### Ralstonia solanacearum, a widespread bacterial plant pathogen in the post-genomic era

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**Ralstonia solanacearum**

**Taxonomy:** Bacteria; Proteobacteria; β subdivision; Ralstonia group; genus Ralstonia

**Microbiological properties:** Gram-negative, aerobic, motile rod

**Disease symptoms:** Agent of bacterial wilt of solanaceous plants characterized by a sudden wilt of the whole plant. Typically, stem cross-sections oozes a slimy bacterial exudate. *R. solanacearum* is also the agent of Moko disease of banana and brown rot of potato.

**Disease control:** Pathogen-free seed and transplants. Existence of a few resistant and tolerant plant varieties. Prophylactic sanitation practices and cultural rotations.

Since the last *Ralstonia solanacearum* pathogen profile was published ten years ago (Genin & Boucher, 2002), the studies concerning this plant pathogen have definitely taken the genomic and post-genomic avenue. This was pioneered by the first sequenced and annotated genome for a major plant bacterial pathogen (Salanoubat et al., 2002) and followed by many more genomes in the years after. All molecular features studied have now a genomic flavor. In the future, this will help to connect the classical field pathology and diversity studies with gene content of specific strains. This contributes to a global understanding of *R. solanacearum* virulence mechanisms. Figure 1 highlights some features specific to *R. solanacearum* and also displays different plant bioassays.

A classification in four phylotypes

In pace with the development of molecular tools, classification of *R. solanacearum* has undergone many changes during the past 10 years. In 2005, Fegan and Prior proposed a new hierarchical classification based upon the analysis of the sequence of the ITS (Internal Transcribed Spacer) region, the *hrpB* and the endoglucanase (*egl*) genes. The analysis of 140 *R. solanacearum* strains isolated from all over the world revealed a subdivision of the species into four phylotypes, which are correlated with the strains' geographical origins. Phylotype I includes strains originating primarily from Asia, Phylotype II those from America, Phylotype III those from Africa, and Phylotype IV those from Indonesia, Australia and Japan. This phylotype IV also contains the two close relatives of *R. solanacearum*: *Ralstonia syzygii* and the blood disease bacterium (BDB) strains. A multiplex PCR based upon sequence information from the ITS region has been developed to rapidly identify the phylotype to which a strain belongs (Fegan & Prior, 2005). Each phylotype can be further subdivided in groups of strains named sequevars, or sequence variants, according to the egl nucleotidic sequence. More than 50 sequevars have been defined so far.

This classification was confirmed by comparative genomic hybridization of a set of 18 strains, representing the biodiversity of *R. solanacearum* on a microarray representative of the GMI1000 reference strain genome (Guidot et al., 2007). Genomic data for nine new *R. solanacearum* strains also confirmed this classification (Remenant et al., 2010, Remenant et al., 2011, Remenant et al., 2012). Thanks to the overwhelming phylogenetic data on phylotype II strains, it has been...
suggested that this phylotype should be divided in two subgroups IIA and IIB (Castillo & Greenberg, 2007, Cellier et al., 2012). One can’t exclude that deeper sampling in the other phylotypes may also result in a similar refinement of classification.

The geographic isolation, and not host preference, has been the main driver of the separation of *R. solanacearum* strains into four phylotypes (Castillo & Greenberg, 2007, Wicker et al., 2012). Using coalescent genealogy reconstruction, Wicker et al. (2012) suggested that *R. solanacearum* originated from the Australian/Indonesian region where phylotype IV strains are found. A subgroup of the ancestral strains from this region, from the Australian/Indonesian region, a subgroup of the ancestral strains probably spread throughout the present Austral-Eastern Africa and Madagascar, and differentiated later in phylotype III and phylotype I (predicted with an East African/Asian origin). Another subgroup of ancestral strains migrated to the actual Brazil and differentiated later into the subgroups IIA and IIB at a time similar to that of the phylotype I/III differentiation, possibly before the fragmentation of Gondwana (Castillo & Greenberg, 2007, Wicker et al., 2012).

A species complex, let’s keep it simple

A species complex is defined as a cluster of closely-related isolates whose individual members may represent more than one species. The term ‘species complex’ was first applied to *R. solanacearum* by Gillings et al. (1993) to reflect the phenotypic and genotypic variability within the species. Taghavi et al. (1996) then confirmed the concept of the *R. solanacearum* species complex by including *R. syzygii* and BDB strains into the *R. solanacearum* phylogeny. Studies of DNA-DNA similarity revealed that the relatedness between *R. solanacearum* isolates is often just under the 70% threshold level commonly expected within a species (Roberts et al., 1990). By comparing different strains to the phylotype I strain GMI1000 by microarray hybridization, the most divergent strains still have 68-69% of their genes hybridizing with the GMI1000 oligonucleotides (Guidot et al, 2007). More recently, Remenant et al. (2010; 2011) used Average Nucleotide Identity (ANI) to evaluate genetic distances between the eight sequenced genomes from this species complex, calculate genomic distances between all sequenced genomes in the species complex. From the results, based on the ANI>95% cut-off (Konstantinidis & Tiedje, 2005, Konstantinidis et al., 2006) the authors suggested that the *R. solanacearum* species complex should be restructured into three different species: one containing phylotype I and III, a second containing phylotype II, and a third containing phylotype IV including *R. syzygii* and BDB strains (Remenant et al., 2011). The ANI provides a more robust and accurate measurement of the genetic distance than the DNA-DNA hybridization (Konstantinidis & Tiedje, 2005). However, as pointed out by Konstantinidis and Tiedje (2005), the ANI should not be considered as the sole argument for species definition (Konstantinidis & Tiedje, 2005). Ecological niche occupation which is a justifiable measurement of the phenotypic potential of a bacterial strain, is another important argument for species definition (Konstantinidis et al., 2006, Konstantinidis & Tiedje, 2005). A simple inspection of the ecological niches occupied by strains from the *R. solanacearum* species complex indicates that all strains share phenotypic potential, as they are all soil-borne and plant-xylem infecting bacteria. In addition, all *R. solanacearum* strains from the four phylotypes with shared phenotypic potential as they are able to infect tomato plants and cause the same symptoms (Remenant et al., 2010). Another important consideration is the (likely

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allopatric) divergence of related strains accompanied by important genome reduction and ecological specialization like it is the case for \textit{R. syzygii}, the BDB strains and \textit{R. solanacearum} phylotype IV strains (i.e. PSI07) (Remenant \textit{et al.}, 2011). Despite a high ANI score (>98%), we could argue that these strains should not be the same species, exactly like \textit{Burkholderia mallei} and \textit{B. pseudomallei} are justified as different species (Konstantinidis \textit{et al.}, 2006).

