Molecular Plant Pathology

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

Journal:	*Molecular Plant Pathology*
Manuscript ID:	MPP-PP-13-026.R1
Manuscript Type:	Pathogen Profile
Date Submitted by the Author:	n/a
Complete List of Authors:	Peeters, Nemo; INRA UMR441 Laboratoire des Interactions Plantes Micro-organismes (LIPM), 24 chemin de borde rouge, Auzeville CS52627; CNRS UMR2594 Laboratoire des Interactions Plantes Micro-organismes (LIPM), 24 chemin de borde rouge, Auzeville CS52627 Guidot, Alice; INRA UMR441 Laboratoire des Interactions Plantes Micro-organismes (LIPM), 24 chemin de borde rouge, Auzeville CS52627; CNRS UMR2594 Laboratoire des Interactions Plantes Micro-organismes (LIPM), 24 chemin de borde rouge, Auzeville CS52627 Vailleau, Fabienne; INRA UMR441 Laboratoire des Interactions Plantes Micro-organismes (LIPM), 24 chemin de borde rouge, Auzeville CS52627; CNRS UMR2594 Laboratoire des Interactions Plantes Micro-organismes (LIPM), 24 chemin de borde rouge, Auzeville CS52627; Université de Toulouse ; INP ; ENSAT, 18 chemin de Borde Rouge Valls, Marc; Department of Genetics, University of Barcelona and Centre for Research in Agricultural Genomics (CRAG)., Edifici CRAG, Campus UAB
Keywords:	Ralstonia solanacearum

1

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

Nemo Peeters[1,2*], Alice Guidot[1,2], Fabienne Vailleau[1,2, 3] and Marc Valls[4]

*corresponding author, nemo.peeters@toulouse.inra.fr, +33 561285592

1-INRA UMR441 Laboratoire des Interactions Plantes Micro-organismes (LIPM) 24 chemin de Borde Rouge – Auzeville CS 52627 31326 CASTANET TOLOSAN CEDEX, FRANCE

2-CNRS UMR2594 Laboratoire des Interactions Plantes Micro-organismes (LIPM) 24 chemin de Borde Rouge – Auzeville CS 52627 31326 CASTANET TOLOSAN CEDEX, France

3-Université de Toulouse ; INP ; ENSAT ; 18 chemin de Borde Rouge, 31326 Castanet Tolosan, France.

4-Department of Genetics, University of Barcelona and Centre for Research in Agricultural Genomics (CRAG). Edifici CRAG, Campus UAB, 08193 BELLATERRA, CATALONIA, SPAIN

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 solanaceaous plants characterized by a sudden wilt of the whole plant. Typically, stem cross-sections ooze 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.*~~*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.*~~*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

Formatted: Font: Italic

Formatted: Font: Not Italic

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Formatted: Line spacing: Multiple 1.15 li

Field Code Changed

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 alsonot 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.*et al.*, 2012).

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

A species complex, let's keep it simple

Formatted: Normal, Line spacing: single

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.*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 complexcalculate 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.*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 (20056), 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.*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.*et al.*, 2010). Another important consideration is the (likely

Field Code Changed

Formatted: English (U.S.)

Formatted: Font: Not Italic

Formatted: Font: Italic

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

~~We believe that the distinction of these three genomic groups is a good description of the *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 *R. solanacearum* and *R. pickettii*. 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 *R. solanacearum* subspecies *solanacearum*; strains in phylotype I and III *R. solanacearum* subspecies *sequeirae* with the type strain GMI1000; strains assigned to phylotype IV including the *R. syzygii* and BDB strains, *R. solanacearum* subspecies *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, *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 *et al.*, 2012).~~

~~Dissemination~~

~~Epidemiological analyses revealed that two major routes allow dissemination of *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 *Pelargonium* production (Norman *et al.*, 2009). Infected *Pelargonium* material has also been linked to the introduction of potato brown-rot strains into Europe (Janse *et al.*, 2004, Janse, 2012) and Taiwan (Cellier *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 *Pelargonium* also introduced new *R. solanacearum* strains to Northern Florida in 2001 probably originating from Martinique (Hong *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 *R. solanacearum*~~

Formatted: English (U.S.)

