The large, diverse and robust arsenal of *Ralstonia solanacearum* type III effectors and their *in planta* functions

David Landry¹, Manuel González-Fuente¹, Laurent Deslandes¹ and Nemo Peeters¹*

¹ Laboratoire des Interactions Plantes Micro-organismes (LIPM), INRAE, CNRS, Université de Toulouse, F-31326 Castanet-Tolosan, France

¶ These authors contributed equally to this work.

* Corresponding author

E-mail: nemo.peeters@inrae.fr

Keywords: *Ralstonia solanacearum*, type III effectors, virulence, targets, immunity, susceptibility, effectome

Word count: 3752
**Summary**

The type III secretion system with its delivered type III effectors (T3Es) is one of the main virulence determinants of *Ralstonia solanacearum*, a worldwide devastating plant pathogenic bacterium affecting many crop species. The pan-effectome of the *R. solanacearum* species complex has been exhaustively identified and is composed of more than 100 different T3Es. Among the reported strains, their content range from 45 to 76 T3Es. This considerably large and varied effectome could be considered one of the factors contributing to *R. solanacearum* wide host range. In order to understand how *R. solanacearum* uses its T3Es to subvert the host cellular processes, many functional studies have been conducted over the last three decades. It has been shown that *R. solanacearum* effectors, as those from other plant pathogens, can suppress the plant defence mechanisms, modulate the host metabolism or avoid the bacterial recognition through a wide variety of molecular mechanisms. *R. solanacearum* T3Es can also be perceived by the plant and trigger immune responses. Up to date, the molecular mechanisms employed by *R. solanacearum* T3Es to modulate these host processes have been described for a growing number of T3Es, although remain unknown for the majority of them. In this microreview, we summarize and discuss the current knowledge on the characterized *R. solanacearum* species complex T3Es.

**Introduction**

Bacteria from the *Ralstonia solanacearum* species complex (RSSC) are soilborne plant pathogens responsible for bacterial wilt on more than 250 species, Moko and blood diseases of banana, brown rot of potato and Sumatra disease on clove tree (Peeters, Guidot, *et al.*, 2013). Due to its aggressiveness, broad host range, widespread geographical distribution and long-lasting persistence on the soil, *Ralstonia* ranks among the most devastating plant pathogenic bacteria (Mansfield *et al.*, 2012). For a successful infection, RSSC bacteria rely on different virulence determinants including the production of exopolysaccharides and phytohormones,
secretion of cell wall degrading enzymes, detoxification and nutrient-scavenging systems and motility (Genin and Denny, 2012). However, the main virulence determinant of RSSC bacteria is the type III secretion systems (T3SS), a ‘molecular syringe’ that allows the translocation of several type III effector proteins (T3Es) directly into the host cell (Coll and Valls, 2013). These T3Es, referred to as ‘Ralstonia injected proteins’ (Rips), are able to subvert the defences and modify the metabolism of the host to accommodate the bacterial needs.

The RSSC type III effectome, a large and varied arsenal

Since the first RSSC T3E genes were cloned in the 90s (Carney and Denny, 1990; Arlat et al., 1994; Guéneron et al., 2000), different approaches have been conducted to systematically identify at the genome-scale the full T3E repertoire of several RSSC strains. Two main strategies were conducted: (1) Sequence-based approaches, looking for sequence homology with previously described effector genes and/or for the presence of certain 25-nucleotide cis elements in their promoters, the hrpII box or the plant-inducible promoter (PIP) box motifs (Salanoubat et al., 2002; Cunnac, Boucher and Genin, 2004; Gabriel et al., 2006; Peeters, Carrère, et al., 2013; Sabbagh et al., 2019). (2) Regulation-based strategies, exploiting that T3E gene expression is controlled by HrpB, an AraC family member of transcriptional regulators (Genin et al., 1992; Cunnac, Boucher and Genin, 2004). Regulation-based strategies include gene expression studies (Cunnac et al., 2004; Occhialini et al., 2005) and genetic screens using random transposon-insertion mutagenesis (Mukaihara et al., 2004). Verification of the T3SS-dependency of the secretion or translocation is typically required to confirm the bona fide T3E status of in silico predicted or candidate T3Es (Lonjon et al., 2018). Most translocation analyses exploit the adenylate cyclase (Cya) reporter system (Cunnac et al., 2004; Mukaihara and Tamura, 2009; Mukaihara, Tamura and Iwabuchi, 2010). T3SS-dependent secretion analyses compare the secreted proteins, detected by immunoblotting or mass spectrometry, of wild-type
compared to *hrp* mutant strains (Tamura, Murata and Mukaihara, 2005; Solé *et al.*, 2012; Lonjon *et al.*, 2016; Sabbagh *et al.*, 2019).

A recent genomic study on 140 RSSC strains identified the pan-effectome of the species complex, consisting of 102 T3E and 16 hypothetical T3E genes (Sabbagh *et al.*, 2019). RSSC strains carry on average 64 T3E genes (minimum 45 in *R. syzygii* subsp. *syzygii* strain R24 and maximum 76 in *R. pseudosolanacearum* strain Rs-10-244). This contrasts with other plant pathogenic bacteria such as *Pseudomonas syringae* or *Xanthomonas campestris*, with an average of 31 (min 3, max 53) and 23 (min 12, max 28) T3E genes respectively (Roux *et al.*, 2015; Dillon *et al.*, 2019). The existence of several paralog families, such as the RipG (former GALA), RipS (SKWP), RipA (AWR), RipH (HLK) or RipP (PopP) families, can be considered as a remarkable feature of the RSSC. Not a single known RSSC strain does not carry multiple copies of these paralog T3E families. This contributes to the large size of the RSSC pan-effectome. The T3E repertoires of different RSSC strains are quite diverse, with only 16 core T3Es (i.e., T3Es present in at least 95% of sequenced strains), what represents the 13.6% of the RSSC pan-effectome (Sabbagh *et al.*, 2019). This core-effectome is larger than in *P. syringae* (4 core T3Es, 5.7% of its pan-effectome) or *X. campestris* (3 core T3Es, 8.6% of its pan-effectome) (Roux *et al.*, 2015; Dillon *et al.*, 2019). Several studies have tried to connect the T3E diversity to the host specificity of RSSC strains (Ailloud *et al.*, 2015; Cho *et al.*, 2019; Sabbagh *et al.*, 2019). Although some host specificity determinants could be identified, the power of such studies has usually been largely limited by the lack of exhaustive strain host range empirical data.

**Many T3Es, but for what purpose?**

As model root and vascular plant pathogens, RSSC bacteria are among the pathogens with a larger number of functionally characterized T3Es. Some effectome-scale experiments have tried to shed light on the function of RSSC T3Es through systematic determination of their
ability to induce hypersensitive response (Wroblewski et al., 2009), inhibit plant defences (Nakano and Mukaihara, 2019a), or identification of their plant targets (González-Fuente et al., 2019). However, most of our current knowledge on their function comes from smaller-scale experiments in which often one or a few T3Es are studied. Up to date, we have counted more than 50 different RSSC T3Es that have been characterized with varying degree of detail (Figure 1, Table 1). One of the main factors complicating this task is the observed genetic redundancy among different RSSC T3Es (Angot et al., 2006; Solé et al., 2012; Chen et al., 2014). This redundancy is likely to ensure a more robust virulence strategy for the bacteria (Ghosh and O’Connor, 2017) although it makes the functional dissection of single effectors more complicated, particularly for the paralog families. Nevertheless, some members of these families can still have specific and non-redundant functions (Angot et al., 2006; Turner et al., 2009; Wang et al., 2016).

