microbial biotechnology

Open Access

Opinion

# Current knowledge on the *Ralstonia solanacearum* type III secretion system

**Núria S. Coll[1] and Marc Valls[1,2]***

[1]*Centre for Research in Agricultural Genomics (CRAG).Edifici CRAG, Campus UAB, 08193 Bellaterra, Catalonia, Spain.*

[2]*Department of Genetics, Universitat de Barcelona, Av. Diagonal 643 Annex, 08028 Barcelona, Catalonia, Spain.*

*Ralstonia solanacearum* was ranked in a recent survey the second most important bacterial plant pathogen, following the widely used research model *Pseudomonas syringae* (Mansfield *et al*., 2012). The main reason is that bacterial wilt caused by *R. solanacearum* is the world's most devastating bacterial plant disease (http://faostat.fao.org), threatening food safety in tropical and subtropical agriculture, especially in China, Bangladesh, Bolivia and Uganda (Martin and French, 1985). This is due to the unusually wide host range of the bacterium, its high persistence and because resistant crop varieties are unavailable. In addition, *R. solanacearum* has been established as a model bacterium for plant pathology thanks to pioneering molecular and genomic studies (Boucher *et al*., 1985; Salanoubat *et al*., 2002; Cunnac *et al*., 2004b; Occhialini *et al*., 2005; Mukaihara *et al*., 2010). As for many bacterial pathogens, the main virulence determinant in *R. solanacearum* is the type III secretion system (T3SS) (Boucher *et al*., 1985; 1994), which injects a number of effector proteins into plant cells causing disease in hosts or a hypersensitive response in resistant plants. In this article we discuss the current state in the study of the *R. solanacearum* T3SS, stressing the latest findings and future perspectives.

Received 16 December, 2012; revised 26 February, 2013; accepted 27 February, 2013. *For correspondence. E-mail marcvalls@ub.edu; Tel. (+34) 935636600; Fax (+34) 935636601. Work conducted at the Centre for Research in Agricultural Genomics (CRAG). Edifici CRAG, Campus UAB, 08193 Bellaterra, Catalonia, Spain.
*Microbial Biotechnology* (2013) **6**(6), 614–620
doi:10.1111/1751-7915.12056
**Funding Information** Our research is supported by Grants SGR1427 and CONES2010-0030 from Comissionat per Universitats i Recerca of the Catalan Government (M. V.); HF2008-0021 and AGL2010-21870 from the Ministerio de Ciencia, Tecnología e Innovación of the Spanish Government (M. V.); and PCDMC-321738 from EU-Marie Curie Actions (N. S. C.).

## A regulatory cascade controls T3SS expression

Synthesis of the T3SS machinery – encoded by some 20 *hrp/hrc* genes – is tightly controlled in all species studied, probably due to its high metabolic cost. *Ralstonia solanacearum* is the only bacterial species for which a regulatory cascade linking T3SS gene expression to plant host contact has been described (Brito *et al*., 2002). In *R. solanacearum hrp/hrc* gene induction is triggered upon recognition of an unidentified non-diffusible cell wall component by the outer membrane receptor PrhA (Aldon *et al*., 2000), which transfers the activation signal through a cascade of transcriptional regulators (Brito *et al*., 2002). HrpG is a central regulator in this cascade (Brito *et al*., 1999; Valls *et al*., 2006), whose downstream activator HrpB directly controls transcription of the T3SS genes and its associated effectors (Genin *et al*., 1992; Occhialini *et al*., 2005). Interestingly, these two regulators have homologues in various *Xanthomonas ssp.* and *Burkholderia ssp.* strains, including the human pathogen *B. pseudomallei* (Wengelnik and Bonas, 1996; Zou *et al*., 2006; Li *et al*., 2011; Lipscomb and Schell, 2011), whereas the PrhA receptor and the upper regulators in the cascade are not conserved in other species.