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

~~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~~unclear~~.~~ ~~This~~ 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 ~~However, not all *R. solanacearum* exhibit broad host range; Some strains only infect a small selection of plant hosts, like~~ Moko strains ~~(~~infecting banana and *Heliconia*~~)~~ ~~and~~ or the brown-rot strains ~~(~~infecting potato and tomato. However, host specialization in the *R. solanacearum* species complex is rarely thoroughly described. For example, pathogenicity tests under controlled conditions found that most Moko-strains are also virulent to susceptible tomato and potato (Cellier ~~et al.~~*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~~to~~ 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 repertories of hundreds of strains for which pathogenicity traits were defined (Guidot ~~et al.~~*et al.*, 2007, Cellier ~~et al.~~*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 ~~repertory~~ associated ~~to~~ with these pathogenicity ~~on potato~~traits (Guidot ~~et al.~~*et al.*, 2007, Cellier ~~et al.~~*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

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Formatted: Font: Italic

Field Code Changed

Field Code Changed

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.~~*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 ~~e~~emerging strains are the phylotype IIB4-NPB~~BP~~ (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.~~*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 have 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~~that followed a previous banana crop (Wicker ~~et al.~~*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~~

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

~~virulence-related genes)~~ on a worldwide collection of 89 *R. solanacearum* strains representative of the four phylotypes and the 51 *egl*-based sequevars and concluded that recombination play a major role in *R. solanacearum* genome evolution. ~~Interestingly, t~~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) ~~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.~~*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.~~*et al.*, 2010, Remenant ~~et al.~~*et al.*, 2011, Remenant ~~et al.~~*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.~~*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.~~*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~~euvesicatoria*, another tomato pathogen prevalent in Cameroon (Remenant ~~et al.~~*et al.*, 2010). ~~(Remenant *et al.*, 2010a).~~ Interestingly, recombination 'hot spots' were detected in the GMI1000 genome correlating with the presence of Chi-like signature sequences (Fall ~~et al.~~*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.~~*et al.*, 2008, Guidot ~~et al.~~*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

Formatted: Font: Italic

Formatted: Font: Italic

Field Code Changed

Field Code Changed

Formatted: Font: Not Italic, French (France)

Formatted: French (France)

Field Code Changed

Formatted: French (France)

Formatted: French (France)

Field Code Changed

Formatted: French (France)

Formatted: French (France)

Field Code Changed

Formatted: English (U.S.)

Formatted: English (U.S.)

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.~~*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 pili, 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~~ a role during plant ~~colonization~~invasion, 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 diplays 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~~Fig. 2). PhcA activates EPS~~, pectinase and~~ and ~~cellulose~~cellulase-encoding 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)(Bhatt, 2004, Ralstonia solanacearum iron scavenging~~

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Field Code Changed

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

~~by the siderophore 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~~3-hydroxy-palmitoylmethylester~~ (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~~VsrA/VsrD can also activate *XpsR* transcription~~ and, consequently EPS synthesis (Huang *et al.*, 1998, Schell *et al.*, 1994). In addition, VrsD 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.~~*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 (*vsrB* and *vsrD*) 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 ~~VrsC~~ VsrC (Garg *et al.*, 2000, Huang ~~et al.~~*et al.*, 1995).

Regulation of the T3SS exemplifies that, during evolution, ~~regulation~~ horizontally transferred operons can co-opt transcriptional regulators present in the recipient bacterium ~~has been often over-imposed by natural selection on horizontally transferred operons~~ (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*~~spp are totally unrelated to those found in *Pectobacterium* ~~*ssp*~~spp and *Pseudomonas* ~~*ssp*~~spp (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~~Fig 2). HrpG and HrpB are both genetically and functionally conserved in *Xanthomonas* ~~*spp*~~spp (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.~~*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~~Fig. 2). *In vitro* transcriptomic studies have revealed that the *hrp* regulators control additional functions other that 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~~Fig. 2). These encoded ~~known virulence determinants such as pectinolytic and cellulase activities –some of which are common targets of PhcA-, and others~~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 ~~aminoacid~~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~~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~~ Additional ~~regulatory influences~~inputs ~~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 ~~this~~ these are LrpE, a leucine-rich repeat protein found to negatively regulate expression of *hrp* genes three- to fivefold (Murata *et al.*, 2006). Similarly, ~~and the influence of the *prKLM* operon.~~ Aabsence 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.~~*et al.*, 2005). Interestingly, EPS production is also slightly down-regulated by HrpG through an increase of the levels of *EpsR* (Valls ~~et al.~~*et al.*, 2006) (~~fig~~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.~~*et al.*, 2002, Mole ~~et al.~~*et al.*, 2007): First, early in colonization, expression of the T3SS~~type 3~~ would be induced by plant cell contact and PhcA would ~~be~~ not be induced due to low bacterial density, allowing swimming motility to be active. In the second step, when bacterial numbers increase~~boost~~ 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