The renaming proposition of Remenant and colleagues is interesting but should be re-evaluated in the light of more whole genome sequence data to clearly evaluate whether there is a continuum or clustered genetic relatedness in this species complex. For instance, the sequence of strain ACH0732, which is not clearly associated with a specific phylotype (Fegan & Prior, 2005) would indeed be especially informative.

We believe that the distinction of these three genomic groups is a good description of the \textit{R. solanacearum} diversity. However, assigning new species names to these groups would confusingly reinforce the genetic frontier between these strains, undistinguishable for the non-expert from the distance between for example \textit{R. solanacearum} and \textit{R. picketti}. Moreover, three different species names for the solanaceous bacterial wilt causing strains could complicate the dialogue between farmers, field pathologists and scientists. An alternate proposition is to generate subspecies, using the names proposed by Remenant and colleagues, we propose the following subspecies: Strains assigned to phylotype II, which includes the type strain K60, should be named \textit{R. solanacearum} subspecies \textit{solanacearum}; strains in phylotype I and III \textit{R. solanacearum} subspecies \textit{haywardii}, with the type strain GM11000; strains assigned to phylotype IV including the \textit{R. syzygii} and BDB strains, \textit{R. solanacearum} subspecies \textit{haywardii}, with the type strain PSI07. This proposition combines the advantages of identifying the proximity between phylotypes I and III on one hand and phylotype IV, \textit{R. syzygii} and BDB on the other hand, without blocking further strain identification in an artificial species level differentiation. This could be exemplified by the strain ACH0732, which could not be classified in one of the four described phylotypes (Fegan & Prior, 2005), highlighting the continuum of genetic diversity in this species complex (Wicker \textit{et al.}, 2012).

Dissemination

Epidemiological analyses revealed that two major routes allow dissemination of \textit{R. solanacearum} strains in the environment: waterways at a local scale and transport of infected materials at both local and worldwide scales. The severe wilt disease occurring in 1955 on ginger in northern Australia originated from latently infected rhizomes imported from China (Hayward & Pegg, 2012). Numerous studies were conducted on the origin of potato brown-rot strains in North America and demonstrated that all introductions in the last few years have been linked to worldwide \textit{Pelargonium} production (Norman \textit{et al.}, 2009). Infected \textit{Pelargonium} material has also been linked to the introduction of potato brown-rot strains into Europe (Janse \textit{et al.}, 2004, Janse, 2012) and Taiwan (Cellier \textit{et al.}, 2012). The severe wilt disease occurring in 1955 on ginger in northern Australia originated from latently infected rhizomes imported from China (Hayward & Pegg, 2012). Infected \textit{Pelargonium} also introduced new \textit{R. solanacearum} strains to Northern Florida in 2001 probably originating from Martinique (Hong \textit{et al.}, 2008). One to four years later, these strains were detected in different irrigation ponds on tomato farms and ornamental nurseries situated several miles away from the first infected farm. This observation suggested that dissemination of \textit{R. solanacearum}
strains may have occurred through the underground river system common to Northern Florida (Hong et al., 2008). Many reports have demonstrated that R. solanacearum can survive in waterways for many years and even at low temperatures (4°C) for at least one month (Elphinstone, 2005, Caruso et al., 2005). The bacterium can enter into a viable but non-culturable (VBNC) state (Alvarez et al., 2008). R. solanacearum cells maintained in river water for four years are still able to cause infections in tomato plants and this is even true for some VBNC cells (Alvarez et al., 2008, Caruso et al., 2005).

Host range

Host range specificity in R. solanacearum is intricate. The diverse strains in the R. solanacearum species complex exhibit an bacterium is characterized by an unusually large host range, being able to infect more than 250 plant species in 54 monocot and dicot botanical families (Elphinstone, 2005). Host specialization has been reported for some strains, for example the IIB-3 and IIB-4 Moko strains are also virulent to susceptible tomato and potato. However, host specialization in the R. solanacearum species complex is rarely thoroughly described. For example, pathogenicity tests under controlled conditions found that many Moko-strains are also virulent to susceptible tomato and potato (Cellier et al., 2012).

Brown-rot strains are clustered into phylotype IIB-1 and IIB-2, historically known as race 3 biovar 2. Only IIB-1 strains maintain a high level of virulence under cold temperatures. However, strains from other phylogenetic groups were also isolated from diseased potato plants in Europe, the Mediterranean and in the highland of West Cameroon (Cellier & Prior, 2010, Toukam et al., 2009). This highlights the complexity of the genetic basis for pathogenicity on potato. Many works were conducted to tentatively identify the genes associated with host specificity. For that purpose, the authors used the methodology of comparative genomic hybridization on pangenomic microarrays representative of R. solanacearum genes was used to compare gene repertoires of hundreds of strains for which pathogenicity traits were defined (Guidot et al., 2007, Cellier et al., 2012).

These works were conducted with potato pathogenic strains and banana pathogenic strains but comparative genomic hybridization of a set of potato pathogenic strains and non-potato pathogenic strains on pangenomic microarrays representative of R. solanacearum genes did not find any genes associated with host specificity on potato traits (Guidot et al., 2007, Cellier et al., 2012). (Guidot et al., 2007, Cellier et al., 2012). Strains causing Moko disease are found in both phylotypes IIA (IIA-6 and IIA-24) and IIB (IIB-3 and IIB-4) with strains that are pathogenic for tomato and potato. Interestingly, pathogenicity tests under controlled conditions found that most IIB-3 and IIB-4 Moko-strains are also virulent to susceptible tomato and potato. This illustrates again the complexity of the plant / bacterial interaction specificity.

In order to better characterize the specificity of the interaction between R. solanacearum and solanaceous plants, a recent analysis has been conducted in controlled conditions to study the pathogen interaction between a collection of three solanaceous Solanaceae (tomato, eggplant and pepper) representative of the bacterial wilt resistance genetic resources and a collection of 12 strains representative of the known phylogenetic diversity of R. solanacearum (Lebeau et al., 2011). Interestingly, although all plants belong to the same family, Solanaceae, they interact differently with the 12 R. solanacearum strains. Six interaction phenotypes were defined and named pathoprofiles
based on the aggressiveness of the strains on the host plants. Intermediate phenotypes correspond to latent infection of the plants (bacterial colonization of the xylem tissue with few or no wilting symptoms). No pathoprofile is phylotype specific and none of the plants of this collection were resistant to all tested $R.\ solanacearum$ strains (Lebeau et al., 2011).