Similarly to other pathogens, RSSC T3Es collectively contribute to the pathogen fitness in the plant through different and not always well characterized mechanisms (Toruño, Stergiopoulos and Coaker, 2016). These include the interference with the plant basal defence responses, alteration of the plant metabolism and avoidance of the specific recognition of other T3Es. However, some RSSC T3Es can also be recognized by specific plant genotypes and induce strong immune responses.

**Interference with plant basal immunity**

The subversion of basal defences is one of the most studied functions of pathogen effectors. Several RSSC T3Es are known to interfere with different host cellular processes involved in these basal defence responses. RipP2 (former PopP2) relies on its acetyltransferase activity to acetylate the WRKY domain of the plant homonymous transcription factors, what prevents their association with DNA and subsequent expression of defence-related genes (Le Roux et al., 2015). RipAY is selectively activated by eukaryotic thioredoxins to degrade the
host glutathione, which plays an important role in plant immunity (Fujiwara et al., 2016, 2020; Mukaihara et al., 2016; Sang et al., 2018). RipAR and RipAW rely on their E3 ubiquitin ligase activity to inhibit plant defence responses (Nakano, Oda and Mukaihara, 2017). Also linked to ubiquitination, RipG (former GALA) family of T3Es presents a eukaryotic F-box domain required for the interaction with Arabidopsis components of the Skp, Cullin, F-box containing (SCF) complex contributing to Ralstonia virulence (Angot et al., 2006; Remigi et al., 2011).

RipAL is a chloroplastic effector with a lipase domain required for the induction of jasmonic acid (JA) production and suppression of salicylic acid (SA) signalling (Nakano and Mukaihara, 2018). The inhibition of SA-mediated defences seems also to be the role of RipR (former PopS) and RipG1 and RipG3, although the molecular mechanisms behind this inhibition still remain unknown (Jacobs et al., 2013; Medina-Puche et al., 2019). RipAB (former PopB) downregulates the calcium signalling pathway and inhibits the plant basal defences (Zheng et al., 2019). Finally, RipN contains a Nudix hydrolase domain required to alter the NADH/NAD+ ratio in planta and to inhibit the plant defence responses (Sun et al., 2019).

In addition to these functionally characterized RSSC T3Es, other basal defence inhibiting T3Es have been identified in large-scale screenings. 16 additional RSSC T3Es have been reported as suppressors of the flg22-induced reactive oxygen species (ROS) production, a marker typically associated with pathogen-associated molecular pattern (PAMP)-triggered immunity (Sang and Macho, 2017): RipA5 (former AWR5), RipAD, RipAF1, RipD, RipE1, RipI, RipQ, RipAC (former PopC), RipAL, RipAP, and RipAU; and in lesser extent, RipH1 (former HLK1), RipM, RipS1 (former SKWP1), RipAN and RipB (Nakano and Mukaihara, 2019a; Jeon et al., 2020).

**Targeting plant metabolism**

Plant pathogenic bacterial T3Es can also interfere with different host metabolic processes to promote the bacterial survival, release nutrients and facilitate the infection (Macho,
RSSC bacteria thrive in the xylem manipulating the composition of the xylem sap (Lowe-Power et al., 2018). This manipulation can occur through different mechanisms, including the T3SS, as RSSC bacteria are able to inject T3Es into living cells surrounding the vasculature (Vasse et al., 2000; Henry et al., 2017). Indeed, some RSSC T3Es display different activities that could modulate the plant metabolism. The better characterized example is RipTAL (former Brg11), which presents homology with Xanthomonas spp. transcription activator-like (TAL) effectors (de Lange et al., 2013). RipTAL induces the expression of plant genes involved in the synthesis of polyamines evading their native translational regulation mechanisms (Wu et al., 2019). It is hypothesized that this RipTAL-induced boost of the plant polyamine levels prevents the proliferation of possible Ralstonia competitors (Wu et al., 2019).

RipA5 acts as an inhibitor of the conserved target of rapamycin (TOR) pathway in yeast and plant cells (Popa et al., 2016). As a key regulator of the switch between growth and stress responses (Dobrenel et al., 2016), RipA5-mediated inhibition of the plant TOR pathway leads to reduced nitrate reductase activity (Popa et al., 2016). Last, RipTPS possess trehalose-6-phosphate synthase activity in yeast (Poueymiro et al., 2014). As trehalose-6-phosphate is a key regulatory molecule in plant metabolism (Baena-González and Lunn, 2020), RipTPS could potentially interfere with this regulation but, so far, this activity has not been shown in planta.

**Contribution to virulence through (as of yet) unknown mechanisms**

In addition to the beforementioned RSSC T3Es for which functional roles could be assigned, other T3E genes have been also identified as contributors to bacterial virulence on difference hosts. These additional T3E genes have been identified through pathogenicity or competitive index assays with single or multiple gene mutants. These tests allow to pinpoint the involvement in virulence but do not provide further information about the underlying molecular mechanisms. This is the case of RipA2 and RipD, which contribute to virulence in tomato (Cunnac et al., 2004); or RipAA and RipG7, important in the early and late stages of
infection of the model legume species *Medicago truncatula* respectively (Turner *et al.*, 2009; Wang *et al.*, 2016). RipAC, RipAF1, RipAK, RipAV, RipAY, RipD, RipP2, RipR, RipS4, RipY, RipTAL contribute to bacterial fitness in eggplant (Macho *et al.*, 2010). For RipD and RipP2, this contribution to fitness was also demonstrated in tomato and bean, and in the case of RipAA, exclusively in tomato (Macho *et al.*, 2010). The RipA family members contribute collectively to virulence in both eggplant and tomato (Solè *et al.*, 2012), and the RipH family members also contribute to virulence in tomato (Chen *et al.*, 2014). RipAM, RipAN and RipBH contribute significantly to virulence in potato (Zheng *et al.*, 2019), and RipAC acts similarly in tomato (Yu *et al.*, 2020).

**Effectors triggering plant immune responses**

Through evolution, plants have evolved mechanisms to recognize specific RSSC T3Es and induce a strong defence response often associated with hypersensitive response (HR) (Balint-Kurti, 2019). This is precisely what was observed on petunia with RipX (former PopA), the first RSSC T3E to have been characterized (Arlat *et al.*, 1994). This same phenotype was later observed in tobacco (Belbahri *et al.*, 2002; Racapé *et al.*, 2005), and could be explained by a RipX-mediated inhibition of the gene expression of the ATP synthase F1 subunit α (Sun *et al.*, 2020). RipAA and RipP1 (former AvrA and PopP1 respectively) trigger strong HR responses in diverse *Nicotiana* spp. (Carney and Denny, 1990; Robertson *et al.*, 2004; Poueymiro *et al.*, 2009; Chen *et al.*, 2018). Additionally, RipP1 also triggers HR on petunia St40 line (Lavie *et al.*, 2002), and RipAA, in pepper CW300 and RNaKy accessions (Wroblewski *et al.*, 2009). RipP2 was the first RSSC T3E for which the corresponding immune receptor was identified in Arabidopsis: Recognition of *R. solanacearum* 1 (RRS1) (Deslandes *et al.*, 1998, 2003). It was later shown that this recognition also involves the Resistance to *Pseudomonas syringae* 4 (RPS4) immune receptor (Gassmann, Hinsch and Staskawicz, 1999; Narusaka *et al.*, 2009; Williams *et al.*, 2014). The RPS4/RRS1-dependent immunity is activated
by RipP2 acetylation of RRS1 C-terminal WRKY domain representing an integrated decoy that mimics RipP2 virulence targets (Tasset et al., 2010; Le Roux et al., 2015; Sarris et al., 2015). RipAT and RipAV induce HR-like phenotypes when expressed in most lettuce and certain pepper and tomato cultivars (Wroblewski et al., 2009). RipA1, RipA2, RipA3 and RipA5 trigger HR responses with varying intensities on different *Nicotiana* spp. (Solé et al., 2012; Jeon et al., 2020). RipTPS produces an HR specifically on *N. tabacum* independently of its enzymatic activity (Poueymiro et al., 2014). RipAX2 (former Rip36) elicits immunity on wild and cultivated eggplants in a Zn-finger domain-dependent (Nahar et al., 2014) and independent (Morel et al., 2018) manner respectively. RipAB triggers HR in *N. benthamiana* but only when localized in the nucleus (Zheng et al., 2019). RipB induces chlorosis in different *Nicotiana* spp. in a Recognition of XopQ1 (Roq1)-dependent manner (Nakano and Mukaihara, 2019b). RipBN triggers resistance in tomato in a *Pseudomonas tomato race 1* (*Ptr1*)-dependent manner (Mazo-Molina et al., 2019). RipE1 triggers immune responses mediated by both SA and JA in *N. benthamiana* and Arabidopsis (Sang et al., 2020). RipE1 also triggers HR in *N. tabacum* and *N. benthamiana* in a Suppressor of G2 allele of *skp1* (*SGT1*)-dependent manner for the latter (Jeon et al., 2020). Last, RipI triggers immune responses in tomato and cell death in yeast and *N. benthamiana*, the latter through interaction with the plant basic helix-loop-helix 93 (bHLH93) transcription factor (Deng et al., 2016; Zhuo et al., 2020).