Formatted: Font: Italic

Field Code Changed

Formatted: Font: Not Italic

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.~~ D~~Several d~~ifferent 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.~~*et al.*, 2004a, Cunnac ~~et al.~~*et al.*, 2004b, Occhialini ~~et al.~~*et al.*, 2005), (iii) the search for atypical protein motifs indicating a potential function ~~not~~ in ~~the~~ eukaryotic host ~~cell~~ cells (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.~~*et al.*, 2010, Poueymiro & Genin, 2009). This latter work ~~is now being~~could now be expanded with the availability of several new genomic sequences spanning the four phylotypes representing the *R. solanacearum* diversity (Remenant ~~et al.~~*et al.*, 2010, Salanoubat ~~et al.~~*et al.*, 2002, Remenant ~~et al.~~*et al.*, 2011, Xu *et al.*, 2011, Gabriel *et al.*, 2006). This would allow the evaluation of common and specific Rip ("*Ralstonia* injected proteins" ~~(~~Cunnac ~~et al.~~*et al.*, 2004b, Mukaihara ~~et al.~~*et al.*, 2010)~~Cunnac, 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}{Mukaihara, 2010, Genome-Wide Identification of a Large Repertoire of Ralstonia solanacearum Type III Effector Proteins by a New Functional Screen}). We have extended the usage of the Rip nomenclature « *R. solanacearum* proteins injected into plant cells » already used (Cunnac *et al.*, 2004b, Mukaihara *et al.*, 2010) and have now defined more than hundred "Rip genes" in the *R. solanacearum* species complex (unpublished data).~~

As already pointed out when the first *R. solanacearum* genome was published (Salanoubat ~~et al.~~*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.~~*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~~sp~~. specific Transcription Activator-Like T3E (Fall ~~et al.~~*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, Solé *et al.*, 2012)~~. Interestingly most of these

Formatted: English (U.S.)

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Field Code Changed

Formatted: Underline

Formatted: Underline

Formatted: Underline

Formatted: Highlight

Field Code Changed

Field Code Changed

gene families are conserved among the different *R. solanacearum* strains (Sole ~~et al.~~*et al.*, 2012, Remigi ~~et al.~~*et al.*, 2011), see also the MAGe genome browser displaying the sequenced *R. solanacearum* strains (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.~~*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 GMI1000 causes wilting and is undistinguishable from the K60 tobacco-pathogenic strain (Poueymiro ~~et al.~~*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.~~*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.~~*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. ~~:o~~On tomato, mutants in the T3E *RSp0304* (*HopD1* homolog) and *AWR2*, show a decreased disease progression (Cunnac ~~et al.~~*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.~~*et al.*, 2012).

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

For the GALAs, another well studied T3E family, it was demonstrated that they are collectively required on tomato and ~~Arabidopsis~~*A~~rabidopsis~~. thaliana* for a full-disease phenotype (Angot ~~et al.~~*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.~~*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.~~*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.~~*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.~~*et al.*, 2004b). This low yield of T3Es with virulence functions ~~out of an important number of~~compared to the large T3E repertoire~~s~~ (Poueymiro & Genin, 2009), could be explained by functional redundancy (Sole ~~et al.~~*et al.*, 2012, Angot ~~et al.~~*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~~to~~ 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.~~*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 phylotype 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.~~*et al.*, 1998, Deslandes ~~et al.~~*et al.*, 2002, Deslandes ~~et al.~~*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

Field Code Changed

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Field Code Changed

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 we~~It 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.~~*et al.*, 2003). This ~~could~~ suggest that cross-talk can occur~~s~~ between resistance to *R. solanacearum* and developmental pathways (Godiard ~~et al.~~*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 5 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) T~~t~~omato cultivar 'Hawaii 7996'~~. This cultivar was described~~ has been described ~~as one of the most stable sources of~~as ~~resistance~~ resistant 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~~2~~) 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)~~(Qian *et al.*, 2012)~~. In eggplant, four QTLs have been identified in a RIL population~~, these QTLs~~ that exhibit ~~ing~~ 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.~~*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.~~*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.~~*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.

Formatted: Font: Italic

Field Code Changed

Formatted: Font: Not Italic

Formatted: Normal, Line spacing: single

Formatted: Line spacing: Multiple 1.15 li

Field Code Changed

Field Code Changed

15

Moreover, salicylic acid (SA) was identified as a key element of *wat1*-mediated resistance to *R. solanacearum* (Denance et al.*et al.*, 2012). Several other studies identified WRKY transcription factors as important players in modulating the plant response towards *R. solanacearum* attack. Indeed, Mukhtar *et al.* (2008) showed that Arabidopsis*Arabidopsis. thaliana* plants lacking a functional *WRKY27* gene exhibited an enhanced tolerance to *R. solanacearum* strains GMI1000 and Rd-15 (Mukhtar *et al.*, 2008)(Mukhtar *et al.*, 2008). Similar situations were previously observed for the ethylene-insensitive *EIN2-1* gene (Hirsch *et al.*, 2002) and the *NWS1* gene (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 *R. solanacearum* resistance: Overexpression of *CaWRKY40* in tobacco enhanced resistance to *R. solanacearum,* whereas silencing of *CaWRKY40* in pepper attenuated the resistance (Dang *et al.*, 2012). On the other hand, *CaWRKY58*-overexpressing tobacco plants showed an enhanced susceptibility to the same strain, and *CaWRKY58*-silenced pepper plants displayed and enhanced resistance (Wang *et al.*, 2013b). In another work, Feng *et al.* (2012) identified the abscisic acid (ABA) signaling pathway as important for biological control of Bacterial wilt in *A. thaliana*. This ABA-dependent defense mechanism was shown to be independent of SA, JA and ethylene in the biological control exerted by an *hrpB* mutant of *R. solanacearum* (Feng *et al.*, 2012).