This study highlights why $R.\ solanacearum$ is so difficult to control on a long-term basis for breeders.

Emergence of strains with a new host range

$R.\ solanacearum$ is described as a highly flexible organism capable of rapid adaptation to environmental changes and new hosts and to counteract plant resistance. However, characterization of emerging strains in $R.\ solanacearum$ is difficult and has rarely been reported. The most studied case of $R.\ solanacearum$ recently emerging strains are the phylotype IIB4-NPB (Non-Pathogenic on Banana) strains in Martinique (Wicker et al., 2009a, Wicker et al., 2009b). These strains belong to phylotype IIB-4 group in which Moko-disease causing strains also cluster, but they are not pathogenic to banana. The epidemiological data demonstrate that phylotype IIB-4NPB strains constitute an emerging population in Martinique. This genetic group was absent in $R.\ solanacearum$ collections from the French West Indies until the first strain was isolated in 1999. These strains show a previously unknown host range in $R.\ solanacearum$, including cucurbits, ornamental plants and Solanaceae. Importantly, they seem to have expanded their host range from Anthurium-Cucurbitaceae in 1999-2002 to Anthurium-Cucurbitaceae-Solanaceae in 2002-2003 (Wicker et al., 2009a). Moreover, they were recovered from solanaceous wild species and several weeds as well as in the water throughout Martinique, demonstrating their rapid spread over the island. The factors that have favored this emergence of strains with novel host specificity are still unclear. The banana/vegetable rotations in Martinique fields have probably a role to play. Indeed the isolation of IIB-4NPB strains from wilted tomatoes or wilted cucurbits was only reported on fields with a preceding banana crop (Wicker et al., 2009b). Interestingly, IIB-4NPB strains were also isolated in Brazil from Cucurbits (Cellier et al., 2012). Because Brazil is a Moko-disease area, it is also possible that IIB-4NPB strains emerged in Brazil and established in Martinique through movement of contaminated ornamental material such as Anthurium.

Generation of biodiversity

In bacteria, polymorphism created by mutations is redistributed among strains by recombination and horizontal gene transfers. The major contribution of recombination in the evolutionary dynamics of $R.\ solanacearum$ has recently been demonstrated is debated. Using multilocus sequence analysis (MLSA) and estimations of linkage disequilibrium between eight loci in 58 strains from the four phylotypes, Castillo and Greenberg (2007) concluded that $R.\ solanacearum$ is an essentially clonal organism. Clonality was specifically found when housekeeping genes were analyzed. However, when virulence related genes were analyzed, high levels of recombination between $R.\ solanacearum$ strains were detected (Castillo & Greenberg, 2007). More recently, by Wicker et al. (2012), The authors conducted MLSA (Multilocus Sequence Analysis) with nine loci (seven housekeeping and two
virulence-related genes) on a worldwide collection of 89 *R. solanacearum* strains representative of the four phylotypes and the 51 epi-based seqeuvar and concluded that recombination play a major role in *R. solanacearum* genome evolution. Interestingly, they detected Phylotype IV as a gene donor for the majority of the recombination events. Interestingly, phylotype I, which is known to affect the highest number of hosts, appeared the most recombinogenic lineage. The only clonal group was phylotype IIB. Interestingly, Phylotype I, which is known to affect the highest number of hosts, appeared the most recombinogenic lineage. This result reinforces the hypothesis that phylotype I strains possess the highest potential for adaptation to new hosts and environments. These findings are in opposite with the conclusion made by Castillo and Greenberg (2007). Using multilocus sequence analysis (MLSA) and estimations of linkage disequilibrium between eight loci in 58 strains from the four phylotypes, Castillo and Greenberg (2007) concluded that *R. solanacearum* is an essentially clonal organism. However, most (24 on 58) of the strains analyzed by Castillo and Greenberg (2007) belonged to phylotype IIB (phylotype II Group A in Castillo & Greenberg, 2007) which is a clonal group according to Wicker et al. (2012). This could be part of the reason why Castillo & Greenberg (2007) arrived at this conclusion. Clonality was specifically found when housekeeping genes were analyzed. However, when virulence-related genes were analyzed, high levels of recombination between *R. solanacearum* strains were detected (Castillo & Greenberg, 2007).

Another important mechanism in the evolution of *R. solanacearum* genomes is horizontal gene transfer (HGT) (Remenant et al., 2010, Coupat et al., 2008, Guidot et al., 2009, Fall et al., 2007). Analysis of the genomic sequences of nine *R. solanacearum* genomes revealed numerous genomic islands, many of them were surrounded by mobile elements such as IS (Insertion Sequence) or bacteriophages, suggesting an horizontal acquisition (Remenant et al., 2010, Remenant et al., 2011, Remenant et al., 2012).

Hierarchical clustering based on the variable genes within the genomic islands among 18 *R. solanacearum* strains indicated that they were acquired by ancestral strains and were then transmitted vertically within phylotypes (Guidot et al., 2007). Methods based on phylogenetic reconstruction of gene families with prokaryote homology detected 151 genes (13.3%) of foreign origin in the *R. solanacearum* GMI1000 genome (Fall et al., 2007). The small plasmid carrying the Type IV secretion system detected in the genome of the CMR15 strain was possibly acquired from *Xanthomonas citri pv. citri*, another tomato pathogen prevalent in Cameroon (Remenant et al., 2010). Interestingly, recombination ‘hot spots’ were detected in the GMI1000 genome correlating with the presence of Chi-like signature sequences (Fall et al., 2007).

The frequency of gene transfer between phylogenetically-distant bacteria is expected to be low. Nonetheless, HGT between strains from the four phylotypes have been shown to be possible in the lab (Coupat et al., 2008, Guidot et al., 2009). Coupat and colleagues (2008) (Coupat et al., 2008) demonstrated that 80% of *R. solanacearum* strains are naturally transformable by plasmids and/or genomic DNA, and that large DNA fragments ranging from 30 to 90 kb can be transferred between strains. The potential to exchange virulence genes by HGT could play a major role in rapid pathogenicity evolution of *R. solanacearum* strains. The role of HGT in enhancing the aggressivity on
Tomato of *R. solanacearum* strains has been experimentally demonstrated (Coupat-Goutaland *et al.*, 2011).

