; Tans-Kersten et al. 2004). At a later stage of infection, at a high cell density, the accumulation of 3-OH PAME leads to activation of PhcA and, subsequently, production of EPS and activation of potent CWDEs (cellulases and pectin methylesterase).

Loss of pathogenicity of the T3SS-deficient mutant of this bacterium shows that type III effectors are involved in its pathogenicity, depending on interactions between type III effectors and the host plant. Genome sequence analysis was performed on GMI1000 (Salanoubat et al. 2002) and *R. solanacearum* strain UW551 (Gabriel et al. 2006), which are nonpathogenic to tobacco plants, and has been analyzed in another two strains (Boucher, personal communication). Based on genomic analysis, screening of type III effectors controlled by HrpB and genes induced in tomato during growth has been conducted using *in vivo* expression technology, resulting in isolation of many candidate type III effector genes (Cunnac et al. 2004; Mukaihara et al. 2004) and pathogenicity-related genes (Brown and Allen 2004). However, involvement of these candidates in interactions with host plants remains to be elucidated, especially with regard to changes in the host response that are involved in virulence. Moreover, the mechanisms of proliferation in intercellular spaces immediately after invasion also remain unclear. In this review, we focus on the global regulation of the pathogenicity of *R. solanacearum* OE1-1 (OE1-1), which is pathogenic to tobacco plants, from proliferation in intercellular spaces to invasion of xylem vessels.

Proliferation of the bacteria in intercellular spaces is the quantitative determinant of *R. solanacearum* pathogenicity

OE1-1 belongs to biovar 4 and race 1, and is pathogenic to solanaceous plants such as tobacco plants. Infiltration with OE1-1 induces necrotic lesions in tobacco leaves at 72 h after infiltration (Hikichi et al. 1999). The *hrp*B-deficient mutant of OE1-1 lost its pathogenicity and ability to induce necrotic lesions in infiltrated tobacco leaves, suggesting that the pathogenicity of OE1-1 is dependently of *hrp* genes (Kanda et al. 2003a). Moreover, populations of mutants in the inoculated area were retained equally after inoculation, and were not detected in any other region. Transcripts of *hsr*203J and *hin*1, marker genes of plant-microbe interactions (Kiba et al. 2003) detected 8 h after infiltration of OE1-1, were not detected in the mutant-infiltrated tobacco leaves. *hrp* mutants, which are deficient in the type III secretion machinery, lost their ability to proliferate in host plants immediately after invasion, resulting in a loss of ability to induce host responses and the provocation of disease.

Of the various mini *Tn*5-mutants of OE1-1, we selected a folate auxotroph, RM, in which the transposon was inserted into *pabB*, encoding para-aminobenzoate synthase component I. It lost its ability to vigorously proliferate in intercellular spaces along with its systemic infectivity and virulence (Shinohara et al. 2005). Complementing RM with *pabB* allowed the mutant to proliferate in intercellular spaces and cause disease. In tobacco plants pretreated with folate, RM was able to vigorously proliferate in intercellular spaces and cause disease. Interestingly, when directly inoculated into xylem vessels, the mutant proliferated and was virulent. Moreover, the mutant proliferated well in stem fluids but not intercellular fluids, suggesting that the folate concentration within intercellular spaces may be a limiting factor for bacteria proliferation. Therefore, folate biosynthesis contributes to vigorous proliferation of the bacteria in intercellular spaces. These results suggest that proliferation of the bacteria in intercellular

spaces is required for its systemic infectivity, leading to its virulence.

OE1-1 suppresses *popA* expression immediately after invasion into host plants to escape host defenses