**Effectors preventing other effectors to be recognized in planta**

The recognition of RSSC T3Es and subsequent strong immune responses can also be counteracted through the action of other T3Es, sometimes referred as ‘metaeffectors’ (Kubori et al., 2010). This could allow the bacteria to conserve effectors with potent virulence functions for which a given host has already developed specific recognition capabilities. This is the case of RipAY, which can inhibit the previously mentioned RipE1-triggered immunity (Sang et al., 2020). RipAY inhibits RipE1-mediated activation of the SA signalling pathway probably
through degradation of the plant cellular glutathione (Mukaihara et al., 2016; Sang et al., 2018, 2020). It has also been proposed that RipAC suppresses RipE1-triggered immunity, inhibiting in this case the SGT1-mediated MAPK activation (Yu et al., 2020). RipAK is able to prevent *Ralstonia*-induced HR in *N. tabacum* by inhibiting the plant catalase activity (Sun et al., 2017). Whether this HR is induced by RipAA, RipB and/or RipP1, responsible for RSSC incompatibility in *N. tabacum* (Poueymiro et al., 2009; Nakano and Mukaihara, 2019b), is still unknown.

**Conclusions and perspectives**

In this microreview, we have summarized the current knowledge about RSSC T3Es. Despite being one of the largest and most studied bacterial plant pathogen effectomes, a majority of RSSC T3Es remain up to date poorly characterized. This will undoubtedly change in the near future as more and more RSSC T3Es are currently being characterized by several research groups worldwide. Nevertheless, from what it is currently known, we can already see that the large RSSC effectome is highly diversified in terms of molecular functions, subcellular localizations and host targeted processes. RSSC T3Es act in the host plasma membrane, cytoplasm, nucleus, chloroplasts or peroxisomes, and interfere with the plant gene expression regulation at the transcriptional and translational level, metabolism, ubiquitination, phytohormone production and signalling, redox homeostasis or calcium signalling. This functional repertoire, coupled with genetic and functional redundancy, confers RSSC bacteria with a strong, varied and robust set of weaponry against their hosts. It is thus tempting to hypothesize that this T3E diversity contributes to the adaptability of *Ralstonia* as a species complex to a wide range of plant hosts. It could also be noticed that this large cornucopia of T3Es could be a key factor in the appearance of RSSC strains adapted to new host plants, like the recently identified strains virulent on cucurbitaceous crops (Wicker et al., 2007), coffee plant (Lopes, Rossato and Boiteux, 2015), fig tree (Jiang et al., 2016), African daisy (Weibel
et al., 2016) or roses (Tjou-Tam-Sin et al., 2017). Future work will help to elucidate whether
the so far uncharacterized T3Es target similar processes to the previously described or if, on the
 contrary, they interfere with completely different plant processes. This is key to understand
whether the strength of RSSC effectomes comes rather from its high diversity (i.e., RSSC
bacteria target simultaneously many different plant processes) or from its redundancy (i.e.,
RSSC bacteria target a few key plant processes with redundant T3Es). The characterization of
new T3Es will also allow determining the plant processes that RSSC bacteria specifically target
in order to establish a successful infection. Interestingly, nine out the sixteen RSSC core T3Es
have been shown to contribute to virulence in different hosts: RipA2, RipAB, RipAM, RipAN,
RipAY, RipG5, RipG6, RipH2 and RipR. From these nine T3Es, functional information is only
available for five of them: RipG5 and RipG6 interact with components of the E3 ubiquitin
ligase complex (Angot et al., 2006; Remigi et al., 2011), RipR inhibits SA-mediated defence
responses (Jacobs et al., 2013), RipAY degrades plant glutathione (Fujiwara et al., 2016, 2020;
Mukaihara et al., 2016; Sang et al., 2018) and RipAB downregulates the calcium-signalling
pathway (Zheng et al., 2019). These different processes, together with the unknown ones
targeted by the other core T3Es, could represent the minimum plant processes that *Ralstonia*
needs to modulate. This ‘basal arsenal’ could be complemented with accessory T3Es that could
have additive effects, targeting same or different processes. However, this characterization
might prove quite complex as these plant processes, and their modulation by *Ralstonia* T3Es,
might vary substantially among different organs and host species. The diverse and sometimes
large host range of RSSC strains and the functional diversity and redundancy of its effectome
are therefore some of the causes of RSSC adaptability and aggressiveness, but also some of the
major factors complicating its systematic and exhaustive study. A valuable tool that will open
a wide variety of possibilities in the decipherment of RSSC T3E functions is the generation of
a strain devoid of all its effectors, as it has been performed on the *P. syringae* strain DC3000.
(Cunnac et al., 2011). This should be completed soon on the RSSC strain OE1-1 (K. Onishi, Kochi University, Kochi, Japan, April 2020, personal communication). The fact that RSSC bacteria can infect both model and agronomically important crop species confers a practical perspective to this information gathered over the last decades. This should certainly contribute to the design of effective and sustainable control measures against the devastating RSSC.

Acknowledgements

DL and MGF were supported by PhD fellowships from the French Ministry of National Education and Research and the French Laboratory of Excellence project ‘TULIP’ (ANR-10-LABX-41; ANR-11-IDEX-0002-02) respectively. The LIPM is supported by the French Laboratory of Excellence project ‘TULIP’ (ANR-10-LABX-41; ANR-11-IDEX-0002-02).

None of the co-authors have a conflict of interest to declare.

References

Ailloud, F. et al. (2015) ‘Comparative genomic analysis of Ralstonia solanacearum reveals candidate genes for host specificity’, *BMC genomics*, 16, p. 270. doi: 10.1186/s12864-015-1474-8.

Angot, A. et al. (2006) ‘Ralstonia solanacearum requires F-box-like domain-containing type III effectors to promote disease on several host plants.’, *Proceedings of the National Academy of Sciences of the United States of America*. United States, 103(39), pp. 14620–14625. doi: 10.1073/pnas.0509393103.

Arlat, M. et al. (1994) ‘PopA1, a protein which induces a hypersensitivity-like response on specific Petunia genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*’, *The EMBO journal*, 13(3), pp. 543–53. Available at: http://www.nebi.nlm.nih.gov/pubmed/8313899.

Baena-González, E. and Lunn, J. E. (2020) ‘SnRK1 and trehalose 6-phosphate – two ancient pathways converge to regulate plant metabolism and growth’, *Current Opinion in Plant*
Balint-Kurti, P. (2019) ‘The plant hypersensitive response: concepts, control and consequences’, Molecular plant pathology, 20(8), pp. 1163–1178. doi: 10.1111/mpp.12821.