## A regulatory network with connections to many cellular processes

In addition to the activation by the presence of plant cell wall components, expression of the T3SS genes is also induced by metabolic and environmental inputs. It has been known for a long time that *hrpB* expression is repressed when the bacterium grows in complete medium, as compared with a minimal medium that is thought to mimic plant apoplastic fluids (Arlat *et al*., 1992; Genin *et al*., 2005). More recently, it was found that other regulatory circuits impact *hrp* gene expression. The global regulator PhcA, which activates expression of many virulence activities including motility, plant cell wall degradation, and exopolysaccharide synthesis (Genin and Denny, 2012) has been reported to repress *hrpB* expression by two orders of magnitude during growth in complete medium (Genin *et al*., 2005). PhcA can also bind directly to the promoter of upstream regulators in the *Hrp*cascade but it only downregulates their transcription to one half of

the normal levels (Yoshimochi *et al*., 2008). Recent findings showed that PrhG – a HrpG paralogue – also influences expression of the HrpB regulon (Plener *et al*., 2010) and that this pathway is modulated by an unrelated virulence operon (Zhang *et al*., 2011). Thus, the actual view is that of a complex network of regulators controlling *hrp* gene expression in connection with a number of environmental and physiological cues.

The *hrp* regulatory system thus integrates different inputs but it also brings about various output responses by co-regulating transcription of the T3SS and effector genes to that of genes likely associated to metabolic adaptation to parasitic life in the plant (Occhialini *et al*., 2005; Valls *et al*., 2006). Indeed, transcriptomic studies have revealed that HrpG controls expression of some 400 genes, half of them independently of the downstream regulator HrpB. Some of these additional genes encode lectins and enzymes that degrade plant polysaccharides or drive the synthesis of polyamines or phytohormones (Valls *et al*., 2006). Further analyses may detect additional targets of the T3SS regulatory system that have escaped our notice due to experimental or technical limitations. In this sense, it is expected that RNA sequencing experiments can identify small RNAs involved in virulence controlled by the *hrp* regulators, as has been found in *Xanthomonas campestris*, which bears a closely-related regulatory system (Chen *et al*., 2011; Schmidtke *et al*., 2012).

### T3SS regulation *in planta*

An experimental limitation of the above described regulatory circuits is that they were all defined based on experiments carried out *in vitro* using synthetic media. Recent research has focused on determining their relevance and expression timing *in planta* during infection. The creation of a gene delivery system to integrate gene constructs in a permissive site of the *R. solanacearum* chromosome (Monteiro *et al*., 2012b) has been key to monitor transcription in these conditions. This tool enables the analysis of promoter output from single-copy fusions to fluorescent or luminescent reporters during plant infection, as the constructs remain stably integrated in the modified strains. Surprisingly, the master T3SS regulator *hrpB* was found to be transcribed in bacteria growing inside wilting plants, causing expression of *hrp* genes under these conditions (Monteiro *et al*., 2009; 2012a). These findings have been recently validated by an independent transcriptome analysis approach, which has confirmed that half of the HrpB regulon is induced in bacteria recovered from wilting plants (Jacobs *et al*., 2012). These results are in contradiction with the widespread view that the T3SS is only required during the first stages of host colonization. This notion was based on the observations that the T3SS genes are induced immediately after plant contact (Kamoun and Kado, 1990; Thwaites *et al*., 2004; Ortiz-Martin *et al*., 2010) and that this system is involved in suppression of host defence responses to promote bacterial multiplication early after infection (Deslandes and Rivas, 2012). Thus, it will be interesting to ascertain whether the T3SS remains active in late stages of disease development in other plant pathogens or if this is a particularity of *R. solanacearum*, and to elucidate what is the functional importance of the T3SS – if any – during the *R. solanacearum* saprophytic life cycle.

### A large effector repertoire

One of the key questions in bacterial pathogenicity is defining the whole inventory of the type III effectors (T3E) present in a given strain or species. The pioneering genome sequencing and annotation of *R. solanacearum* strain GMI1000 identified a first set of effector candidate genes based on homology to known effectors from other species or presence of domains typically eukaryotic (Salanoubat *et al*., 2002). The existence of well-defined T3SS transcriptional regulators greatly contributed to complete the list. Two approaches were followed to identify candidate effectors co-regulated with the T3SS: (i) the search for promoters with a HrpB binding sequence, similar to the PIP box described in *X. campestris* (Cunnac *et al*., 2004a; Koebnik *et al*., 2006) and (ii) transcriptomic studies using HrpB-deficient and overexpressing strains (Occhialini *et al*., 2005). Translocation analyses with the *cyaA* reporter or T3SS-dependent secretion to the medium have been used to validate most effector candidates (Cunnac *et al*., 2004b; Tamura *et al*., 2005; Mukaihara *et al*., 2010; Solé *et al*., 2012), so that the reference strain GMI1000 is thought to bear 72 type III effectors (Poueymiro and Genin, 2009; Mukaihara *et al*., 2010). Compared with animal pathogens, bacterial plant pathogens contain larger numbers (~ 30–40) of effectors, but the *R. solanacearum* effector repertoire is exceptionally large, probably due to its wide host range.