R. solanacearum secretes PopA, an extracellular Hrp protein and harpin, which contain high proportions of glycine and alanine but no cysteine and are secreted through the T3SS (Arlat et al. 1994). PopA purified from GMI1000 induced an HR-like response in infiltrated-tobacco leaves. *popA* consists of an operon with *popB* and *popC*; PopB has functional nuclear localization signals and PopC has a leucine-rich repeat (Guenoron et al. 2000). PopB and PopC are also secreted through the T3SS. Expression of *popABC* is also regulated by HrpB, similar to that of *hrp* genes including *hrpY*, which encodes a component of *hrp* pili. RT-PCR analysis showed that *popA* in OE1-1 was expressed 3 h after invasion (HAI), but not before, in intercellular spaces, though *hrpY* was expressed immediately after invasion (Kanda et al. 2003b). Pathogenicity analysis using a *popABC* operon-deleted mutant of OE1-1 (*ΔABC*) showed that *popABC* is not directly involved in the pathogenicity of OE1-1. A transformant of *ΔABC*, Papa, which constitutively expresses *popA* in intercellular spaces, expressed *popA* by 0.5 h after inoculation. The transformant could no longer proliferate or spread in intercellular spaces, and was no longer virulent. Moreover, the HR and expression of HR-related genes were not induced in Papa–infiltrated leaves. These results suggest that the expression of *popA* in Papa immediately after invasion triggers the suppression of bacterial proliferation and movement, resulting in loss of virulence. However, Papa retained its virulence when directly inoculated into xylem vessels, suggesting that tobacco plants can recognize PopA when expressed early in disease development, and respond with an effective defense in intercellular spaces (Kanda et al. 2003b). Therefore, the bacteria suppress *popA* expression to escape host defenses immediately after invasion of the host plant. Taken together, these findings suggest that proliferation of the bacteria in intercellular spaces is the quantitative determinant of *R. solanacearum* pathogenicity and is dependent on *hrp* genes.

Global regulation of genes in the *hrp* regulon at early stages of the infection process of OE1-1

After invasion into intercellular spaces of the root cortex, *R. solanacearum* recognizes plant cell signals through PrhA, which is located on the bacterial outer membrane. The signals are transferred to *hrp*B expression via the signal cascade PrhA-PrhR/PrhI-PrhJ-HrpG (Aldon et al. 2000; Brito et al. 1999 & 2002; Cunnac et al. 2004). By co-cultivating OE1-1 carrying the *lacZ* reporter gene with *Arabidopsis thaliana* in liquid medium, we were able to investigate the regulation of pathogenicity gene expression, including *hrp* genes, at early stages of the infection process of OE1-1. The eps genes responsible for EPS production are known to be induced at a late stage of infection in a cell density-dependent manner by

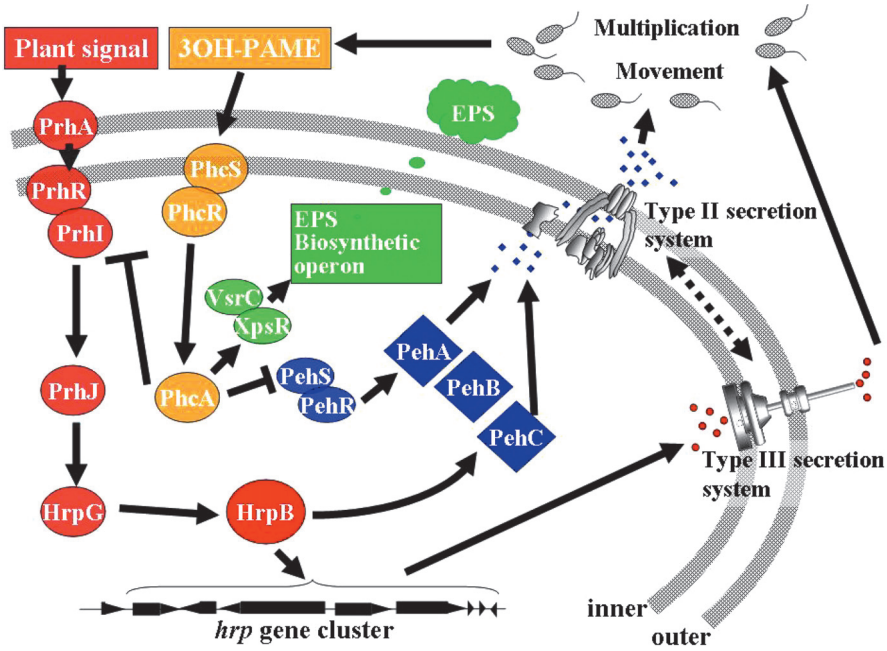

Figure 1. Scheme showing regulation of pathogenicity-related genes in *R. solanacearum* OE1-1. Symbols are: →, positive regulation; ⊣, negative regulation; ↔, interactions.