Belbahri, L. et al. (2002) ‘A local accumulation of the Ralstonia solanacearum PopA protein in transgenic tobacco renders a compatible plant-pathogen interaction incompatible’, The Plant Journal, 28(4), pp. 419–430. doi: 10.1046/j.1365-313X.2001.01155.x.

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’, Journal of bacteriology, 172(9), pp. 4836–43. doi: 10.1128/jb.172.9.4836-4843.1990.

Chen, L. et al. (2014) ‘Involvement of HLK effectors in Ralstonia solanacearum disease development in tomato.’, Journal of General Plant Pathology, 80(1), pp. 79–84. doi: 10.1007/s10327-013-0490-2.

Chen, L. et al. (2018) ‘Involvement of avirulence genes avrA and popP1 of Japanese Ralstonia solanacearum strains in the pathogenicity to tobacco’, Physiological and Molecular Plant Pathology, 102, pp. 154–162. doi: 10.1016/j.pmpp.2017.12.007.

Cho, H. et al. (2019) ‘Prediction of Host-Specific Genes by Pan-Genome Analyses of the Korean Ralstonia solanacearum Species Complex’, Frontiers in Microbiology, 10. doi: 10.3389/fmicb.2019.00506.

Coll, N. S. and Valls, M. (2013) ‘Current knowledge on the Ralstonia solanacearum type III secretion system.’, Microbial biotechnology, 6(6), pp. 614–20. doi: 10.1111/1751-7915.12056.

Cunnac, S. et al. (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.’, Molecular Microbiology, 53(1), pp. 115–128. doi: 10.1111/j.1365-2958.2004.04118.x.
Cunnac, S. et al. (2011) ‘Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in Pseudomonas syringae’, *Proceedings of the National Academy of Sciences of the United States of America*, 108(7), pp. 2975–80. doi: 10.1073/pnas.1013031108.

Cunnac, S., Boucher, C. and 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’, *Journal of Bacteriology*, 186(8), pp. 2309–2318. doi: 10.1128/JB.186.8.2309-2318.2004.

Deng, M.-Y. et al. (2016) ‘The phytopathogenic virulent effector protein RipI induces apoptosis in budding yeast Saccharomyces cerevisiae’, *Toxicon: official journal of the International Society on Toxinology*, 121, pp. 109–118. doi: 10.1016/j.toxicon.2016.09.006.

Deslandes, L. et al. (1998) ‘Genetic characterization of RRS1, a recessive locus in Arabidopsis thaliana that confers resistance to the bacterial soilborne pathogen Ralstonia solanacearum.’, *Molecular plant-microbe interactions : MPMI*, 11(7), pp. 659–67. doi: 10.1094/MPMI.1998.11.7.659.

Deslandes, L. 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.’, *Proceedings of the National Academy of Sciences of the United States of America*. United States, 100(13), pp. 8024–9. doi: 10.1073/pnas.1230660100.

Dillon, M. M. et al. (2019) ‘Molecular Evolution of Pseudomonas syringae Type III Secreted Effector Proteins’, *Frontiers in Plant Science*, 10. doi: 10.3389/fpls.2019.00418.

Dobrenel, T. et al. (2016) ‘TOR Signaling and Nutrient Sensing’, *Annual Review of Plant Biology*, 67(1), pp. 261–285. doi: 10.1146/annurev-arplant-043014-114648.

Fujiwara, S. et al. (2016) ‘RipAY, a Plant Pathogen Effector Protein, Exhibits Robust γ-Glutamyl Cyclotransferase Activity When Stimulated by Eukaryotic Thioredoxins.’, *The
Fujiwara, S. et al. (2020) ‘Characterization of the mechanism of thioredoxin-dependent activation of γ-glutamylcyclotransferase, RipAY, from Ralstonia solanacearum’, Biochemical and Biophysical Research Communications, 523(3), pp. 759–765. doi: 10.1016/j.bbrc.2019.12.092.

Gabriel, D. W. et al. (2006) ‘Identification of open reading frames unique to a select agent: Ralstonia solanacearum race 3 biovar 2’, Molecular plant-microbe interactions : MPMI, 19(1), pp. 69–79. doi: 10.1094/MPMI-19-0069.

Gassmann, W., Hinsch, M. E. and Staskawicz, B. J. (1999) ‘The Arabidopsis RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes’, The Plant journal : for cell and molecular biology, 20(3), pp. 265–77. doi: 10.1046/j.1365-313x.1999.t01-1-00600.x.

Genin, S. et al. (1992) ‘Evidence that the hrpB gene encodes a positive regulator of pathogenicity genes from Pseudomonas solanacearum’, Molecular microbiology, 6(20), pp. 3065–76. doi: 10.1111/j.1365-2958.1992.tb01764.x.

Genin, S. and Denny, T. P. (2012) ‘Pathogenomics of the Ralstonia solanacearum species complex’, Annual review of phytopathology, 50, pp. 67–89. doi: 10.1146/annurev-phyto-081211-173000.

Ghosh, S. and O’Connor, T. J. (2017) ‘Beyond Paralogs: The Multiple Layers of Redundancy in Bacterial Pathogenesis.’, Frontiers in cellular and infection microbiology, 7, p. 467. doi: 10.3389/fcimb.2017.00467.

González-Fuente, M. et al. (2019) ‘EffectorK, a comprehensive resource to mine for pathogen effector targets in the Arabidopsis proteome.’, bioRxiv, p. 2019.12.16.878074. doi: 10.1101/2019.12.16.878074.
Guéneron, M. et al. (2000) ‘Two novel proteins, PopB, which has functional nuclear localization signals, and PopC, which has a large leucine-rich repeat domain, are secreted through the hrp-secretion apparatus of Ralstonia solanacearum’, Molecular microbiology, 36(2), pp. 261–77. doi: 10.1046/j.1365-2958.2000.01870.x.

Henry, E. et al. (2017) ‘Direct and Indirect Visualization of Bacterial Effector Delivery into Diverse Plant Cell Types during Infection’, The Plant Cell, 29(7), pp. 1555–1570. doi: 10.1105/tpc.17.00027.

Jacobs, J. M. et al. (2013) ‘Ralstonia solanacearum Requires PopS, an Ancient AvrE-Family Effector, for Virulence and To Overcome Salicylic Acid-Mediated Defenses during Tomato Pathogenesis’, mBio, 4(6), pp. 1–12. doi: 10.1128/mBio.00875-13.

Jeon, H. et al. (2020) ‘Ralstonia solanacearum Type III Effectors with Predicted Nuclear Localization Signal Localize to Various Cell Compartments and Modulate Immune Responses in Nicotiana spp.’, The plant pathology journal, 36(1), pp. 43–53. doi: 10.5423/PPJ.OA.08.2019.0227.

Jiang, Y. et al. (2016) ‘First report of bacterial wilt caused by Ralstonia solanacearum on fig trees in China.’, Forest Pathology. Edited by S. Woodward, 46(3), pp. 256–258. doi: 10.1111/efp.12267.

Kubori, T. et al. (2010) ‘Legionella Metaeffector Exploits Host Proteasome to Temporarily Regulate Cognate Effector’, PLoS Pathogens. Edited by C. E. Stebbins, 6(12), p. e1001216. doi: 10.1371/journal.ppat.1001216.

de Lange, O. et al. (2013) ‘Breaking the DNA-binding code of Ralstonia solanacearum TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease’, New Phytologist, 199(3), pp. 773–786. doi: 10.1111/nph.12324.

Lavie, M. et al. (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
Lonjon, F. et al. (2016) ‘Comparative Secretome Analysis of Ralstonia solanacearum Type 3 Secretion-Associated Mutants Reveals a Fine Control of Effector Delivery, Essential for Bacterial Pathogenicity’, Molecular & Cellular Proteomics, 15(2), pp. 598–613. doi: 10.1074/mcp.M115.051078.