A pan-genomic analysis of *R. solanacearum* will determine the super-effector repertoire and help define core and variable effectors in this species, providing evolutionary cues on host range determination. A recent study comprising 19 *P. syringae* strains yielded a super-repertoire of 57 effector genes (Baltrus *et al*., 2011). Considering that the average effector number per strain analysed is considerably lower in *P. syringae* compared with *R. solanacearum* (15–30 in *P. syringae* versus 72 in *R. solanacearum* GMI1000), it is reasonable to expect that the super-effector repertoire of *R. solanacearum* will be correspondingly larger. Up to date, the genomes of 11 *R. solanacearum* strains have been sequenced (GMI1000, RS1000, UW551, Po82, CFBP2957, PSI07,

 

CMR15, Molk2, IPO1609, K60 and Y45) and many others are on their way. These genomes, representative of the whole range of strains composing the *R. solanacearum* species complex, will facilitate pan-genomic analyses in the near future and shed light on effector conservation and function in this species. It will be interesting to ascertain whether in *R. solanacearum* divergent repertoires can be found in strains that are pathogenic on the same host, as it is the case for *P. syringae* (Baltrus *et al*., 2011; Lindeberg *et al*., 2012).

The minimal functional set of core effectors has not been yet determined in *R. solanacearum*. In *P. syringae* DC3000 it has been recently shown to comprise AvrPtoB, HopE1, HopG1, HopAM1, AvrE, HopM1, HopAA1 and HopN1 (Cunnac *et al*., 2011). These effectors function together in host immune suppression, chlorosis and lesion formation, in addition to bacterial growth. Among these, HopG1 is the most widespread in *R. solanacearum* sequenced strains, being only absent in PSI07, K60 and Y45 HopAA1 is the second most represented, as it can be found in GMI1000, Po82, Molk2, IPO1602, CFBP2957 and CMR15. AvrE homologues are identified in Po82, Molk2, IPO1602 and CFBP2957, although the picture is more complex, as distantly-related orthologues may be present in other strains. Finally, an AvrPtoB homologue is only present in Molk2 and the remaining four *P. syringae* predicted core effectors (HopM1, HopN1, HopE1, HopAM1) are absent in *R. solanacearum*. The fact that only half of the *P. syringae* core effectors have members in *R. solanacearum* may indicate that the core effectome in this species is constituted by either functional analogues with no sequence similarity to their *P. syringae* counterparts or by a total different set of activities. Functional genetics studies will clarify in the future which of these hypotheses is true.

## Type III effector function

Deciphering effector function is essential to understand the molecular interactions between pathogens and their hosts in terms of host specificity and pathogenicity. In *P. syringae*, it has been suggested that a small subset of core effectors target antimicrobial vesicle trafficking in plants, whereas a larger and more variable set would interfere with plant kinase-based pathogen recognition pathways (Lindeberg *et al*., 2012). Whether these two strategies to defeat plant immune processes are conserved in *R. solanacearum* remains an open question.