**Pathogenicity determinants**

The main pathogenicity determinant in *R. solanacearum* is the type 3 secretion system (Boucher *et al.*, 1985, Coll & Valls, 2013), a syringe-like membrane appendix that injects the so-called “effector proteins” (type 3 effector proteins, or T3E hereafter) into the plant cell cytosol to favour infection (Erhardt *et al.*, 2010, Tampakaki *et al.*, 2010). Mutants defective in any of the >20 *hrp* or *hrc* genes, encoding structural or regulatory proteins of this molecular syringe, are non-pathogenic (Boucher *et al.*, 1985). Exopolysaccharide (EPS), a loose slime of heterogeneous composition (Orgambide *et al.*, 1991) also plays an important role in *R. solanacearum* pathogenicity. EPS strongly contributes to the occlusion of the xylem vessels that eventually causes the plant wilting symptoms. EPS is also important for plant colonization (Araud-Razou *et al.*, 1998, Husain & Kelman, 1958, Kao *et al.*, 1992, Denny & Baek, 1991). Besides these two major virulence determinants mentioned, *R. solanacearum* produces an array of additional factors that also contribute to colonization and/or to symptom appearance. These are exhaustively reviewed (Genin & Denny, 2012) and include, among others, type II-secreted plant cell-wall-degrading enzymes, motility or attachment appendages, aerotaxis transducers, cellulases and pectinases. For instance: type 4 pilus, involved in twitching motility, biofilm formation and root attachment; and the flagella, responsible for swimming motility, were both shown to contribute to virulence on tomato (Kang *et al.*, 2002, Tans-Kersten *et al.*, 2001).

Interestingly, motility and attachment seem to play their role during plant colonization, as most mutants affected in these capacities are hypopathogenic when inoculated in the soil but behave like wild type strains when directly inoculated in plant stems (Meng *et al.*, 2011, Yao & Allen, 2007). A recent report also involves aggregation due to the Flp Pili in pathogenicity, as a mutant deficient in these pili displays wild-type swimming or twitching motility but is impaired in its ability to cause wilting of potato plants (Wairuri *et al.*, 2012).

**Regulation of virulence genes**

Bacterial plant pathogens possess sophisticated regulatory circuits to finely control the energy-consuming expression of virulence determinants. An exhaustive comparative review on virulence regulatory modules in different bacterial pathogens can be found elsewhere (Mole *et al.*, 2007). The pathways controlling transcription of the main *R. solanacearum* virulence genes are well known (Genin, 2010, Genin & Denny, 2012, Schell, 2000). The LysR-family transcriptional regulator PhcA plays a central role, as it directly or indirectly regulates many of these genes (Fig. 2). PhcA activates EPS, pectinase and celluloseencoding genes and represses swimming motility, T3SS and siderophore expression (Genin & Denny, 2012). PhcA represses transcription of a pectinase-encoding gene via PehR, although it also slightly activates expression of other pectinase genes (Fig. 2) (Brumbley & Denny, 1990, Clough *et al.*, 1997a).
by the siderophile staphyloferrin B is controlled by PhcA, the global virulence regulator (Genin, 2005). Control of the Ralstonia solanacearum Type III secretion system (Hrp) genes by the global virulence regulator PhcA. Interestingly, transcription of the global regulator PhcA is controlled by a Ralstonia-specific cell-density dependent mechanism that involves 3-hydroxy palmitic acid methyl ester 12-hydroxy palmitoyl methyl ester (3-OH PAME) produced by the inner membrane protein PhcB (Flavier et al., 1997, Genin & Denny, 2012). At low cell densities, PhcA expression is repressed by PhcR by a post-transcriptional mechanism (Fig. 2). When the amounts of 3-OH PAME build up due to confinement or bacterial densities above 10^7 cells/ml, the molecule activates the two-component system PhcS/PhcR. PhcR is then phosphorylated and PhcA expression de-repressed (Clough et al., 1997, Schell, 2000). One of the main outcomes of PhcA activation in high-cell density conditions is the production of large amounts of EPS. This control is exerted through induction of XpsR, which directly activates transcription of the eps operon (Huang et al., 1995). Interestingly, it was found that the two-component regulatory system VsrA/VsrD is also required to fully activate xpsR transcription and, consequently EPS synthesis (Huang et al., 1998, Schell et al., 1994). In addition, VsrD directly affects swimming motility by repressing transcription of the flagellum genes (Fig. 2). Another two-component system, VsrB/VsrC, has also been described to control EPS synthesis and repress transcription of the pectinase pglA, adding another layer of control on EPS synthesis (Huang et al., 1995). Although the Vsr genes respond to still-unknown signals, it was found that the Erwinia amylovora homologues of vsrA/D (hrpX and hrpY) are induced in conditions that mimic the plant apoplast (Wei et al., 2000). It is also remarkable that members of both systems (vrsB and vrsD) were identified in a genetic screen amongst 153 R. solanacearum K60 genes induced during growth in tomato (Brown & Allen, 2004). XpsR is thus a central switch in EPS regulation, as it integrates inputs from both VsrAD and PhcA to directly regulate the eps promoter and is required for both its negative control by EpsR and its positive control by VsrC (Garg et al., 2000, Huang et al., 1995).