Lonjon, F. et al. (2018) ‘In Vitro and In Vivo Secretion/Translocation Assays to Identify Novel Ralstonia solanacearum Type 3 Effectors’, in, pp. 209–222. doi: 10.1007/978-1-4939-7604-1_17.

Lopes, C. A., Rossato, M. and Boiteux, L. S. (2015) ‘The Host Status Of Coffee (Coffea arabica) To Ralstonia solanacearum Phylotype I Isolates’, Tropical Plant Pathology, 40(1), pp. 1–4. doi: 10.1007/s40858-014-0001-9.

Lowe-Power, T. M. et al. (2018) ‘Metabolomics of tomato xylem sap during bacterial wilt reveals Ralstonia solanacearum produces abundant putrescine, a metabolite that accelerates wilt disease’, Environmental microbiology, 20(4), pp. 1330–1349. doi: 10.1111/1462-2920.14020.

Macho, A. P. et al. (2010) ‘A competitive index assay identifies several Ralstonia solanacearum type III effector mutant strains with reduced fitness in host plants’, Molecular plant-microbe interactions : MPMI, 23(9), pp. 1197–205. doi: 10.1094/MPMI-23-9-1197.

Macho, A. P. (2016) ‘Subversion of plant cellular functions by bacterial type-III effectors: beyond suppression of immunity.’, New Phytologist, 210(1), pp. 51–57. doi: 10.1111/nph.13605.

Mansfield, J. et al. (2012) ‘Top 10 plant pathogenic bacteria in molecular plant pathology.’, Molecular plant pathology, 13(6), pp. 614–29. doi: 10.1111/j.1364-3703.2012.00804.x.

Mazo-Molina, C. et al. (2019) ‘The Ptr1 Locus of Solanum lycopersicoides Confers
Resistance to Race 1 Strains of Pseudomonas syringae pv. tomato and to Ralstonia pseudosolanacearum by Recognizing the Type III Effectors AvrRpt2 and RipBN’, *Molecular Plant-Microbe Interactions*, 32(8), pp. 949–960. doi: 10.1094/MPMI-01-19-0018-R.

Medina-Puche, L. *et al.* (2019) ‘A novel pathway linking plasma membrane and chloroplasts is co-opted by pathogens to suppress salicylic acid-dependent defences’, *bioRxiv*, p. 837955. doi: 10.1101/837955.

Meyer, D. *et al.* (2006) ‘PopF1 and PopF2, two proteins secreted by the type III protein secretion system of *Ralstonia solanacearum*, are translocators belonging to the HrpF/NopX family’, *Journal of bacteriology*, 188(13), pp. 4903–17. doi: 10.1128/JB.00180-06.

Morel, A. *et al.* (2018) ‘The eggplant AG91-25 recognizes the Type III-secreted effector RipAX2 to trigger resistance to bacterial wilt (*Ralstonia solanacearum* species complex).’, *Molecular plant pathology*, pp. 0–3. doi: 10.1111/mpp.12724.

Mukaihara, T. *et al.* (2004) ‘Genetic screening of Hrp type III-related pathogenicity genes controlled by the HrpB transcriptional activator in *Ralstonia solanacearum*’, *Molecular Microbiology*, 54(4), pp. 863–875. doi: 10.1111/j.1365-2958.2004.04328.x.

Mukaihara, T. *et al.* (2016) ‘*Ralstonia solanacearum* Type III Effector RipAY Is a Glutathione-Degrading Enzyme That Is Activated by Plant Cytosolic Thioredoxins and Suppresses Plant Immunity’, *mBio*, 7(2). doi: 10.1128/mBio.00359-16.

Mukaihara, T. and Tamura, N. (2009) ‘Identification of novel *Ralstonia solanacearum* type III effector proteins through translocation analysis of hrpB-regulated gene products’, *Microbiology*, 155(7), pp. 2235–2244. doi: 10.1099/mic.0.027763-0.

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’, *Molecular Plant-Microbe Interactions*, 23(3), pp. 251–262. doi: 10.1094/MPMI-23-3-0251.
Nahar, K. et al. (2014) ‘*Ralstonia solanacearum* type III secretion system effector Rip36 induces a hypersensitive response in the nonhost wild eggplant *Solanum torvum*, *Molecular Plant Pathology*, 15(3), pp. 297–303. doi: 10.1111/mpp.12079.

Nakano, M. and Mukaihara, T. (2018) ‘*Ralstonia solanacearum* Type III Effector RipAL Targets Chloroplasts and Induces Jasmonic Acid Production to Suppress Salicylic Acid-Mediated Defense Responses in Plants’, *Plant & cell physiology*, 59(12), pp. 2576–2589. doi: 10.1093/pcp/pcy177.

Nakano, M. and Mukaihara, T. (2019a) ‘Comprehensive Identification of PTI Suppressors in Type III Effector Repertoire Reveals that *Ralstonia solanacearum* Activates Jasmonate Signaling at Two Different Steps’, *International journal of molecular sciences*, 20(23). doi: 10.3390/ijms20235992.

Nakano, M. and Mukaihara, T. (2019b) ‘The type III effector RipB from *Ralstonia solanacearum* RS1000 acts as a major avirulence factor in *Nicotiana benthamiana* and other *Nicotiana* species.’, *Molecular plant pathology*, 20(9), pp. 1237–1251. doi: 10.1111/mpp.12824.

Nakano, M., Oda, K. and Mukaihara, T. (2017) ‘*Ralstonia solanacearum* novel E3 ubiquitin ligase (NEL) effectors RipAW and RipAR suppress pattern-triggered immunity in plants’, *Microbiology*, 163(7), pp. 992–1002. doi: 10.1099/mic.0.000495.

Narusaka, M. et al. (2009) ‘RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens’, *The Plant Journal*, 60(2), pp. 218–226. doi: 10.1111/j.1365-313X.2009.03949.x.

Occhialini, A. et al. (2005) ‘Genome-Wide Analysis of Gene Expression in *Ralstonia solanacearum* Reveals That the *hrpB* Gene Acts as a Regulatory Switch Controlling Multiple Virulence Pathways’, *Molecular Plant-Microbe Interactions*, 18(9), pp. 938–949. doi: 10.1094/MPMI-18-0938.
Peeters, N., Guidot, A., et al. (2013) ‘Ralstonia solanacearum, a widespread bacterial plant pathogen in the post-genomic era.’, *Molecular Plant Pathology*, 14(7), pp. 651–662. doi: 10.1111/mpp.12038.

Peeters, N., Carrère, S., et al. (2013) ‘Repertoire, unified nomenclature and evolution of the Type III effector gene set in the Ralstonia solanacearum species complex.’, *BMC genomics*, 14, p. 859. doi: 10.1186/1471-2164-14-859.

Popa, C. *et al.* (2016) ‘The effector AWR5 from the plant pathogen *Ralstonia solanacearum* is an inhibitor of the TOR signalling pathway’, *Scientific Reports*, 6(1), p. 27058. doi: 10.1038/srep27058.

Poueymiro, M. *et al.* (2009) ‘Two type III secretion system effectors from *Ralstonia solanacearum* GMI1000 determine host-range specificity on tobacco’, *Molecular plant-microbe interactions : MPMI*, 22(5), pp. 538–50. doi: 10.1094/MPMI-22-5-0538.

Poueymiro, M. *et al.* (2014) ‘A *Ralstonia solanacearum* Type III Effector Directs the Production of the Plant Signal Metabolite Trehalose-6-Phosphate’, *mBio*. Edited by D. Buettner and R. Kahmann, 5(6), pp. 1–9. doi: 10.1128/mBio.02065-14.