PhcA. While the expression of *prhA* was constitutive, other genes in the *hrp* regulon were dependent on cell density. Cell densities required for maximum expression of these genes were less than those required for *eps* genes, indicating that expression of these genes are repressed after they reach the maximum. By transposon mutagenesis, PhcA was demonstrated to be a negative regulator of genes in the *hrp* regulon (unpublished observation).

The expression of *phcA* itself is known to be activated in a cell density-dependent manner through quorum sensing. By creating *lacZ* operon fusions to *prhA*, *prhIR*, *prhJ*, *hrpG*, *hrpB* and *popA* in both OE1-1 and OE1-1 *phcA* backgrounds, we found that PhcA negatively regulated expression of *prhIR* (unpublished observation). Taken all together, infection processes are summarized in Figure 1. After invasion into intercellular spaces, OE1-1 induces expression of *hrpB* in response to plant signals and activates the *hrp* regulon, which constructs T3SS. OE1-1 can proliferate in intercellular spaces with the aid of secreted proteins through T3SS. When cell densities reach the threshold, *phcA* expression is activated. Finally, repression of *prhIR* expression by PhcA results in repression of *hrpB*-regulated genes and activation of *eps* genes results in EPS production.

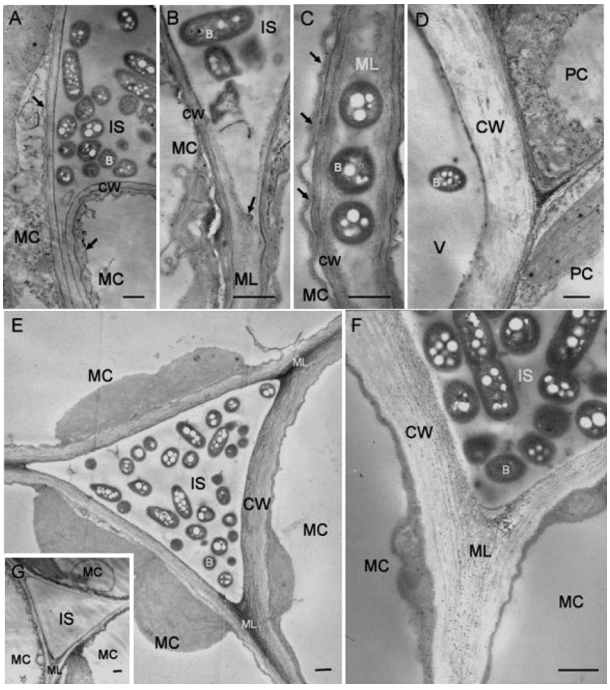

Figure 2. Electron micrographs showing tobacco leaf tissues at 3 days after infiltration with *R. solanacearum* OE1-1 (A–D) and Shin (E–F). (G) Mesophyll cells and an intercellular space in a mock-inoculated leaf. B, bacteria; CW, cell wall; MC, mesophyll cells; ML, middle lamella; V, xylem vessel; PC, xylem parenchyma cell. Bars＝0.5 μm.

Contribution of the type II secretion system to invasion of OE1-1 into xylem vessels, leading to systemic infection

After proliferation in intercellular spaces, the bacteria systemically infect the host plants through xylem vessels and produce EPS, which is involved in quantitatively controlling virulence (Schell 2000). A mutant of OE1-1, Shin, derived from EZ::TN<KAN-2> transposon-insertion retained its ability to grow in intercellular spaces and produce EPS, but lost its systemic infectivity in host plants (manuscript submitted). The transposon was inserted into *sdpK*, encoding a protein involved in construction of the T2SS and consisting of the *sdpGHIJK* operon. The mutant lost its ability to secrete polygalacutonase (Peh), PehA, PehB, and PehC and its Peh activity. The T2SS-mutant also showed reduced virulence when directly inoculated into xylem vessels. Complementing Shin with the *sdpGHIJK* operon allowed the mutant to compensate T2SS function and systemically infect the host plants, resulting in disease. Interestingly, the transformant of Shin with the *sdpGHIJK* operon was more virulent than OE1-1. Therefore, the consortium of CWDEs produced by the bacteria in xylem vessels may also be involved in quantitatively controlling virulence, in addition to EPS, as well as playing a role in invasion of the vessels. Moreover, when directly inoculated into xylem vessels, the T2SS-mutant showed less virulence in tomato plants than tobacco plants, suggesting that the contribution of CWDEs to virulence may quantitatively vary in different host species.