Up to date 23 *R. solanacearum* T3E have been assigned a function *in planta* using biochemical and/or pathology assays (Table 1). To study the contribution of each individual effector to bacterial fitness *in planta*, three methods have been used: (i) to measure growth of *R. solanacearum* mutant strains inside of natural hosts (tomato, eggplant); (ii) to measure growth of *P. syringae* heterologously expressing *R. solanacearum* T3E in Arabidopsis (Solé *et al*., 2012); (iii) competitive index assays between co-inoculated wt and mutant strains, which have proved to be a highly sensitive method to detect minor contributions to pathogenicity (Macho *et al*., 2010). These methods have revealed that several effectors promote growth in *R. solanacearum* natural hosts (Table 1): AvrPphF, AWR1, AWR2, PopP2 and Rip34 (HopD1-like) in tomato; AvrPphF, AWR1, AWR2, Rsp0842 (PopC-like), PopP2, SKWP4, Rip19 (AvrBs3-like), Rip39, Rip64, Rip3, Rip55 and Rip23 in eggplant; and AvrPphF, PopP2, Rsp0842 (PopC-like) and Rip34 (HopD1-like) in bean. Interestingly, two members of the AWR family show contrasting phenotypes, restricting growth in Arabidopsis and tomato (AWR4) or eggplant and Arabidopsis (AWR5), which may indicate a certain degree of recognition of these T3S in certain host cellular contexts. Other *R. solanacearum* T3E have been ascribed an avirulence function: AvrA is considered the major determinant leading to resistance of tobacco to some strains (Carney and Denny, 1990; Robertson *et al*., 2004; Poueymiro *et al*., 2009). Other avirulence reactions are triggered by PopP1 in resistant tobacco plants and in petunia (Lavie *et al*., 2002), PopP2 in Arabidopsis (Deslandes *et al*., 2002; 2003; Bernoux *et al*., 2008) and AWR2 and AWR5 in various contexts (Solé *et al*., 2012). Together, these results evidence that the interaction of *R. solanacearum* with its different plant hosts partly results from a combination of synergistic and antagonistic interactions between specific effectors within a single strain.

The characterization of the molecular/biochemical function of the increasingly large number of *R. solanacearum* effectors remains a major challenge. So far only a limited number of its T3E have been biochemically characterized (Table 1). Several members of the GALA family (Gala1, Gala3, Gala5, Gala6 and Gala7) have been shown to interact with SKP1-like proteins, and are thought to mimic plant E3 ubiquitin ligases (Angot *et al*., 2006). PopP2 has been shown to trigger re-localization of the cysteine protease RD19 to the nucleus, where it is thought to form a protein complex with the atypical WRKY-containing NB-LRR protein RRS1-R leading to disease resistance (Deslandes *et al*., 2002; 2003; Bernoux *et al*., 2008). However, direct interaction has only been shown for PopP2/RRS1-R and RRS1-R/RD19, but not for PopP2 and RD19. Recent work suggests that RRS1-R activation of the plant immune responses upon PopP2 recognition involves perception of PopP2 auto-acetylation (Tasset *et al*., 2010). Finally, the harpin-like T3E PopA has been shown to localize to the membrane of tobacco cells, where it forms ion-conducting pores, likely facilitating translocation of bacterial proteins into the cytoplasm of plant cells (Racapé *et al*., 2005).

 

**Table 1.** List of *R. solanacearum* type III effector with a defined role *in planta* or for which the mode of action has been (partly) elucidated.