Regulation of the T3SS exemplifies that, during evolution, horizontally transferred operons can co-opt transcriptional regulators present in the recipient bacterium. It has been found that horizontally transferred operons can be reconfigured by natural selection to express genes in response to signals mimicking the plant apoplast (Cases & de Lorenzo, 2001). For instance, although the genes encoding the T3SS are highly conserved across species, the pathways controlling their transcription in R. solanacearum and Xanthomonas ssp are totally unrelated to those found in Pectobacterium ssp and Pseudomonas ssp (Tang et al., 2006). In R. solanacearum, HrpB, an AraC-type regulator, and HrpG, its upstream OmpR-like two-component response regulator, control hrp/hrc gene expression. HrpB directly triggers transcription of the T3SS genes, probably binding to the so-called hrpII box found in promoter regions (Cunnac et al., 2004a, Cunnac et al., 2004b, Genin et al., 1992) and its expression is controlled by HrpG (Brito et al., 1999) (Fig. 2). HrpG and HrpB are both genetically and functionally conserved in Xanthomonas ssp (Wengelnik & Bonas, 1996, Li et al., 2011, Zou et al., 2006), but unique to R. solanacearum are the upstream regulators that trigger the specific induction of HrpG when the bacterium detects a plant cell wall component (Aldon et al., 2000). PrhA is the outer membrane receptor that perceives this signal (Aldon et al., 2000) and transfers it to the membrane-associated proteins PrhI and PrhR to trigger hrp/hrc expression through the consecutively induced transcriptional regulators PrhJ, HrpG and HrpB (Brito et al., 2002) (Fig. 2). In vitro transcriptomic studies have revealed that the hrp regulators control additional functions other than the T3SS and most of its associated effectors. It was found that HrpB was also involved in regulation of chemotaxis and biosynthesis of various low-molecular-weight chemical compounds,
such as the Hrp-Dependent Factor (HDF), which may induce a cell-density LuxR system (Delaspre et al., 2007, Occhialini et al., 2005). HrpG was found to control an even larger set of T3SS-unrelated genes independently of HrpB (Fig. 2). These encoded known virulence determinants such as pectinolytic and cellulase activities—some of which are common targets of PhcA—and other genes likely involved in plant pathogen interactions, including adhesion factors (lectins), the only predicted catalase enzyme in the genome and an ethylene-forming enzyme that produces this plant hormone (Valls et al., 2006). HrpG was also found to slightly affect known virulence determinants such as pectinolytic and cellulase activities, some of which are common targets of PhcA (Fig. 2). A recent study has also involved HrpG in the control of the last step of methionine synthesis (Plener et al., 2012). It was proposed that HrpG promotes production of MetE, which synthesises methionine without the need of vitamin B12, as a way to ensure the biosynthesis of this amino acid in the vitamin-poor environment encountered in planta. Thus, HrpG occupies a central node in pathogenicity regulation, since in addition to controlling a panoply of virulence genes, it integrates both plant-cell-dependent induction and metabolic cues that affect transcription of the T3SS (Fig. 2). Examples of the latter metabolic signals are the repression of T3SS genes by casamino acids and their induction during growth in minimal media (Arlat et al., 1992, Genin et al., 2005). Recently, PrhG was identified in strain GMI1000 as a HrpG paralogue that also activates HrpB expression. Interestingly, prhG is induced during growth in minimal medium but not by plant cells, so that this regulator controls expression of the T3SS under minimal medium conditions (Plener et al., 2010).

Other regulatory influences on influencing the expression of the T3SS have also been described, but the regulators involved and their mechanisms of action remain poorly understood. Examples of these are LrpE, a leucine-rich repeat protein found to negatively regulate expression of hrp genes three- to fivefold (Murata et al., 2006). Similarly, the influence of the prKLM operon, Absence of any of the latter three genes encoded in the prKLM operon in on strain OE1-1 decreased prhG expression and consequently that of hrpB and the PopA effector by 10-fold (Zhang et al., 2011). However, these genes do not encode transcriptional regulators and must influence the hrp regulon indirectly by an unknown mechanism.

As previously mentioned, the global regulator PhcA modulates T3SS gene expression and it does so at two different levels: by slightly inhibiting prhI/R transcription and by strongly inhibiting hrpB gene expression through an unknown mechanism acting on HrpG (Yoshimochi et al., 2009a, Yoshimochi et al., 2009b, Genin et al., 2005). Interestingly, EPS production is also slightly down-regulated by HrpG through an increase of the levels of EpsR (Valls et al., 2006) (Fig. 2). These examples illustrate that cross-talk occurs between regulatory cascades at various levels regulatory cascades have cross-talks at various levels to form a complex network co-regulating virulence activities in response to environmental signals. All this knowledge led to the corollary that in R. solanacearum, the infection process takes place in two steps (Schell, 2000, Brito et al., 2002, Mole et al., 2007): First, early in colonization, expression of the T3SS type 3 would be induced by plant cell contact and PhcA would not be induced due to low bacterial density, allowing swimming motility to be active. In the second step, when bacterial numbers increase inside the xylem, the PhcA regulator would be expressed, triggering EPS and cellulase production and repressing the T3SS and siderophore. However, recent in planta expression data using promoter::reporter fusions integrated in the bacterial chromosome and quantitative RT-PCR have challenged this model (Monteiro et al., 2012b, Monteiro et al., 2012a). These studies showed that the hrpB and T3E transcripts are abundant at late stages of plant colonization, when bacterial numbers are high and plants already wilted. More
recently, transcriptome analyses have confirmed this finding, showing half of the *hrpB*-regulated
genes are induced in bacteria recovered from the xylem of wilting tomato plants (Jacobs et al., 2012).
All these observations illustrate our limitations to predict the behaviour of bacterial virulence genes
in real field conditions and suggest the existence of still-unknown inducing signals.

T3E repertoire

Over the last 10 years, T3E biology has boomed. Over this time, several groups have achieved a very
good description of the repertoire of T3Es. Different methods have been applied to define
the set of T3Es in *R. solanacearum*: (i) the search for orthologues of already known T3Es, (ii) the
identification of T3Es through gene regulation studies (Cunnac et al., 2004a, Cunnac et al.,
2004b, Occhiolini et al., 2005), (iii) the search for atypical protein motifs indicating a potential
function not in the eukaryotic host cell-wall of *cell-cell* (Angot et al., 2006) and finally (iv) a functional screen for
type 3 injected protein (Mukaihara et al., 2010). Altogether, these efforts generated a
comprehensive list of T3Es for two closely-related phylotype I strains (Mukaihara et al., 2010,
Poueymiro & Genin, 2009). This latter work is now being expanded with the availability
of several new genomic sequences spanning the four phylotypes representing the *R. solanacearum
species complex* (unpublished data).