Racapé, J. *et al.* (2005) ‘Ca2+-dependent lipid binding and membrane integration of PopA, a harpin-like elicitor of the hypersensitive response in tobacco’, *Molecular microbiology*, 58(5), pp. 1406–20. doi: 10.1111/j.1365-2958.2004.04910.x.

Remigi, P. *et al.* (2011) ‘Functional diversification of the GALA type III effector family contributes to Ralstonia solanacearum adaptation on different plant hosts’, *New Phytologist*, 192(4), pp. 976–987. doi: 10.1111/j.1469-8137.2011.03854.x.

Robertson, A. E. *et al.* (2004) 'Relationship Between Avirulence Gene (<i>avrA</i>) Diversity in *Ralstonia solanacearum* and Bacterial Wilt Incidence', *Molecular Plant-Microbe Interactions*, 17(12), pp. 1376–1384. doi: 10.1094/MPMI.2004.17.12.1376.

Roux, B. *et al.* (2015) ‘Genomics and transcriptomics of *Xanthomonas campestris* species
challenge the concept of core type III effectome’, *BMC genomics*. BMC Genomics, 16(1), p. 975. doi: 10.1186/s12864-015-2190-0.

Le Roux, C. C. *et al.* (2015) ‘A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity’, *Cell*. United States, 161(5), pp. 1074–1088. doi: 10.1016/j.cell.2015.04.025.

Sabbagh, C. R. R. *et al.* (2019) ‘Pangenomic type III effector database of the plant pathogenic *Ralstonia* spp.’, *PeerJ*, 7, p. e7346. doi: 10.7717/peerj.7346.

Salanoubat, M. *et al.* (2002) ‘Genome sequence of the plant pathogen *Ralstonia solanacearum*’, *Nature*, 415(6871), pp. 497–502. doi: 10.1038/415497a.

Sang, Y. *et al.* (2018) ‘The *Ralstonia solanacearum* type III effector RipAY targets plant redox regulators to suppress immune responses’, *Molecular Plant Pathology*, 19(1), pp. 129–142. doi: 10.1111/mpp.12504.

Sang, Y. *et al.* (2020) ‘Intra-strain elicitation and suppression of plant immunity by *Ralstonia solanacearum* type-III effectors in *Nicotiana benthamiana*’, *Plant Communications*. Elsevier Ltd, p. 100025. doi: 10.1016/j.xplc.2020.100025.

Sang, Y. and Macho, A. P. (2017) ‘Analysis of PAMP-Triggered ROS Burst in Plant Immunity.’, *Methods in molecular biology (Clifton, N.J.)*, 1578, pp. 143–153. doi: 10.1007/978-1-4939-6859-6_11.

Sarris, P. F. *et al.* (2015) ‘A Plant Immune Receptor Detects Pathogen Effectors that Target WRKY Transcription Factors.’, *Cell*. United States, 161(5), pp. 1089–1100. doi: 10.1016/j.cell.2015.04.024.

Solé, M. *et al.* (2012) ‘The awr Gene Family Encodes a Novel Class of *Ralstonia solanacearum* Type III Effectors Displaying Virulence and Avirulence Activities’, *Molecular Plant-Microbe Interactions*, 25(7), pp. 941–953. doi: 10.1094/MPMI-12-11-0321.

Sun, T. *et al.* (2020) ‘*Ralstonia solanacearum* elicitor RipX Induces Defense Reaction by
 Suppressing the Mitochondrial \textit{atpA} Gene in Host Plant’, \textit{International Journal of Molecular Sciences}, 21(6), p. 2000. doi: 10.3390/ijms21062000.

Sun, Y. \textit{et al.} (2017) ‘The \textit{Ralstonia solanacearum} effector RipAK suppresses plant hypersensitive response by inhibiting the activity of host catalases’, \textit{Cellular Microbiology}, 19(8), p. e12736. doi: 10.1111/cmi.12736.

Sun, Y. \textit{et al.} (2019) ‘The \textit{Ralstonia solanacearum} effector RipN suppresses plant PAMP-triggered immunity, localizes to the endoplasmic reticulum and nucleus, and alters the NADH/NAD$^+$ ratio in Arabidopsis’, \textit{Molecular Plant Pathology}, 20(4), pp. 533–546. doi: 10.1111/mpp.12773.

Tamura, N., Murata, Y. and Mukaihara, T. (2005) ‘Isolation of \textit{Ralstonia solanacearum hrpB} constitutive mutants and secretion analysis of \textit{hrpB}-regulated gene products that share homology with known type III effectors and enzymes’, \textit{Microbiology}, 151(9), pp. 2873–2884. doi: 10.1099/mic.0.28161-0.

Tasset, C. C. \textit{et al.} (2010) ‘Autoacetylation of the \textit{Ralstonia solanacearum} effector PopP2 targets a lysine residue essential for RRS1-R-mediated immunity in Arabidopsis.’, \textit{PLoS pathogens}. United States, 6(11), p. e1001202. doi: 10.1371/journal.ppat.1001202.

Tjou-Tam-Sin, N. N. A. \textit{et al.} (2017) ‘First Report of Bacterial Wilt Caused by \textit{Ralstonia solanacearum} in Ornamental Rosa sp.’, \textit{Plant Disease}, 101(2), pp. 378–378. doi: 10.1094/PDIS-02-16-0250-PDN.

Toruño, T. Y., Stergiopoulos, I. and Coaker, G. (2016) ‘Plant-Pathogen Effectors: Cellular Probes Interfering with Plant Defenses in Spatial and Temporal Manners.’, \textit{Annual Review of Phytopathology}, 54(1), pp. 419–441. doi: 10.1146/annurev-phyto-080615-100204.

Turner, M. \textit{et al.} (2009) ‘Dissection of bacterial Wilt on \textit{Medicago truncatula} revealed two type III secretion system effectors acting on root infection process and disease development’, \textit{Plant physiology}, 150(4), pp. 1713–22. doi: 10.1104/pp.109.141523.
Vasse, J. et al. (2000) ‘The hrpB and hrpG Regulatory Genes of *Ralstonia solanacearum* Are Required for Different Stages of the Tomato Root Infection Process’, *Molecular Plant-Microbe Interactions*, 13(3), pp. 259–267. doi: 10.1094/MPMI.2000.13.3.259.

Wang, K. et al. (2016) ‘Functional assignment to positively selected sites in the core type III effector RipG7 from *Ralstonia solanacearum*’, *Molecular plant pathology*, 17(4), pp. 553–64. doi: 10.1111/mpp.12302.

Weibel, J. et al. (2016) ‘A Ralstonia solanacearum Strain from Guatemala Infects Diverse Flower Crops, Including New Asymptomatic Hosts Vinca and Sutera, and Causes Symptoms in Geranium, Mandevilla Vine, and New Host African Daisy (*Osteospermum ecklonis*)’, *Plant Health Progress*, 17(2), pp. 114–121. doi: 10.1094/PHP-RS-16-0001.

Wicker, E. et al. (2007) ‘*Ralstonia solanacearum* Strains from Martinique (French West Indies) Exhibiting a New Pathogenic Potential’, *Applied and Environmental Microbiology*, 73(21), pp. 6790–6801. doi: 10.1128/AEM.00841-07.

Williams, S. J. et al. (2014) ‘Structural Basis for Assembly and Function of a Heterodimeric Plant Immune Receptor’, *Science*, 344(6181), pp. 299–303. doi: 10.1126/science.1247357.

Wroblewski, T. et al. (2009) ‘Comparative large-scale analysis of interactions between several crop species and the effector repertoires from multiple pathovars of *Pseudomonas* and *Ralstonia*’, *Plant physiology*, 150(4), pp. 1733–49. doi: 10.1104/pp.109.140251.