| Gene name | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| GMI1000 | RS1000 | Alternative name | Protein name | Family | Predicted domains | Role *in planta* | Hosts tested | Mode of action | References |
| RSc0608 | rip5 | avrA | AvrA | – | – | Avirulence/Promotes growth | Nicotiana spp./ Tomato | – | Carney and Denny (1990); Robertson *et al*. (2004); Turner *et al*. (2009); Macho *et al*. (2010) |
| RSp0822 | rip40 | – | AvrPphF | HopF2/AvrPphF | – | Promotes growth | Tomato, Eggplant, Bean | – | Macho *et al*. (2010) |
| Rsc2139 | – | – | Awr1 | AWR | – | Promotes growth | Tomato, Eggplant | – | Solé *et al*. (2012) |
| RSp0099 | rip29 | hpx31/ripA | Awr2 | | – | Avirulence/Promotes growth | Nicotiana spp./ Tomato, Eggplant, Arabiodpsis | – | |
| RSp0847 | rip45 | hpx4 | Awr4 | | – | Restricts growth | Arabidopsis | – | |
| RSp1024 | rip56 | hpx10 | Awr5 | | – | Avirulence/Restricts growth | Nicotiana spp./ Tomato, Eggplant, Arabiodpsis | – | |
| RSp0914 | rip53 | gala1 | Gala1 | GALA | LRR repeats – F-box | – | – | Interaction with SKP1-like proteins | Angot *et al*. (2006) |
| RSp0028 | rip28 | gala3 | Gala3 | | | – | – | | |
| RSc1801 | rip18 | hpx16 | Gala5 | | | – | – | | |
| RSc1356 | rip13 | hpx13 | Gala6 | | | – | – | | |
| RSc1357 | rip14 | hpx14 | Gala7 | | | Host specificity factor | Medicago truncatula | | |
| RSp0877 | rip49 | popA | PopA | – | Harpin | | Nicotiana | Formation of plasma membrane ion-conducting pores | Racapé *et al*. (2005) |
| Rsp0842 | – | – | – | PopC | LRR | Promotes growth | Eggplant, Bean | – | Macho *et al*. (2010) |
| RSc0826 | rip7 | popP1 | PopP1 | YopJ/AvrRxv | Ser/Thr acetyltransferase, functional NLS | Avirulence | Petunia | – | Lavie *et al*. (2002) |
| RSc0868 | rip8 | popP2 | PopP2 | | | Avirulence/Promotes growth | Arabidopsis/Tomato, Eggplant, Bean | Nuclear relocalization of RRS1-R and RD19, binds RRS1-R | Deslandes *et al*. (2002); Deslandes *et al*. (2003); Bernoux *et al*. (2008); Macho *et al*. (2010) |
| RSc1839 | rip20 | hpx30 | Skwp4 | SKWP | Heat/armadillo-related repeats | Promotes growth | Eggplant | – | Macho *et al*. (2010) |
| RSc1815 | rip19 | hpx17 | Rip19 | AvrBs3 | central repeat | Promotes growth | Eggplant | – | |
| RSp0304 | rip34 | hpx25 | Rip34 | HopD1/AvrPphD | – | Promotes growth | Tomato, Eggplant, Bean | – | |
| RSp0732 | rip39 | hpx27 | Rip39 | HopAV1 | Coiled-coil | Promotes growth | Eggplant | – | |
| RSp1281 | rip64 | hpx24 | Rip64 | HopR1 | – | Promotes growth | Eggplant | – | |
| RSc0257 | rip3 | – | Rip3 | – | Ankyrin repeat | Promotes growth | Eggplant | – | |
| RSp1022 | rip55 | hpx21 | Rip55 | – | – | Promotes growth | Eggplant | – | |
| RSc2359 | rip23 | hpx28 | Rip23 | – | – | Promotes growth | Eggplant | – | |

Despite all our current knowledge on *R. solanacearum* T3E derived from the combination of genomic, biochemical and pathology data obtained in the last two decades, there is still a considerable number of effectors with no assigned function. These are usually effectors with no similarity to known proteins or domains or no apparent role in virulence or avirulence. The lack of assigned function *in planta* for many effectors is likely due to redundancy and specialized functionality restricted to certain host plant contexts. To dissect such complex interface between a pathogen and its host a novel genetic screening (insertional mutagenesis and depletion, iMAD) has been successfully used (O'Connor *et al*., 2012). This method systematically combines bacterial and plant mutations, and would be extremely helpful to characterize the interaction of *R. solanacearum* with its multiple hosts. Still, 30% of *R. solanacearum* T3Es have no counterpart in other bacteria (Mukaihara *et al*., 2010), making this species a good model to explore novel effector functions.

**Acknowledgements**

We thank M. Huarte, L. Gutarra and S. Genin for their advice and stimulating discussions. Our research is supported by Grants SGR1427 and CONES2010-0030 from Comissionat per Universitats i Recerca of the Catalan Government (M. V.); HF2008-0021 and AGL2010-21870 from the Ministerio de Ciencia, Tecnología e Innovación of the Spanish Government (M. V.); BP_B 00030 from the Catalan Government; and PCDMC-321738 from EU-Marie Curie Actions (N. S. C.).

**Conflict of interest**

None declared.

**References**

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

Angot, A., Peeters, N., Lechner, E., Vailleau, F., Baud, C., Gentzbittel, L., *et al*. (2006) *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. *Proc Natl Acad Sci U S A* **103:** 14620–14625.