As already pointed out when the first *R. solanacearum* genome was published (Salanoubat et al.,
2002), there are some striking features specific to this bacterium. First, it seems that the T3E
repertoire is larger than in other plant pathogenic bacteria; secondly *R. solanacearum* seems to be
the recipient of a diverse set of T3Es likely acquired by HGT. Indeed, the GALA T3E (Kajava et al.,
2008) or the PPR-containing T3E (Salanoubat et al., 2002), harboring typical eukaryotic features
like the F-box domain (Ho et al., 2008) and PPR motifs (Delannoy et al., 2007), could originate from
an ancestral eukaryotic donor. On the other hand many T3Es could have originated from a HGT from
other pathogenic bacteria since homologs exist in various other animal and plant bacterial pathogens
(Poueymiro & Genin, 2009). This is very likely to be the case for the *Xanthomonas* spp., specific
transcriptional activator-like T3E (Fall et al., 2007). Recently a detailed characterization of these
*R. solanacearum* TAL-like T3Es showed that they are indeed nuclear targeted and can function as
transcriptional activators in plant cells (Li et al., 2013). Another particular feature of the
*R. solanacearum* effector repertoire is the abundance of duplicated T3E genes. Indeed several T3E
genes are present as gene families (Sole et al., 2012, Remigi et al., 2011, Poueymiro & Genin,
2009). (Poueymiro & Genin, 2009, Remigi et al., 2011, Sole et al., 2012). Interestingly most of these
gene families are conserved among the different *R. solanacearum* strains (Sole *et al.*, 2012, Remigi *et al.*, 2011), see also the MAGe genome browser displaying the sequenced *R. solanacearum* strains ([https://www.genoscope.cns.fr/agc/microscope/mage](https://www.genoscope.cns.fr/agc/microscope/mage)). "The presence of these gene families, arising from indicating early gene duplications in a common ancestor, probably are likely to undergo followed by diversification of the function of the new paralogous T3Es functional diversification to provide adaptation on different host plants, like has been shown for the GALA family (Remigi *et al.*, 2011).

**Effector-triggered Immunity**

Historically, the first biological function identified for T3Es has been their contribution to the Effector Triggered Immunity or ETI (Jones & Dangl, 2006). The T3E PopP1 induces a cultivar-specific HR-like response on petunia (Lavie *et al.*, 2002, Arlat *et al.*, 1994). Furthermore, PopP1 (Robertson *et al.*, 2004), and recently PopP1 together with AvrA (Poueymiro *et al.*, 2009) have been shown to contribute to the HR-mediated resistance in tobacco. Indeed, upon tobacco root inoculation, the double PopP1 AvrA mutant in GM1000 causes wilting and is undistinguishable from the K60 tobacco-pathogenic strain (Poueymiro *et al.*, 2009). The closely-related T3E PopP2 was shown to be responsible for the RRS1-R-mediated resistance in the Nd-1 Arabidopsis ecotype (Deslandes *et al.*, 1998). PopP2 interacts directly with RRS1-R in the nucleus of plant cells, leading to an asymptomatic and much reduced bacterial colonization (Deslandes *et al.*, 2002, Deslandes *et al.*, 2003). PopP2 has been shown to interact with the Arabidopsis Arabidopsis thaliana protein RD19, redirecting its localization from lytic vacuoles to the nucleus, where both physically interact. Although its exact role has yet to be defined, RD19 is required for the PopP2/RRS1-R-mediated resistance (Bernoux *et al.*, 2008). Furthermore it has been shown that this T3E, belonging to the widespread YopJ/AvrXv family displays an acetyltransferase activity. This activity results in an autoacetylation of PopP2 required for an effective PopP2/RRS1-R resistance (Tasset *et al.*, 2010). *R. solanacearum* could harbor other T3Es triggering plant immunity. Indeed Agrobacterium Agrobacterium tumefaciens-mediated expression of AWR5 induces HR-like symptoms in *Nicotiana tabacum* (Sole *et al.*, 2012). Interestingly, in the same T3E family, the multiple mutant awr1-5 displays an increased pathogenicity on Arabidopsis Col-0, suggesting the possibility that, at least one of these AWRs is actually recognized by an R-gene, triggering a weak ETI in this host (Sole *et al.*, 2012).

**T3E Virulence functions**

Other T3Es have been characterized on the basis of their contribution to disease. Although the exact molecular mechanisms have yet to be described, different T3Es have different contributions to disease on different host plants. *coQ* on tomato, mutants in the T3E RSp0304 (HopD1 homolog) and AWR2, show a decreased disease progression (Cunnac *et al.*, 2004b). The AWR T3E family is collectively needed on both tomato and eggplant for a full pathogenicity. Interestingly AWR2 can restore the wild-type phenotype of the multiple awr(1-5) mutant on eggplant (Sole *et al.*, 2012).
For the GALAs, another well studied T3E family, it was demonstrated that they are collectively required on tomato and ArabidopsisArabidopsis, thaliana for a full-disease phenotype (Angot et al., 2006). More recently this result was shown to be more complex with a redundancy of GALA2, GALA3, GALA6 and GALA7 on Arabidopsis, whereas only GALA7 and GALA3 seem to be able to restore full virulence of the quadruple mutant gala2 gala3 gala6 gala7 on tomato (Remigi et al., 2011). In this same family it was also showed that the single gala7 mutant (and none of the other single mutants) is avirulent on the legume host Medicago truncatula (Angot et al., 2006). GALA T3Es could potentially control host protein stability since they are likely to form E3-ubiquitin ligases inside the host cell (Angot et al., 2007). Although structurally different, several other T3Es have similarities with ubiquitin-ligases. This is the case for RSc1349, homologue of the Shigella flexneri IpaH ubiquitin ligase (Singer et al., 2008), or the MolK2 specific RSMK00763 T3E homolog of the P. syringae AvrPtoB (Poueymiro & Genin, 2009). On M. truncatula, GALA7 together with AvrA are both required for the early infection steps of intact roots (Turner et al., 2009). GALA7, but not AvrA, is also required for disease development when colonization is facilitated by cutting the root tips (Turner et al., 2009). Another early/late disease development differential role has been shown for the T3SS secreted harpin PopA. Indeed, in the Japanese strain OE1-1, constitutive early expression of this T3E prevents the natural root infection of N. tabacum, but not the bacterial multiplication inside stem-inoculated plants (Kanda et al., 2003).

Classical plant pathoassays enabled to identify only a few T3Es with a virulence function (Cunnac et al., 2004b). This low yield of T3Es with virulence functions out of an important number of compared to the large T3E repertoires (Poueymiro & Genin, 2009), could be explained by functional redundancy (Sole et al., 2012, Angot et al., 2006) but could also be explained by the fact that some T3Es have only a marginal contribution to virulence, hence undetectable with classical wilt scoring. For this purpose a novel assay based on mixed inoculations was developed (Macho et al., 2010). The principle is to compare the ability of two strains to multiply in the host when they are co-inoculated, compared to their ability to multiply when inoculated individually. Interestingly, two T3Es: Rsp0304 and PopP2, were shown involved for efficient bacterial multiplication in three host plants, eggplant, tomato and bean (Macho et al., 2010).