Wu, D. et al. (2019) ‘A Plant Pathogen Type III Effector Protein Subverts Translational Regulation to Boost Host Polyamine Levels’, *Cell Host & Microbe*, 26(5), pp. 638-649.e5. doi: 10.1016/j.chom.2019.09.014.

Yu, G. et al. (2020) ‘A bacterial effector protein prevents MAPK-mediated phosphorylation of SGT1 to suppress plant immunity’, *bioRxiv*, p. 641241. doi: 10.1101/641241.

Zheng, X. et al. (2019) ‘A systematic screen of conserved *Ralstonia solanacearum* effectors...
reveals the role of RipAB, a nuclear-localized effector that suppresses immune responses in potato’, *Molecular plant pathology*, 20(4), pp. 547–561. doi: 10.1111/mpp.12774.

Zhuo, T. *et al.* (2020) ‘The *Ralstonia solanacearum* effector RipI induces a defence reaction by interacting with the bHLH93 transcription factor in *Nicotiana benthamiana*,’ *Molecular Plant Pathology*. doi: 10.1111/mpp.12937.

**Table and figure legends**

**Table 1.** List of functionally characterized *Ralstonia solanacearum* species complex type III effectors.

**Figure 1.** *Ralstonia solanacearum* species complex (RSSC) bacteria deploy an arsenal of type III effectors (T3Es) to alter the plant metabolism and interfere with plant immune responses.