*in vitro*Gnotobiotic pathosystems to study root infection

As *R. solanacearum* infects plants *via* their roots, *in vitro*gnotobiotic models artificial inoculation systems constitute useful tools enabling to access the host roots systems. Such bioassays, in which axenic plantlets are inoculated with *R. solanacearum* were described on tomato (Monteiro et al.*et al.*, 2012b, Vasse *et al.*, 1995), petunia (Zolobowska & Van Gijsegem, 2006), *M. truncatula* (Vailleau et al.*et al.*, 2007), and *A. thaliana* (Digonnet *et al.*, 2012). They enable to study the early steps of infection, to follow colonization step by step, and to observe "root symptoms". *R. solanacearum*-plant interaction at the root level was first studied in tomato (Monteiro et al.*et al.*, 2012b, Vasse et al.*et al.*, 1995). The authors observed the bacterium penetration *via* the root extremities and at the axils of secondary root (Vasse et al.*et al.*, 1995)(Vasse et al., 1995). Ten years later, the set up of *in vitro*a gnotobiotic experiments on petunia allowed the identification of new root lateral structures (RLS) after the inoculation with *R. solanacearum* (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 (Zolobowska & Van Gijsegem, 2006). In the *M. truncatula* *in vitro*gnotobiotic pathosystem, it was described that *R. solanacearum* penetrates specifically *via* the root tips (Turner et al.*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 (Turner et al.*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 (Turner et al.*et al.*, 2009). For the interaction involving *A. thaliana* and *R. solanacearum*, well described and characterized at the whole plant level (Deslandes et al.*et al.*, 1998), an *in vitro*an axenic plant bioassay system has been recently developed to study the early steps of root colonization by *R. solanacearum* (Digonnet et al.*et al.*, 2012). The authors

Field Code Changed

Field Code Changed

Formatted: Font: Not Italic

Formatted: Normal, Line spacing: single

Formatted: Line spacing: Multiple 1.15 li

Formatted: Font: Not Italic

Field Code Changed

Formatted: English (U.S.)

Field Code Changed

Field Code Changed

Formatted: English (U.S.)

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.~~*et al.*, 2012). Contrary to the *M. truncatula* ~~*in vitro*~~ system (Vailleau ~~et al.~~*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 were 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.

~~in the plant side~~

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~~a better description of the species complex, deciphering the role of the T3Es during infection, A~~integrative a~~nalysis of ~~global~~ *in planta* expression ~~profiling~~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.~~*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?

Field Code Changed

Formatted: Normal, Line spacing: single

Formatted: Line spacing: Multiple 1.15 li

Formatted: Normal, Line spacing: single

Formatted: Line spacing: Multiple 1.15 li

Acknowledgements:

We thank Freddy Monteiro and Irene Van Dijk for their assistance in the elaboration of figure 2.

We want to apologize to colleagues whose work could not be cited because of space limitation.

We also thank our colleagues and two anonymous reviewers for their constructive comments.

Bibliography

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

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

Angot, A., Vergunst, A., Genin, S. and Peeters, N. (2007) Exploitation of eukaryotic ubiquitin signaling pathways by effectors translocated by bacterial type III and type IV secretion systems. *PLoS Pathog,* **3,** e3.

Araud-Razou, I., Vasse, J., Montrozier, H., Etchebar, C. and Trigalet, A. (1998) Detection and visualization of the major acidic exopolysaccharide of Ralstonia solanacearum and its role in tomato root infection and vascular colonization. *Eur. J. Plant Pathol.,* **104,** 795–809.

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

Arlat, M., Van Gijsegem, F., Huet, J. C., Pernollet, J. C. and Boucher, C. A. (1994) PopA1, a protein which induces a hypersensitivity-like response on specific Petunia genotypes, is secreted via the Hrp pathway of Pseudomonas solanacearum. *EMBO J.* **13,** 543-553.

Ben, C., Debelle, F., Berges, H., Bellec, A., Jardinaud, M. F., Anson, P., *et al.* (2013) MtQRRS1, a R-locus required for *Medicago truncatula* quantitative Resistance to *Ralstonia solanacearum*. *New Phytol..,* **in press**.

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

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

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

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

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

Carmeille, A., Caranta, C., Dintinger, J., Prior, P., Luisetti, J. and Besse, P. (2006) Identification of QTLs for Ralstonia solanacearum race 3-phylotype II resistance in tomato. *Theor. Appl. Genet.,* **113,** 110-121.