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

Baltrus, D.A., Nishimura, M.T., Romanchuk, A., Chang, J.H., Mukhtar, M.S., Cherkis, K., *et al*. (2011) Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates. *PLoS Pathog* **7:** e1002132.

Bernoux, M., Timmers, T., Jauneau, A., Briere, C., de Wit, P.J., Marco, Y., and Deslandes, L. (2008) RD19, an Arabidopsis cysteine protease required for RRS1-R-mediated resistance, is relocalized to the nucleus by the *Ralstonia solanacearum* PopP2 effector. *Plant Cell* **20:** 2252–2264.

Boucher, C., Genin, S., and Van Gijsegem, F. (1994) Conservation of secretion pathways for pathogenicity determinants for plant and animal bacteria. *Trends Microbiol* **1**: 175–180.

Boucher, C.A., Barberis, P.A., Trigalet, A.P., and Demery, D.A. (1985) Transposon mutagenesis of *Pseudomonas solanacearum*: isolation of Tn5-induced avirulent mutants. *J Gen Microbiol* **131:** 2449–2457.

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

Brito, B., Aldon, D., Barberis, P., Boucher, C., and 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.

Carney, B.F., and Denny, T.P. (1990) A cloned avirulence gene from *Pseudomonas solanacearum* determines incompatibility on *Nicotiana tabacum* at the host species level. *J Bacteriol* **172:** 4836–4843.

Chen, X.L., Tang, D.J., Jiang, R.P., He, Y.Q., Jiang, B.L., Lu, G.T., and Tang, J.L. (2011) sRNA-Xcc1, an integron-encoded transposon- and plasmid-transferred trans-acting sRNA, is under the positive control of the key virulence regulators HrpG and HrpX of *Xanthomonas campestris* pathovar *campestris*. *RNA Biol* **8:** 947–953.

Cunnac, S., Boucher, C., and Genin, S. (2004a) 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., and Genin, S. (2004b) 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.

Cunnac, S., Chakravarthy, S., Kvitko, B.H., Russell, A.B., Martin, G.B., and Collmer, A. (2011) Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in *Pseudomonas syringae*. *Proc Natl Acad Sci U S A* **108:** 2975–2980.

Deslandes, L., and Rivas, S. (2012) Catch me if you can: bacterial effectors and plant targets. *Trends Plant Sci* **17:** 644–655.

Deslandes, L., Olivier, J., Theulieres, F., Hirsch, J., Feng, D.X., Bittner-Eddy, P., *et al*. (2002) Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes. *Proc Natl Acad Sci U S A* **99:** 2404–2409.

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., *et al*. (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to

 

the plant nucleus. *Proc Natl Acad Sci U S A* **100:** 8024–8029.

Genin, S., and Denny, T.P. (2012) Pathogenomics of the *Ralstonia solanacearum* species complex. *Annu Rev Phytopathol* **50:** 67–89.

Genin, S., Gough, C.L., Zischek, C., and Boucher, C.A. (1992) Evidence that the *hrpB* gene encodes a positive regulator of pathogenicity genes from *Pseudomonas solanacearum*. *Mol Microbiol* **6:** 3065–3076.

Genin, S., Brito, B., Denny, T.P., and Boucher, C. (2005) Control of the *Ralstonia solanacearum* Type III secretion system (Hrp) genes by the global virulence regulator PhcA. *FEBS Lett* **579:** 2077–2081.

Jacobs, J.M., Babujee, L., Meng, F., Milling, A., and Allen, C. (2012) The in planta transcriptome of *Ralstonia solanacearum*: conserved physiological and virulence strategies during bacterial wilt of tomato. *mBio* **3:** e00114-12.

Kamoun, S., and Kado, C.I. (1990) A plant-inducible gene of *Xanthomonas campestris* pv. *campestris* encodes an exocellular component required for growth in the host and hypersensitivity on nonhosts. *J Bacteriol* **172:** 5165–5172.

Koebnik, R., Kruger, A., Thieme, F., Urban, A., and Bonas, U. (2006) Specific binding of the *Xanthomonas campestris* pv. *vesicatoria* AraC-type transcriptional activator HrpX to plant-inducible promoter boxes. *J Bacteriol* **188:** 7652–7660.