Observation of tobacco leaf tissues at 3 days after infiltration with *R. solanacearum* OE1-1 under electron microscope showed that OE1-1 existed in the intercellular space between two connecting mesophyll cells, and the separation of the mesophyll cells and plasmalemma was observed (Figure 2A, arrows). Degradation of the middle lamella in close proximity to the bacteria was also observed (Figure 2B, arrowhead). OE1-1 also existed in the degraded middle lamella, and the separation of the plasmalemma was observed (Figure 2C, arrowheads). Furthermore, OE1-1 existed in a xylem vessel. (Figure 2D) On the other hand, Shin only existed in an intercellular space (Figure 2E, 2F). Furthermore, the lack of degeneration of the mesophyll cells and middle lamella next to the intercellular space containing bacteria was observed. These results suggest that the T2SS-mutant lost its ability to invade xylem vessels. These findings suggest that the T2SS contributes to invasion of *R. solanacearum* into xylem vessels, leading to systemic infection.

Cooperation of T2SS with T3SS

We also constructed mutants lacking T2SS and/or T3SS in the OE1-1 phenotype conversion (PC) mutant

background. The PC mutants secreted a large amount of proteins even in rich medium. When the secreted proteins from a T2SS-deficient mutant, T3SS-deficient mutant and T2SS/T3SS mutant were compared, we found that several T3SS proteins including PopB were secreted more by the T2SS-deficient mutant than the wild-type (unpublished observation). *popB* consists of an operon with *popA* and *popC*; Expression of *popABC* is also regulated by HrpB and PopB is secreted through the T3SS as well as PopA and PopC (Arlat et al 1994; Kanda et al. 2003b). PopA was extracellularly secreted from the T2SS-deficient mutant as well as the parent strain OE1-1. Furthermore, *pop* operon was expressed in the T2SS-deficient mutant similarly to in the parent strain. These results suggest that T2SS may influence secretion of specific T3SS proteins, such as PopB. Furthermore, expression of several genes which encode proteins secreted through T2SS, including an exo-polygalacturonase gene, *pehC*, was positively regulated by HrpB, suggesting that expression of specific T2SS-secreted proteins, which are involved in the bacteria pathogenicity, is co-regulated with that of type III effectors. Therefore, T2SS seems to cooperate with T3SS during protein secretion of pathogenicity factors (Figure 1).

Conclusions

The infection stages of *R. solanacearum* are divided into two stages: the early stage includes invasion and proliferation in intercellular spaces along with invasion of xylem vessels, while the later stage includes proliferation and production of EPS in the xylem vessels. The pathogenicity of *R. solanacearum* is qualitatively regulated in the early stage, and is dependent on pathogenicity-related genes such as HrpB-regulated and PhcA-negatively regulated genes. In the later stage, on the other hand, it is quantitatively regulated by PhcA-positively regulated genes such as *eps* genes. Moreover, global regulation of *R. solanacearum* pathogenicity is dependent on bacteria cell density.

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References

Aldon D, Brito B, Boucher C, Genin S (2000) A bacterial sensor of plant cell contact controls the transcriptional induction of *Ralstonia solanacearum* pathogenicity genes. *EMBO J 19*: 2304–2314

Arlat M, Gough CL, Zischek C, Barberis P, Trigalet A, Boucher CA (1992) Transcriptional organization and expression of the large *hrp* gene cluster of *Pseudomonas solanacearum. Mol Plant-Microbe Interact* 5: 187–193

Arlat M, Van Gijsegum F, Huet JC, Pernollet JC, Boucher CA (1994) PopA1, a protein which induces a hypersensitive-like response on specific Petunia genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum. EMBO J* 13: 543–553

Bogdanove AJ, Beer SV, Bonas U, Boucher CA, Collmer A, Couplin DL, Cornelis GR, Huang HC, Hutcheson SW, Panopolus NJ, Van Gijisegm F (1996) Unified nomenclature for broadly conserved *hrp* genes of phytopathogenic bacteria. *Mol Microbiol*: 20: 681–683