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

Formatted: Normal, Line spacing: single

Cases, I. and de Lorenzo, V. (2001) The black cat/white cat principle of signal integration in bacterial promoters. *EMBO J,* **20,** 1-11.

Castillo, J. A. and Greenberg, J. T. (2007) Evolutionary dynamics of Ralstonia solanacearum. *Applied and Environmental Microbiology,* **73,** 1225-1238.

Cellier, G., Remenant, B., Chiroleu, F., Lefeuvre, P. and Prior, P. (2012) Phylogeny and Population Structure of Brown Rot- and Moko Disease-Causing Strains of Ralstonia solanacearum Phylotype II. *Appl. Environ. Microbiol.,* **78,** 2367-2375.

Clough, S. J., Lee, K. E., Schell, M. A. and Denny, T. P. (1997) A two-component system in Ralstonia (Pseudomonas) solanacearum modulates production of PhcA-regulated virulence factors in response to 3-hydroxypalmitic acid methyl ester. *J. Bacteriol.,* **179,** 3639-3648.

Coll, N. S. and Valls, M. (2013) Current knowledge on the Ralstonia solanacearum type III secretion system. *Microbial Biotechnology,* **In press**.

Coupat, B., Chaumeille-Dole, F., Fall, S., Prior, P., Simonet, P., Nesme, X*., et al.* (2008) Natural transformation in the Ralstonia solanacearum species complex: number and size of DNA that can be transferred. *FEMS Microbiol. Ecol.,* **66,** 14-24.

Coupat-Goutaland, B., Bernillon, D., Guidot, A., Prior, P., Nesme, X. and Bertolla, F. (2011) Ralstonia solanacearum Virulence Increased Following Large Interstrain Gene Transfers by Natural Transformation. *Mol. Plant-Microb. Interact.,* **24,** 497-505.

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

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

Danesh, D., Aarons, S., McGill, G. E. and Young, N. D. (1994) Genetic dissection of oligogenic resistance to bacterial wilt in tomato. *Mol. Plant-Microbe Interact.,* **7,** 464-471.

Dang, F. F., Wang, Y. N., Yu, L., Eulgem, T., Lai, Y., Liu, Z. Q*., et al.* (2012) CaWRKY40, a WRKY protein of pepper, plays an important role in the regulation of tolerance to heat stress and resistance to Ralstonia solanacearum infection. *Plant Cell Environ.,* **36,** 757-774.

Delannoy, E., Stanley, W. A., Bond, C. S. and Small, I. D. (2007) Pentatricopeptide repeat (PPR) proteins as sequence-specificity factors in post-transcriptional processes in organelles. *Biochem Soc Trans,* **35,** 1643-1647.

Delaspre, F., Nieto Penalver, C. G., Saurel, O., Kiefer, P., Gras, E., Milon, A*., et al.* (2007) The Ralstonia solanacearum pathogenicity regulator HrpB induces 3-hydroxy-oxindole synthesis. *Proc. Natl Acad. Sci. USA,* **104,** 15870-15875.

Denance, N., Ranocha, P., Oria, N., Barlet, X., Riviere, M. P., Yadeta, K. A*., et al.* (2012) Arabidopsis wat1 (walls are thin1)-mediated resistance to the bacterial vascular pathogen, Ralstonia solanacearum, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. *Plant J.,* **73,** 225-239.

Denny, T. P. and Baek, S. R. (1991) Genetic evidence that extracellular polysaccharide is a virulence factor of *Pseudomonas solanacearum*. *Mol. Plant-Microbe Interact.,* **4,** 198-206.

Deslandes, L., Olivier, J., Peeters, N., Feng, D. X., Khounlotham, M., Boucher, C*., et al.* (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc. Natl. Acad. Sci. USA,* **100,** 8024-8029.

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

Deslandes, L., Pileur, F., Liaubet, L., Camut, S., Can, C., Williams, K*., et al.* (1998) Genetic characterization of RRS1, a recessive locus in Arabidopsis thaliana that confers resistance to the bacterial soilborne pathogen Ralstonia solanacearum. *Mol. Plant-Microbe Interact.,* **11,** 659-667.

Digonnet, C., Martinez, Y., Denance, N., Chasseray, M., Dabos, P., Ranocha, P*., et al.* (2012) Deciphering the route of *Ralstonia solanacearum* colonization in *Arabidopsis thaliana* roots during a compatible interaction: focus at the plant cell wall. *Planta,* **236,** 1419-1431.

Elphinstone, J. G. (2005) The current Bacterial Wilt situation: A global overview. In: *Bacterial Wilt Disease and the Ralstonia solanaceaum Species Complex.* (Caitilyn Allen, P. P. a. A. C. H., ed.). The American Phytopathological Society, 3340 Pilot Knob Road St. Paul, Minnesota 55121, U.S.A., pp. 9-28.