Lavie, M., Shillington, E., Eguiluz, C., Grimsley, N., and Boucher, C. (2002) PopP1, a new member of the YopJ/AvrRxv family of type III effector proteins, acts as a host-specificity factor and modulates aggressiveness of *Ralstonia solanacearum*. *Mol Plant Microbe Interact* **15:** 1058–1068.

Li, Y.R., Zou, H.S., Che, Y.Z., Cui, Y.P., Guo, W., Zou, L.F., *et al*. (2011) A novel regulatory role of HrpD6 in regulating *hrp-hrc-hpa* genes in *Xanthomonas oryzae* pv. *oryzicola*. *Mol Plant Microbe Interact* **24:** 1086–1101.

Lindeberg, M., Cunnac, S., and Collmer, A. (2012) Pseudomonas syringae type III effector repertoires: last words in endless arguments. *Trends Microbiol* **20:** 199–208.

Lipscomb, L., and Schell, M.A. (2011) Elucidation of the regulon and *cis*-acting regulatory element of HrpB, the AraC-type regulator of a plant pathogen-like type III secretion system in *Burkholderia pseudomallei*. *J Bacteriol* **193:** 1991–2001.

Macho, A.P., Guidot, A., Barberis, P., Beuzon, C.R., and Genin, S. (2010) A Competitive index assay identifies several *Ralstonia solanacearum* type iii effector mutant strains with reduced fitness in host plants. *Mol Plant Microbe Interact* **23:** 1197–1205.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., *et al*. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol Plant Pathol* **13:** 614–629.

Martin, C., and French, E.R. (1985) *Bacterial Wilt of Potato:* Pseudomonas solanacearum, Vol. **13**. Lima, Peru: International Potato Center.

Monteiro, F., Van Dijk, I., Solé, M., Genin, S., and Valls, M. (2009) Visualising the transcription of *Ralstonia solanacearum* key pathogenicity genes in planta (Poster). In *XIV International Congress on Molecular Plant-Microbe Interactions*. Quebec, Canada.

Monteiro, F., Genin, S., van Dijk, I., and Valls, M. (2012a) A luminescent reporter evidences active expression of *Ralstonia solanacearum* type III secretion system genes throughout plant infection. *Microbiology* **158:** 2107–2116.

Monteiro, F., Sole, M., van Dijk, I., and Valls, M. (2012b) A chromosomal insertion toolbox for promoter probing, mutant complementation, and pathogenicity studies in *Ralstonia solanacearum*. *Mol Plant Microbe Interact* **25:** 557–568.

Mukaihara, T., Tamura, N., and Iwabuchi, M. (2010) Genome-wide identification of a large repertoire of *Ralstonia solanacearum* type III effector proteins by a new functional screen. *Mol Plant Microbe Interact* **23:** 251–262.

O'Connor, T.J., Boyd, D., Dorer, M.S., and Isberg, R.R. (2012) Aggravating genetic interactions allow a solution to redundancy in a bacterial pathogen. *Science* **338:** 1440–1444.

Occhialini, A., Cunnac, S., Reymond, N., Genin, S., and Boucher, C. (2005) Genome-wide analysis of gene expression in *Ralstonia solanacearum* reveals that the *hrpB* gene acts as a regulatory switch controlling multiple virulence pathways. *Mol Plant Microbe Interact* **18:** 938–949.

Ortiz-Martin, I., Thwaites, R., Macho, A.P., Mansfield, J.W., and Beuzon, C.R. (2010) Positive regulation of the Hrp type III secretion system in *Pseudomonas syringae* pv. *phaseolicola*. *Mol Plant Microbe Interact* **23:** 665–681.

Plener, L., Manfredi, P., Valls, M., and Genin, S. (2010) PrhG, a transcriptional regulator responding to growth conditions, is involved in the control of the type III secretion system regulon in *Ralstonia solanacearum*. *J Bacteriol* **192:** 1011–1019.

Poueymiro, M., and Genin, S. (2009) Secreted proteins from *Ralstonia solanacearum*: a hundred tricks to kill a plant. *Curr Opin Microbiol* **12:** 44–52.

Poueymiro, M., Cunnac, S., Barberis, P., Deslandes, L., Peeters, N., Cazale-Noel, A.C., *et al*. (2009) Two type III secretion system effectors from *Ralstonia solanacearum* GMI1000 determine host-range specificity on tobacco. *Mol Plant Microbe Interact* **22:** 538–550.