Boucher CA, Barberis PA, Trigaret PA, Demery DA (1985) Transposon mutagenesis of *Pseudomonas solanacearum: isolation* of *Tn5*-induced avirulent mutants. *J Gen Microbiol* 131: 2449–2457

Boucher CA, Van Gijsegem F, Barberis P, Arlat M, Zischek C (1987) *Pseudomonas solanacearum* genes controlling both pathogenicity on tomato and hypersensitive on tobacco are clustered. *J Bacteriol* 169: 5626–5632

Brito B, Aldon D, Barberis P, Boucher C, Genin S (2002) A signal transfer system through three compartments transduces the plant cell contact-dependent signal controlling *Ralstonia solanacearum hrp* genes. *Mol Plant Microbe Interact* 15: 109–119

Brito B, Marenda M, Barberis P, Boucher C, Genin S (1999) *prhJ* and *hrp*G, two new components of the plant signal-dependent regulatory cascade controlled by PrhA in *Ralstonia solanacearum*. *Mol Microbiol* 31: 237–251

Brown DG and Allen C (2004) *Ralstonia solanacearum* genes induced during growth in tomato: an inside view of bacterial wilt. *Mol Microbiol* 53: 1641–1660

Brumbley SM, Carney BF, Denny TP (1993) Phenotype conversion in *Pseudomonas solanacearum* due to spontaneous inactivation of PhcA, a putative LysR transcriptional regulator. *J Bacteriol* 175: 5477–5487

Cunnac S, Boucher C, Genin S (2004) Characterization of the *cis*-acting regulatory element controlling HrpB-mediated activation of the type III secretion system and effector genes in *Ralstonia solanacearum*. *J Bacteriol* 186: 2309–2318

Cunnac S, Occhialini A, Barberis P, Boucher C, Genin S (2004) Inventory and functional analysis of the large Hrp regulon in *Ralstonia solanacearum*: identification of novel effector proteins translocated to plant host cells through the type III secretion system. *Mol Microbiol* 53: 115–128

Denny TP, Carney BF, Schell MA (1990) Inactivation of multiple virulence genes reduces the ability of *Pseudomonas solanacearum* to cause wilt symptoms. *Mol Plant-Microbe Interact* 3: 293–300

Flavier AB, Clough, SJ, Schell MA, Denny TP (1997) Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol Microbiol* 26: 251–259

Gabriel DW, Allen C, Schell M, Denny TP, Greenberg JT, Duan YP, Flores-Cruz Z, Huang Q, Clifford JM, Presting G, González ET, Reddy J, Elphinstone J, Swanson J, Yao J, Mulholland V, Liu L, Farmerie W, Patnaikuni M, Balogh B, Norman D, Alvarez A, Castillo JA, Jones J, Saddler G, Walunas T, Zhukov A, Mikhailova N (2006) Identification of Open Reading Frames Unique to a Select Agent: *Ralstonia solanacearum* Race 3 Biovar 2. *Mol Plant-Microbe Interact* 19: 69–79

Galan JE, Collmer A (1999) Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* 284: 1322–1328

González ET, Allen C (2003) Characterization of a *Ralstonia solanacearum* operon required for polygalacturonate degradation and uptake of galacturonic acid. *Mol Plant-Microbe Interact* 16: 536–544

Guenoron M, Timmers AC, Boucher C, Arlat M (2000) Two novel proteins, PopB, which has functional nuclear localization signals, and PopC, which has a large luecin-rich repeat domain, are secreted through the Hrp-secretion apparatus of *Ralstonia solanacearum*. *Mol Microbiol* 36: 261–277

Hayward HC (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum.* *Annu Rev Phytopathol* 29: 65–87

Hikichi Y, Nakazawa-Nasu Y, Kitanosono S, Suzuki K, Okuno T (1999) The behavior of genetically *lux*-marked *Ralstonia solanacearum* in grafted tomato cultivars resistant or susceptible to bacterial wilt. *Ann Phytopathol Soc Jpn* 65: 597–603

Huang J, Carney, BF, Denny, TP, Weissinger AK, Schell MA (1995) A complex network regulates expression of *eps* and other virulence genes of *Pseudomonas solanacearum.* *J Bacteriol* 177: 1259–1267

Huang Q, Allen C (1997) An exo-poly-alpha-D-galacturonosidase, PehB, is required for wild-type virulence of *Ralstonia solanacearum.* *J Bacteriol* 179: 7369–7378