Erhardt, M., Namba, K. and Hughes, K. T. (2010) Bacterial nanomachines: the flagellum and type III injectisome. *Cold Spring Harb Perspect Biol,* **2,** a000299.

Fall, S., Mercier, A., Bertolla, F., Calteau, A., Gueguen, L., Perriere, G*., et al.* (2007) Horizontal gene transfer regulation in bacteria as a "spandrel" of DNA repair mechanisms. *PLoS One,* **2,** e1055.

Fegan, M. and Prior, P. (2005) How Complex is the "Ralstonia solanacearum Species Complex". In: *Bacterial Wilt Disease and the Ralstonia solanaceaum Species Complex.* (Allen, C., Prior, P. and Hayward, A. C., eds.). The American Phytopathological Society, 3340 Pilot Knob Road St. Paul, Minnesota 55121, U.S.A., pp. 449-461.

Feng, D. X., Deslandes, L., Keller, H., Revers, F., Favery, B., Lecomte, P*., et al.* (2004) Isolation and characterization of a novel Arabidopsis thaliana mutant unable to develop wilt symptoms after inoculation with a virulent strain of Ralstonia solanacearum. *Phytopathology,* **94,** 289-295.

Feng, D. X., Tasset, C., Hanemian, M., Barlet, X., Hu, J., Trémousaygue, D*., et al.* (2012) Biological control of bacterial wilt in Arabidopsis thaliana involves abscissic acid signalling. *New Phytol.,* **194,** 1035-1045.

Flavier, A. B., Clough, S. J., Schell, M. A. and Denny, T. P. (1997) Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in Ralstonia solanacearum. *Mol. Microbiol.,* **26,** 251-259.

Gabriel, D. W., Allen, C., Schell, M., Denny, T. P., Greenberg, J. T., Duan, Y. P*., et al.* (2006) Identification of open reading frames unique to a select agent: Ralstonia solanacearum race 3 biovar 2. *Mol. Plant-Microbe Interact.,* **19,** 69-79.

Garg, R. P., Huang, J. Z., Yindeeyoungyeon, W., Denny, T. P. and Schell, M. A. (2000) Multicomponent transcriptional regulation at the complex promoter of the exopolysaccharide I biosynthetic operon of Ralstonia solanacearum. *J. Bacteriol.,* **182,** 6659-6666.

Genin, S. (2010) Molecular traits controlling host range and adaptation to plants in Ralstonia solanacearum. *New Phytol.,* **187,** 920-928.

Genin, S. and Boucher, C. (2002) Pathogen profile Ralstonia solanacearum : secrets of a major pathogen unveiled by analysis of its genome. *Mol. Plant Pathol.,* **3,** 111-118.

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

Genin, S. and Denny, T. P. (2012) Pathogenomics of the Ralstonia solanacearum Species Complex. In: *Annu. Rev. Phytopathol., Vol 50.* pp. 67-89.

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

Godiard, L., Sauviac, L., Torii, K. U., Grenon, O., Mangin, B., Grimsley, N. H*., et al.* (2003) ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. *Plant J.,* **36,** 353-365.

Guidot, A., Coupat, B., Fall, S., Prior, P. and Bertolla, F. (2009) Horizontal gene transfer between Ralstonia solanacearum strains detected by comparative genomic hybridization on microarrays. *ISME J.,* **3,** 549-562.

Guidot, A., Prior, P., Schoenfeld, J., Carrere, S., Genin, S. and Boucher, C. (2007) Genomic structure and phylogeny of the plant pathogen Ralstonia solanacearum inferred from gene distribution analysis. *J. Bacteriol.,* **189,** 377-387.

Hernandez-Blanco, C., Feng, D. X., Hu, J., Sanchez-Vallet, A., Deslandes, L., Llorente, F*., et al.* (2007) Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance. *Plant Cell,* **19,** 890-903.

Hirsch, J., Deslandes, L., Feng, D. X., Balague, C. and Marco, Y. (2002) Delayed symptom development in ein2-1, an Arabidopsis ethylene-insensitive mutant, in response to bacterial wilt caused by Ralstonia solanacearum. *Phytopathology,* **92,** 1142-1148.

Ho, M. S., Ou, C., Chan, Y. R., Chien, C. T. and Pi, H. (2008) The utility F-box for protein destruction. *Cellular and Molecular Life Sciences,* **65,** 1977-2000.

Huang, J., Carney, B. F., Denny, T. P., Weissinger, A. K. and Schell, M. A. (1995) A complex network regulates expression of eps and other virulence genes of Pseudomonas solanacearum. *J. Bacteriol.,* **177,** 1259-1267.