During the infection process, conserved bacterial molecules are recognized by plant Pattern Recognition Receptors (PRRs) at the surface of the host cell. They activate basal defence responses to prevent pathogen proliferation. However, RSSC bacteria translocate T3Es into the plant cell to subvert the plant defences and accommodate the bacterial needs. T3Es act on different host pathways. RipAY and RipN alter the glutathione level and NADH/NAD$^+$ ratio respectively. RipAY, RipR, RipAL, RipG1 and RipG3 target at the hormone synthesis and signalling level. Different RipG family members, RipAR and RipAW interfere with ubiquitination processes. The metabolism is also manipulated by RSSC T3Es. RipA5, RipTPS and RipTAL are able to modulate certain metabolic pathways. RipTAL binds to the plant DNA activating the expression of shorter and more efficiently translated transcripts of *arginine decarboxylase* (*ADC*) genes, key enzymes in the biosynthesis of polyamines. This boost in the polyamine level could prevent the proliferation of *Ralstonia* niche competitors. RipP2 relies on its acetyltransferase activity to acetylate defensive WRKY transcription factors, inhibiting their DNA-binding activities and preventing subsequent expression of defence-related genes. The
nuclear T3E RipAB inhibits the expression of Ca$_{2+}$-related defence genes. In addition to these functionally characterized RSSC T3Es, other effectors involved in dampening of basal defence through yet unknown mechanisms have been identified: RipAR, RipAW, RipG family, RipAB, RipA5, RipAD, RipAF1, RipD, RipE1, RipI, RipQ, RipAC, RipAP, RipAU, RipH1, RipM, RipS1, RipAN and RipB. RSSC T3Es can also be perceived in planta by intracellular immune-Nod-Like Receptors (NLRs) leading to the activation of specific defence mechanisms, often associated with a hypersensitive response (HR). RipE1, RipAA, RipP1, RipX, RipP2, RipAT, RipAV, RipA1-A5, RipTPS, RipAX2, RipAB, RipB, RipBN and RipI also induce HR responses on several hosts. Some T3Es can modulated the activity of others and prevent their recognition by the plant surveillance system. Indeed, peroxisome-localized RipAK suppresses effector-triggered HR responses by inhibiting host catalases activities (CATs). RipAY and RipAC inhibit RipE1-mediated HR.
| Effector\(^a\) | Functional annotation\(^b\) | Homolog\(^c\) | Subcellular localization | PTI inhibition\(^d\) | Description | Reference(s) |
|----------------|-----------------------------|-------------|--------------------------|---------------------|-------------|--------------|
| RipA (AWR) family | Cytoplasm (RipA1 and RipA4 also plasmamembrane) | | (+) | Collective contribution to virulence in eggplant and tomato and negative contribution to virulence in *A. thaliana* | Cunnac et al. (2004), Solé et al. (2012) |
| RipA 1 | Cytoplasm and plasmamembrane | | | Cell death in *N. benthamiana* | Solé et al. (2012), Jeon et al. (2020) |
| RipA 2 | Cytoplasm | | | Major contribution to virulence in tomato, eggplant and Arabidopsis and cell death in different *Nicotiana* spp. | Cunnac et al. (2004), Solé et al. (2012) |
| RipA 4 | Cytoplasm | | | Cell death in *N. glutinosa* | Solé et al. (2012) |
| RipA 5 | Cytoplasm | | + | Inhibition of TOR-pathway in yeast and in *N. benthamiana*, negative contribution to virulence in *A. thaliana* and cell death in different *Nicotiana* spp. | Solé et al. (2012), Popa et al. (2016), Nakano & Mukaihara (2019a) |
| RipA A (Avr A) | Cytoplasm | | | Cell death in pepper and different *Nicotiana* spp., contribution to virulence in *M. truncatula* and bacterial fitness in tomato | Macho et al. (2010), Nakano & Mukaihara (2019a), Yu et al. (2020) |
| RipA B | Nucleus | | + | Contribution to virulence in potato and cell death in *N. benthamiana* only when localized in the nucleus | Zheng et al. (2019) |
| RipA C (Pop C) | Nucleus | | + | Contribution to virulence in *A. thaliana* and tomato and bacterial fitness in eggplant and suppression of SGT1-dependent immune response in *A. thaliana* and *N. benthamiana* | Macho et al. (2010), Nakano & Mukaihara (2019a), Yu et al. (2020) |
| RipA D | Cytoplasm and chloroplasts | | + | Inhibition of flg22-induced ROS production in *N. benthamiana* | Jeon et al. (2020) |
| RipA | F1 | Putative ADP-ribosyl transferase | HopF2 (P) | Nucleus and cytoplasm | + | Contribution to bacterial fitness in eggplant and inhibition of flg22-induced ROS production in *N. benthamiana* | Macho et al. (2010), Jeon et al. (2020) |
|------|----|---------------------------------|----------|-----------------------|---|----------------------------------------------------------------|----------------------------------|
| RipA | K | Peroxisomes | HopK (P) | Nucleus and cytoplasm | + | Contribution to bacterial fitness in eggplant and inhibition of plant catalase activity to inhibit plant defence responses in *A. thaliana* and *N. tabacum* | Macho et al. (2010), Sun et al. (2017) |
| RipA | L | Putative lipase domain | Lipase (X) | Chloroplasts | + | Induction of JA production to inhibit SA signalling in *A. thaliana* and pepper | Nakano & Mukaihara (2018, 2019a) |
| RipA | M | Peroxisomes | HopM (P) | Nucleus and cytoplasm | + | Contribution to virulence in potato | Zheng et al. (2019) |
| RipA | N | Peroxisomes | HopN (P) | Nucleus and cytoplasm | + | Contribution to virulence in potato | Zheng et al. (2019), Nakano & Mukaihara (2019a) |
| RipA | P | Ankyrin repeats | HopP (P) | Nucleus and cytoplasm | + | Inhibition of flg22-induced ROS production in *N. benthamiana* | Nakano & Mukaihara (2019a) |
| RipA | R | Ubiquitin ligase domain | Cytoplasm | Nucleus and cytoplasm | + | Inhibition of PTI depending on its E3 ubiquitin ligase activity | Nakano et al. (2016) |
| RipA | T | Ubiquitin ligase domain | Cytoplasm | Nucleus and cytoplasm | + | HR in lettuce and certain pepper and tomato cultivars | Wroblewski et al. (2009) |
| RipA | U | Ubiquitin ligase domain | Cytoplasm | Nucleus and cytoplasm | + | Inhibition of flg22-induced ROS production in *N. benthamiana* | Nakano & Mukaihara (2019a) |
| RipA | V | Ubiquitin ligase domain | Cytoplasm | Nucleus and cytoplasm | + | Contribution to bacterial fitness in eggplant and HR in lettuce and certain pepper and tomato cultivars | Wroblewski et al. (2009), Macho et al. (2010) |
| RipA | W | Ubiquitin ligase domain | Cytoplasm | Nucleus and cytoplasm | + | Inhibition of PTI depending on its E3 ubiquitin ligase activity | Nakano et al. (2016, 2019a) |
| RipA | X2 (Rip 36) | Zn-binding motif | HopH1 (P), XopG (X) | Nucleus and cytoplasm | + | Avirulence in wild and cultivated eggplant | Nahar et al. (2014), Morel et al. (2018) |
| RipA | Y | γ-glutamyl cyclotransferases | HopY (P), XopH (X) | Nucleus and cytoplasm | + | Contribution to bacterial fitness in eggplant, depletion of glutathione in yeast, eggplant and *A. thaliana*, inhibition of SA-mediated defences in *A. thaliana* and *N. benthamiana* and suppression of RipE1-mediated HR in *N. benthamiana* | Macho et al. (2010), Fujiwara et al. (2016, 2020), Mukaihara et al. (2016), Sang et al. (2018, 2020) |
| RipB | Inosineuridine nucleoside N-ribosyl | HopB (P), XopQ (X) | Nucleus and cytoplasm | Nucleus and cytoplasm | + | Roq1-mediated resistance | Nakano & Mukaihara (2019b) |
| Rip         | Description                                                                 | Function                                                                 | Reference                                      |
|-------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------|
| RipB H     | EspL2 (Sa), SheET2 (Y)                                                       | Contribution to virulence in potato                                        | Zheng et al. (2019)                           |
| RipB N     | AvrRp2 (P)                                                                   | Ptr1-mediated resistance                                                 | Mazo-molina et al. (2019)                     |
| RipD        | HopD 1 (P), XopB (X)                                                         | Contribution to bacterial fitness in eggplant, tomato and bean and inhibition of flag22-induced ROS production in *N. benthamiana* | Macho et al. (2010), Jeon et al. (2020)       |
| RipE 1     | HopX 1 (P), XopE (X)                                                         | Induction of SA and JA synthesis to trigger immunity in *N. benthamiana* and *A. thaliana* | Nakano & Mukaihara (2019a), Sang et al. (2020) |
| RipF 1 (Pop F1) | NopX (B/Si)                                                                 | Important for the translocation of effector                               | Meyer et al. (2006)                           |
| RipF 2 (Pop F2) | NodX (B/Si)                                                                 | Important for the translocation of effector                               | Meyer et al. (2006)                           |
| Rip G1     | F-box                                                                       | Collective contribution to virulence in *A. thaliana*, tomato and eggplant, interaction with SKP1-like proteins (except RipG3 and RipG4) | Angot et al. (2006), Remigi et al. (2011), Wang et al. (2016) |
| Rip G3     | F-box and N-myristoylation domains                                             | Inhibition of flag22-induced SA-dependent defence responses in *N. benthamiana* and *A. thaliana* | Medina-Puche et al. (2019)                     |
| Rip G4     | F-Box                                                                        | Inhibition of callose deposition in *A. thaliana*                          | Remigi et al. (2011)                          |
| Rip G7     | F-Box                                                                        | Essential for virulence in late stages of infection in *M. truncatula*. Interaction with *A. thaliana* ASK1,2, 11 and 13 and *M. truncatula* MSKa | Angot et al. (2006), Turner et al. (2009), Wang et al. (2016) |
| Rip H (HL K) | XopP (X)                                                                    | Collective contribution to virulence in tomato                             | Chen et al. (2013), Nakano & Mukaihara (2019a) |
| Family | Domain | Nucleus | Cell death in yeast and N. benthamiana, through interaction with bHLH93 transcription factor, and immune responses in tomato | Deng et al. (2016), Nakano & Mukaihara (2019a), Zhuo (2020) |
|--------|--------|---------|---------------------------------------------------------------------------------|-------------------------------------------------------------|
| RipM   | Nudix hydrolase domain | Nucleus and endoplasmic reticulum | Inhibition of flg22-induced ROS production in N. benthamiana | Nakano & Mukaihara (2019a) |
| RipN   | Putative acetyltransferase | Nucleus | Alteration of the plant NADH/NAD+ ratio and suppression of PTI-defences in A. thaliana | Sun et al. (2019) |
| RipP 1 (Pop P1) | Acetyltransferase | HopZ 2 (P), XopJ 4 (X) | Avirulence factor in different Nicotiana spp. (major contribution in N. glutinosa) and in Petunia lines | Lavie et al. (2002), Pouemyro et al. (2009), Chen et al. (2018) |
| RipP 2 (Pop P2) | Acetyltransferase | AvrA (Sa), HopZ 4 (P), VopA (V), Yop J (Y) | Acetylation of WRKY transcription factors to inhibit PTI-defences and RRS1-R to induce ETI in A. thaliana, avirulence factor in eggplant and contribution to virulence in Arabidopsis and to bacterial fitness in tomato, eggplant and bean | Deslandes et al. (2003), Tasset et al. (2010), Macho et al. (2010), Le Roux et al. (2015), Sarris et al. (2015), Xi'ou et al. (2015) |
| RipQ   | Hop A 1 (P) | + | Inhibition of flg22-induced ROS production in N. benthamiana | Nakano & Mukaihara (2019a) |
| RipR (Pop S) | AvrE/ HopR 1 (P), DspA/E (E), XopAM (X) | | Inhibition of SA-dependent defences and contribution to virulence in Solanum spp. and to bacterial fitness in eggplant | Macho et al. (2010), Jacobs et al. (2013) |
| RipS 1 (SK WP1) | XopA D (X) | + | Inhibition of flg22-induced ROS production in N. benthamiana | Nakano & Mukaihara (2019a) |
| RipS 4 (SK WP4) | XopA D (X) | + | Contribution to bacterial fitness in eggplant | Macho et al. (2010) |
| RipT AL (Brg 11) | Transcription Activator-Like Protein | AvrBs 3/TAL family (X) | Specific binding on DNA from different hosts and induction of synthesis of polyamines in Solanum spp., possibly to inhibit the proliferation of competitors, and contribution to bacterial fitness in eggplant | Macho et al. (2010), de Lange et al. (2013), Wu et al. (2019) |
| RipT PS | Trehalose-6-phosphate synthase | Trehalose-6-phosphate synthase | Synthesis of trehalose-6-phosphate in yeast and enzymatic activity-independent HR in N. tabacum | Pouemyro et al. (2014) |
| Protein | Description | Function | References |
|---------|-------------|----------|------------|
| RipX (Pop A) | Hairpin like protein | Nucleus and plasma membrane | Contribution to bacterial fitness in eggplant | Arlat et al. (1994), Belbahri et al. (2001), Racapé et al. (2005), Sun et al. (2020) |
| RipY | | HR in petunia, N. tabacum and N. benthamiana by affecting negatively the transcription of atpA gene for the latter and formation of ion-conducting pores in vitro | Macho et al. (2010) |

- Former name in brackets.
- Proven or putative functional annotation.
- Homologs characterized in other bacterial genera (A: Acidovorax, B: Bradyrhizobium, E: Erwinia, P: Pseudomonas, Sa: Salmonella, Si: Sinorhizobium, V: Vibrio, X: Xanthomonas and Y: Yersinia).
- Indicated only when the ability to inhibit any classical PAMP-triggered immunity (PTI) response has been proven. In brackets when only some members of a paralog T3E family members inhibit PTI responses.