Racapé, J., Belbahri, L., Engelhardt, S., Lacombe, B., Lee, J., Lochman, J., *et al*. (2005) Ca2+-dependent lipid binding and membrane integration of PopA, a harpin-like elicitor of the hypersensitive response in tobacco. *Mol Microbiol* **58:** 1406–1420.

Robertson, A.E., Wechter, W.P., Denny, T.P., Fortnum, B.A., and Kluepfel, D.A. (2004) Relationship between avirulence gene (*avrA*) diversity in *Ralstonia solanacearum* and bacterial wilt incidence. *Mol Plant Microbe Interact* **17:** 1376–1384.

Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., *et al*. (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature* **415:** 497–502.

Schmidtke, C., Findeiss, S., Sharma, C.M., Kuhfuss, J., Hoffmann, S., Vogel, J., *et al*. (2012) Genome-wide transcriptome analysis of the plant pathogen Xanthomonas identifies sRNAs with putative virulence functions. *Nucleic Acids Res* **40:** 2020–2031.

 

Solé, M., Popa, C., Mith, O., Sohn, K.H., Jones, J.D., Deslandes, L., and Valls, M. (2012) The awr gene family encodes a novel class of *Ralstonia solanacearum* type III effectors displaying virulence and avirulence activities. *Mol Plant Microbe Interact* **25:** 941–953.

Tamura, N., Murata, Y., and Mukaihara, T. (2005) Isolation of *Ralstonia solanacearum hrpB* constitutive mutants and secretion analysis of *hrpB*-regulated gene products that share homology with known type III effectors and enzymes. *Microbiology* **151:** 2873–2884.

Tasset, C., Bernoux, M., Jauneau, A., Pouzet, C., Briere, C., Kieffer-Jacquinod, S., *et al.* (2010) Autoacetylation of the *Ralstonia solanacearum* effector PopP2 targets a lysine residue essential for RRS1-R-mediated immunity in Arabidopsis. *PLoS Pathog* **6:** e1001202.

Thwaites, R., Spanu, P.D., Panopoulos, N.J., Stevens, C., and Mansfield, J.W. (2004) Transcriptional regulation of components of the type III secretion system and effectors in *Pseudomonas syringae* pv. *phaseolicola*. *Mol Plant Microbe Interact* **17:** 1250–1258.

Turner, M., Jauneau, A., Genin, S., Tavella, M.J., Vailleau, F., Gentzbittel, L., and Jardinaud, M.F. (2009) Dissection of bacterial wilt on *Medicago truncatula* revealed two type III secretion system effectors acting on root infection process and disease development. *Plant Physiol* **150**: 1713–1722.

Valls, M., Genin, S., and Boucher, C. (2006) Integrated regulation of the type III secretion system and other virulence determinants in *Ralstonia solanacearum*. *PLoS Pathog* **2:** e82.

Wengelnik, K., and Bonas, U. (1996) HrpXv, an AraC-type regulator, activates expression of five of the six loci in the *hrp* cluster of *Xanthomonas campestris* pv. *vesicatoria*. *J Bacteriol* **178:** 3462–3469.

Yoshimochi, T., Hikichi, Y., Kiba, A., and Ohnishi, K. (2008) The global virulence regulator PhcA negatively controls the *Ralstonia solanacearum hrp* regulatory cascade by repressing expression of the PrhIR signalling proteins. *J Bacteriol* **191:** 3424–3428.

Zhang, Y., Kiba, A., Hikichi, Y., and Ohnishi, K. (2011) prhKLM genes of *Ralstonia solanacearum* encode novel activators of *hrp* regulon and are required for pathogenesis in tomato. *FEMS Microbiol Lett* **317:** 75–82.

Zou, L.F., Wang, X.P., Xiang, Y., Zhang, B., Li, Y.R., Xiao, Y.L., *et al*. (2006) Elucidation of the *hrp* clusters of *Xanthomonas oryzae* pv. *oryzicola* that control the hypersensitive response in nonhost tobacco and pathogenicity in susceptible host rice. *Appl Environ Microbiol* **72:** 6212–6224.

 