Huang, J., Yindeeyoungyeon, W., Garg, R. P., Denny, T. P. and Schell, M. A. (1998) Joint transcriptional control of xpsR, the unusual signal integrator of the Ralstonia solanacearum virulence gene regulatory network, by a response regulator and a LysR-type transcriptional activator. *J. Bacteriol.,* **180,** 2736-2743.

Husain, A. and Kelman, A. (1958) Relation of slime production to mechanism of wilting and pathogenicity of Pseudomonas solanacearum. *Phytopathology,* **48,** 155-164.

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

Jones, J. D. and Dangl, J. L. (2006) The plant immune system. *Nature,* **444,** 323-329.

Kajava, A. V., Anisimova, M. and Peeters, N. (2008) Origin and evolution of GALA-LRR, a new member of the CC-LRR subfamily: from plants to bacteria? *PLoS One,* **3,** e1694.

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

Kang, Y. W., Liu, H. L., Genin, S., Schell, M. A. and Denny, T. P. (2002) Ralstonia solanacearum requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. *Mol. Microbiol.,* **46,** 427-437.

Kao, C. C., Barlow, E. and Sequeira, L. (1992) Extracellular polysaccharide is required for wild-type virulence of Pseudomonas solanacearum. *J. Bacteriol.,* **174,** 1068-1071.

Konstantinidis, K. T., Ramette, A. and Tiedje, J. M. (2006) The bacterial species definition in the genomic era. *Philos Trans R Soc Lond B Biol Sci,* **361,** 1929-1940.

Konstantinidis, K. T. and Tiedje, J. M. (2005) Genomic insights that advance the species definition for prokaryotes. *Proc. Natl. Acad. Sci. USA,* **102,** 2567-2572.

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

Lebeau, A., Daunay, M. C., Frary, A., Palloix, A., Wang, J. F., Dintinger, J*., et al.* (2011) Bacterial Wilt Resistance in Tomato, Pepper, and Eggplant: Genetic Resources Respond to Diverse Strains in the Ralstonia solanacearum Species Complex. *Phytopathology,* **101,** 154-165.

Lebeau, A., Gouy, M., Daunay, M. C., Wicker, E., Chiroleu, F., Prior, P*., et al.* (2013) Genetic mapping of a major dominant gene for resistance to Ralstonia solanacearum in eggplant. *Theor Appl Genet*. **in press**

Li, L., Atef, A., Piatek, A., Ali, Z., Piatek, M., Aouida, M*., et al.* (2013) Characterization and DNA-Binding Specificities of Ralstonia TAL-like Effectors. *Mol Plant*. **in press**

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

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

Mangin, B., Thoquet, P., Olivier, J. and Grimsley, N. H. (1999) Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci. *Genetics,* **151,** 1165-1172.

Meng, F., Yao, J. and Allen, C. (2011) A MotN mutant of Ralstonia solanacearum is hypermotile and has reduced virulence. *J. Bacteriol.,* **193,** 2477-2486.

Mole, B. M., Baltrus, D. A., Dangl, J. L. and Grant, S. R. (2007) Global virulence regulation networks in phytopathogenic bacteria. *Trends Microbiol.,* **15,** 363-371.

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

Monteiro, F., Sole, M., van Dijk, I. and Valls, M. (2012b) A Chromosomal Insertion Toolbox for Promoter Probing, Mutant Complementation, and Pathogenicity Studies in Ralstonia solanacearum. *Mol. Plant-Microb. Interact.,* **25,** 557-568.

Mukaihara, T., Tamura, N. and Iwabuchi, M. (2010) Genome-Wide Identification of a Large Repertoire of Ralstonia solanacearum Type III Effector Proteins by a New Functional Screen. *Mol. Plant-Microb. Interact.,* **23,** 251-262.

Mukhtar, M. S., Deslandes, L., Auriac, M. C., Marco, Y. and Somssich, I. E. (2008) The Arabidopsis transcription factor WRKY27 influences wilt disease symptom development caused by Ralstonia solanacearum. *Plant J.,* **56,** 935-947.

Murata, Y., Tamura, N., Nakaho, K. and Mukaihara, T. (2006) Mutations in the IrpE gene of Ralstonia solanacearum affects Hrp Pili production and virulence. *Mol. Plant-Microb. Interact.,* **19,** 884-895.

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

Orgambide, G., Montrozier, H., Servin, P., Roussel, J., Trigalet-Demery, D. and Trigalet, A. (1991) High heterogeneity of the exopolysaccharides of Pseudomonas solanacearum strain GMI 1000 and the complete structure of the major polysaccharide. *J. Biol. Chem.,* **266,** 8312-8321.

Plener, L., Boistard, P., Gonzalez, A., Boucher, C. and Genin, S. (2012) Metabolic adaptation of Ralstonia solanacearum during plant infection: a methionine biosynthesis case study. *PLoS One,* **7,** e36877.