Huang Q, Allen C (2000) Polygalacturonases are required for rapid colonization and full virulence of *Ralstonia solanacearum* on tomato plants. *Physiol Mol Plant Pathol* 57: 77–83

Kanda A, Ohnishi S, Tomiyama H, Hasegawa H, Yasukohchi M, Kiba A, Ohnishi K, Okuno T, Hikichi Y (2003a) Type III-secretion machinery deficient mutants of *Ralstonia solnacearum* lose their ability to colonize, proliferate and induce host responses immediately after invasion, resulting in loss of their virulence. *J Gen Plant Pathol* 69: 250–257

Kanda A, Yasukohchi M, Ohnishi K, Kiba A, Okuno T, Hikichi Y (2003b) Ectopic expression of *Ralstonia solanacearum* effector protein PopA early in invasion results in loss of virulence. *Mol Plant-Microbe Interact* 16: 447–455

Kang Y, Liu H, Genin S, Schell MA, Denny TP (2002) *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. *Mol Microbiol* 46: 427–437

Kiba A, Tomiyama, H, Takahashi H, Ohnishi K, Okuno T, Hikichi Y (2003) Induction of resistance and expression of defense-related genes in tobacco leaves infiltrated with *Ralstonia solanacearum*. *Plant Cell Physiol* 44: 287–295

Liu H, Kang Y, Genin S, Schell MA, Denny TP (2001) Twitching motility of *Ralstonia solanacearum* requires a type IV pilus system. *Microbiology* 147: 3215–3229

Liu H., Zhang S, Schell MA, Denny TP (2005) Pyramiding unmarked deletions in *Ralstonia solanacearum* shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. *Mol Plant-Microbe Interact* 18: 1296–1305

Mudgett MB, Staskawicz BJ (1998) Protein signaling via type III secretion pathway in phytopathogenic bacteria. *Curr Opin Microbiol* 1: 109–114

Mukaihara T., Tamura N, Murata Y, Iwabuchi M (2004) Genetic screening of Hrp type III-related pathogenicity genes controlled by the HrpB transcriptional activator in *Ralstonia solanacearum*. *Mol Microbiol* 54: 863–875

Roberts PD, Denny TP, Schell MA (1988) Cloning of the *egl* gene of *Pseudomonas solanacearum* and analysis of its role in phytopathogenicity. *J Bacteriol* 170: 1445–1451

Salanoubat M., Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus JC, Cattolico L, Chandler M, Choisne N, Claudel-Renard C, Cunnac S, Demange N, Gaspin C, Lavie M, Moisan A, Robert C, Saurin W, Schiex T, Siguier P, Thebault P, Whalen M, Wincker P, Levy M, Weissenbach J, Boucher CA (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature* 415: 497–502

Schell MA (2000) Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. *Annu Rev Phytopathol* 38: 263–292

Shinohara R, Kanda A, Ohnishi K, Kiba A, Hikichi Y (2005) The contribution of folate biosynthesis to *Ralstonia solanacearum* proliferation in the intercellular spaces. *Appl Environ Microbiol* 71: 417–422

Tans-Kersten J, Brown D, Allen C (2004) Swimming motility, a virulence trait of *Ralstonia solanacearum*, is regulated by FlhDC and the plant host environment. *Mol Plant Microbe Interact* 17: 686–695

Tans-Kersten J, Guan Y, Allen C (1998) *Ralstonia solanacearum* pectin methylesterase is required for growth on methylated pectin but not for bacterial wilt virulence. *Appl Environ Microbiol* 64: 4918–4923

Van Gijsegem F, Gough C, Zischek C, Niqueux E, Arlat M, Genin S, Barberis P, German S, Castello P, Boucher C (1995) The *hrp* gene cluster of *Pseudomonas solanacearum*, which controls the production of a type III secretion system, encodes eight proteins related to components of the bacterial flagellar biogenesis complex. *Mol Microbiol* 15: 1095–1114

Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y (1995) Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff, 1973) comb. Nov., Ralstonia Solanacearum (Smith 1896) comb. Nov. and *Ralstonia eutropha* (Davis, 1969) comb. nov. *Microbiol Immunol* 39: 897–904