Complex mechanisms underlie Bacterial wilt resistance and tolerance

Genetic analysis of resistance to bacterial wilt has been developed both on model and cultivated plants. In A. thaliana, the study of the interaction between two ecotypes, Nd-1 and Kil-0, and two strains of R. solanacearum, GMI1000 and BCCF402 (both phytopathotype I strains), respectively, revealed the implication involvement of RRS1-R. RRS1-R, a TIR-NBS-LRR gene with a WRKY C-terminal domain, has been described as a single recessive resistance gene against strain GMI1000, through the direct recognition of the PopP2 effector (Deslandes et al., 1998, Deslandes et al., 2002, Deslandes et al., 2003). Interestingly, it was demonstrated recently Van der Linden et al., (2013) demonstrated that the gene-for-gene interaction RRS1-R-PopP2 is also involved in Kil-0 tolerance (Van der Linden et al., 2013). Indeed, Kil-0 does not exhibit wilting symptoms after its inoculation with strain BCCF402 of R. solanacearum, despite a high bacterial multiplication in planta. The catalytic triad and the auto-acetylated lysine are conserved in the BCCF402 popP2 allele, but some
allelic variations in both BCCF402 PopP2 and Kil-0 RRS1-R could account for altered protein interactions and/or signal transduction in the plant cell. Still, with can’t be excluded either that this RRS1-R-dependent tolerance in Kil-0 could be dependent on other plant or bacterial factors. A quantitative resistance mechanism was also described in A. thaliana against R. solanacearum (Godiard et al., 2003). Among the three quantitative trait loci (QTLs) identified, one is associated with ERECTA, a leucine-rich-repeat receptor-like kinase (LRR-RLK) involved in development (Torii et al., 1996, Godiard et al., 2003). This could suggest that cross-talk occurs between resistance to R. solanacearum and developmental pathways (Godiard et al., 2003). In the model legume M. truncatula, recombinant inbred line (RIL) population A17 X F83005.5 enabled to identify three QTLs for resistance to R. solanacearum strain GMI1000 (Vailleau et al., 2007). The fine mapping of the major QTL located on the chromosome S of M. truncatula MtQRRS1, allowed the identification of a 64 kb region with a cluster of seven putative R-genes among 15 candidate genes (Ben et al., 2013) in tomato cultivar ‘Hawaii 7996’. This cultivar was described as one of the most stable sources of resistance to bacterial wilt (Danesh et al., 1994, Mangin et al., 1999, Thoquet et al., 1996b, Thoquet et al., 1996a, Wang et al., 2000). The polygenic resistances identified are phylotype- and strain-specific (Carmeille et al., 2006). Recently, Wang et al. (2013) identified two major QTLs for resistance to R. solanacearum (Bwr-6 and Bwr-12) using the tomato cross ‘Hawaii 7996’ X ‘West Virginia 700’ (Wang et al., 2013a). The Bwr12 QTL is specific for resistance to phylotype I, whereas the Bwr-6 QTL is associated with resistance to phylotype I and phylotype II strains. Interestingly, the presence of both QTLs has an additive effect, displaying enhanced resistance. In tobacco, Qian et al. (2012) identified four QTLs associated with resistance to non-characterized, “naturally occurring strains” from bacterial wilt affected areas of China (Qian et al., 2012). In eggplant, four QTLs have been identified in a RIL population, these QTLs that exhibit resistance to different strains of R. solanacearum (Lebeau et al., 2013). Among them, the ERs1 QTL has been described as a major dominant source of resistance towards three phylotype I-strains of R. solanacearum (Lebeau et al., 2013). The further characterization of the genetic components underlying these different resistances to R. solanacearum will prove very useful for the plant breeding community.

Plant signaling in response to Ralstonia solanacearum

A link has been described between A. thaliana secondary cell walls and the outcome of the interaction with R. solanacearum (Hernandez-Blanco et al., 2007). Indeed, a mutation in any of the three secondary cell wall-specific cellulose synthase genes led to a complete resistance to the bacterium. Furthermore, abscisic acid (ABA)-signaling was demonstrated to be involved in this cellulose-synthase-dependent enhanced resistance (Hernandez-Blanco et al., 2007). The role of the cell walls in A. thaliana as barriers against R. solanacearum colonization was also studied through the wat1 (Walls Are Thin1) mutant (Denance et al., 2012). The WAT1 gene is required for secondary cell-wall deposition and the corresponding mutant shows enhanced resistance to R. solanacearum (Denance et al., 2012). Comparing two different inoculation methods of R. solanacearum in A. thaliana leaves, by piercing in the central leaf vein or infiltrating a bacterial suspension in the mesophyll, the authors could conclude that wat1 resistance is localized to the vascular system.
Moreover, salicylic acid (SA) was identified as a key element of \textit{wat1}-mediated resistance to \textit{R. solanacearum} \cite{(Denance et al., 2012)}. Several other studies identified WRKY transcription factors as important players in modulating the plant response towards \textit{R. solanacearum} attack. Indeed, \textit{Mukhtar et al.} (2008) showed that \textit{Arabidopsis} \textit{Arabidopsis, thaliana} plants lacking a functional \textit{WRKY27} gene exhibited an enhanced tolerance to \textit{R. solanacearum} strains GM10000 and Rd-15 \cite{(Mukhtar et al., 2008)}. Similar situations were previously observed for the ethylene-insensitive \textit{EIN2-1} gene \cite{(Hirsch et al., 2002)} and the NWS1 gene \cite{(Feng et al., 2004)}, that appeared to be required for full virulence of the bacteria. Recently, two WRKY transcription factors of pepper were identified as important positive and negative contributors to \textit{R. solanacearum} resistance: Overexpression of \textit{CaWRKY40} in tobacco enhanced resistance to \textit{R. solanacearum}, whereas silencing of \textit{CaWRKY40} in pepper attenuated the resistance \cite{(Dang et al., 2012)}. On the other hand, \textit{CaWRKYS8}-overexpressing tobacco plants showed an enhanced susceptibility to the same strain, and \textit{CaWRKYS8}-silenced pepper plants displayed enhanced resistance \cite{(Wang et al., 2013b)}. In another work, Feng \textit{et al.} (2012) identified the abscisic acid (ABA) signaling pathway as important for biological control of Bacterial wilt in \textit{A. thaliana}. This ABA-dependent defense mechanism was shown to be independent of SA, JA and ethylene in the biological control exerted by an \textit{hpB} mutant of \textit{R. solanacearum} \cite{(Feng et al., 2012)}.

\textit{In vitro} \textit{gnotobiotic} pathosystems to study root infection

As \textit{R. solanacearum} infects plants \textit{via} their roots, \textit{in vitro gnotobiotic} models, \textit{artificial inoculation systems} constitute useful tools enabling to access the host root systems. Such bioassays, in which \textit{axenic} plantlets are inoculated with \textit{R. solanacearum} were described on tomato \cite{(Monteiro et al., 2012b, Vasse et al., 1995)}, petunia \cite{(Zolobowska & Van Gijsegem, 2006)}, \textit{M. truncatula} \cite{(Vailleau et al., 2007)}, and \textit{A. thaliana} \cite{(Digonnet et al., 2012)}. They enable to study the early steps of infection, to follow colonization step by step, and to observe “root symptoms”. \textit{R. solanacearum}-plant interaction at the root level was first studied in tomato \cite{(Monteiro et al., 2012b, Vasse et al., 1995)}. The authors observed the bacterium penetration \textit{via} the root extremities and at the axils of secondary root \cite{(Vasse et al., 1995)} \cite{(Vasse et al., 1995)}. Ten years later, the set up of \textit{in vitro gnotobiotic} experiments on petunia allowed the identification of new root lateral structures \textit{(RLS)} after the inoculation with \textit{R. solanacearum} \cite{(Zolobowska & Van Gijsegem, 2006)}. These structures are T3SS dependent and were demonstrated to be highly efficient colonization sites. However, the involvement of specific T3Es on the formation of these RLS has not been proven \cite{(Zolobowska & Van Gijsegem, 2006)}.

In the \textit{M. truncatula} \textit{in vitro gnotobiotic} pathosystem, it was described that \textit{R. solanacearum} penetrates specifically \textit{via} the root tips \cite{(Turner et al., 2009)}. Bacterial inoculation leads to an arrest of root hair elongation and a reduction in root growth, associated with a browning and a loss of viability of the root tip epidermal cells \cite{(Turner et al., 2009)}. Two T3Es, GALA7 and AvrA have been demonstrated to be partially involved in the induction of the root epidermal cell death phenotype \cite{(Turner et al., 2009)}. For the interaction involving \textit{A. thaliana} and \textit{R. solanacearum}, well described and characterized at the whole plant level \cite{(Deslandes et al., 1998)}, an \textit{in vitro axenic plant bioassay system} has been recently developed to study the early steps of root colonization by \textit{R. solanacearum} \cite{(Digonnet et al., 2012)}. The authors...
showed that the bacteria penetrate *A. thaliana* plantlets at the root apex. Bacteria induced a plasmolysis of epidermal and cortical root cells, accompanied with pectin degradation. The bacteria then move preferentially *via* intercellular spaces to then directly invade the vascular cylinder (Digonnet *et al.*, 2012). Contrary to the *M. truncatula* *in vitro* system (Vailleau *et al.*, 2007), an important bacterial surface colonization can be seen alongside the inoculated plantlets. Thanks to these *gnotobiotic in vitro* systems, it was shown that *R. solanacearum* disturbs the normal root growth upon host infection. Preferential zones of bacterial penetration are the root tips, correlating with the zone where plant exudates are produced, attracting *R. solanacearum* (Yao & Allen, 2006). In the future, these *in vitro* systems will prove important to better characterize the early events of infection (which cells are targeted by the bacteria?) and the chronology of the cellular plant colonization.

**Perspectives**

In this short review, we have tried to encapsulate all the recent research on *R. solanacearum* ranging from strain diversity to gene regulation, T3E biology and plant resistance. We would like to finish by putting the emphasis on the fact that this plant pathogenic bacterium is a research model with interesting specificities, *i.e.* root infection, vascular colonization, large T3E repertoire and broad host range. We think that further research will shed light both on these specificities as well as on general infection strategies common between *R. solanacearum* and other parasites.

In this post-genomic era some of the challenges lying ahead are: more strain sampling for an even better description of the species complex, deciphering the role of the T3Es during infection, integrative analysis of global *in planta* expression of the bacterial genes (RNAseq) together with metabolic modeling towards a systems biology model, experimental evolution to study adaptation to different plant hosts.

**Useful website, tools**

**pRC resource:** The *Ralstonia* chromosome (pRC) plasmids are a set of new integrative plasmids representing useful tools to study gene function in *R. solanacearum* (Monteiro *et al.*, 2012b)

**Stock center:** International Center for Microbial Resources - French Collection for Plant-associated Bacteria CIRM-CFBP, IRHS UMR 1345 INRA-ACO-UA, 42 rue Georges Morel, 49070 Beaucouzé Cedex, France. http://www.angers-nantes.inra.fr/cfbp/

**Ralstonia Genome browser:** https://iant.toulouse.inra.fr/R.solanacearum

**GMI1000 insertion mutant library:**
https://iant.toulouse.inra.fr/R.solanacearumGMI1000/GenomicResources

**MaGe Genome Browser:** https://www.genoscope.cns.fr/agc/microscope/mage/viewer.php?
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Figure legends

Fig. 1 Macro- and microscopic views of *R. solanacearum* and illustration of symptoms associated on plant bioassays. (a) *R. solanacearum* growing on complete BG medium (Boucher et al., 1985). The pink color of the colonies is due to the presence of triphenyl tetrazolium chloride in the medium. (b) Electron microscopy image of *R. solanacearum* rod-shaped cells under division displaying pil structures (by the late Jacques Vasse). (c) Symptoms of Bacterial wilt on *Medicago truncatula* plants. Inoculation in Jiffy pots with two wild-type strains (upper part). Gnotobiotic inoculation with a wild-type strain and an *hrp* mutant (bottom part). (d) Symptoms of Bacterial wilt on *Arabidopsis thaliana* plants. (e) Symptoms of Bacterial wilt on tomato plants. (f) One eggplant with symptoms of Bacterial wilt, and a healthy control plant.

Fig. 2. Major pathways controlling expression of *Ralstonia solanacearum* virulence genes. Circles and squares indicate regulatory proteins, the latter representing the main regulatory hubs. In grey, regulatory inputs sensed by the bacterium and in black pathogenicity activities controlled by this regulatory network. Arrows and T-bars indicate respectively, activation or repression. Black lines stand for control at the transcriptional level and red lines for post-transcriptional effects. Solid lines represent major effects and dotted lines faint transcriptional influences (modulation). For detailed explanations, see text.
Figure 2

254x190mm (96 x 96 DPI)