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

Poueymiro, M., Cunnac, S., Barberis, P., Deslandes, L., Peeters, N., Cazale-Noel, A. C*., et al.* (2009) Two Type III Secretion System Effectors from Ralstonia solanacearum GMI1000 Determine Host-Range Specificity on Tobacco. *Mol. Plant-Microb. Interact.,* **22,** 538-550.

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

Qian, Y. L., wang, X. S., D.Z., W., Zhang, L. N., Zu, C. L., Gao, Z. L*., et al.* (2012) The detection of QTLs controlling bacterial wilt resistance in tobacco (*N. tabacun* L.). *Euphitica,* **in press**.

Remenant, B., Babujee, L., Lajus, A., Medigue, C., Prior, P. and Allen, C. (2012) Sequencing of K60, Type Strain of the Major Plant Pathogen Ralstonia solanacearum. *J. Bacteriol.,* **194,** 2742-2743.

Remenant, B., Coupat-Goutaland, B., Guidot, A., Cellier, G., Wicker, E., Allen, C*., et al.* (2010) Genomes of three tomato pathogens within the Ralstonia solanacearum species complex reveal significant evolutionary divergence. *BMC Genomics,* **11**.

Remenant, B., de Cambiaire, J. C., Cellier, G., Jacobs, J. M., Mangenot, S., Barbe, V*., et al.* (2011) Ralstonia syzygii, the Blood Disease Bacterium and Some Asian R. solanacearum Strains Form a Single Genomic Species Despite Divergent Lifestyles. *Plos One,* **6,** e24356.

Remigi, P., Anisimova, M., Guidot, A., Genin, S. and Peeters, N. (2011) Functional diversification of the GALA type III effector family contributes to Ralstonia solanacearum adaptation on different plant hosts. *New Phytol.,* **192,** 976-987.

Roberts, S. J., Edengreen, S. J., Jones, P. and Ambler, D. J. (1990) Pseudomonas syzygii, sp-nov, the cause of Sumatra disease of cloves. *Syst. Appl. Microbiol.,* **13,** 34-43.

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

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

Schell, M. A. (2000) Control of virulence and pathogenicity genes of Ralstonia solanacearum by an elaborate sensory network. *Annu. Rev. Phytopathol.,* **38,** 263-292.

Schell, M. A., Denny, T. P. and Huang, J. (1994) VsrA, a second two-component sensor regulating virulence genes of Pseudomonas solanacearum. *Mol. Microbiol.,* **11,** 489-500.

Singer, A. U., Rohde, J. R., Lam, R., Skarina, T., Kagan, O., Dileo, R*., et al.* (2008) Structure of the Shigella T3SS effector IpaH defines a new class of E3 ubiquitin ligases. *Nat. Struct. Mol. Biol.,* **15,** 1293-1301.

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

Tampakaki, A. P., Skandalis, N., Gazi, A. D., Bastaki, M. N., Sarris, P. F., Charova, S. N*., et al.* (2010) Playing the "Harp": evolution of our understanding of hrp/hrc genes. *Annu. Rev. Phytopathol.,* **48,** 347-370.

Tang, X., Xiao, Y. and Zhou, J. M. (2006) Regulation of the type III secretion system in phytopathogenic bacteria. *Mol. Plant-Microbe Interact.,* **19,** 1159-1166.

Tans-Kersten, J., Huang, H. Y. and Allen, C. (2001) Ralstonia solanacearum needs motility for invasive virulence on tomato. *J. Bacteriol.,* **183,** 3597-3605.

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

Thoquet, P., Olivier, J., Sperisen, C., Rogowsky, P., Laterrot, H. and Grimsley, N. (1996a) Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii7996. *Mol. Plant-Microbe Interact.,* **9,** 826-836.

Thoquet, P., Olivier, J., Sperisen, C., Rogowsky, P., Prior, P., Anaïs, G*., et al.* (1996b) Polygenic resistance of tomato plants to bacterial wilt in the French West Indies. *Mol. Plant-Microbe Interact.,* **9,** 837-842.

Torii, K. U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R. F*., et al.* (1996) The Arabidopsis ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. *Plant Cell,* **8,** 735-746.

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

Vailleau, F., Sartorel, E., Jardinaud, M. F., Chardon, F., Genin, S., Huguet, T*., et al.* (2007) Characterization of the interaction between the bacterial wilt pathogen Ralstonia solanacearum and the model legume plant Medicago truncatula. *Mol. Plant-Microb. Interact.,* **20,** 159-167.

Valls, M., Genin, S. and Boucher, C. (2006) Integrated regulation of the type III secretion system and other virulence determinants in Ralstonia solanacearum. *Plos Pathogens,* **2,** 798-807.

Van der Linden, L., Bredenkamp, J., Naidoo, S., Fouché-Weich, J., Denby, K. J., Genin, S.*, et al.* (2013) Gene-for-Gene Tolerance to Bacterial Wilt in Arabidopsis. *Mol. Plant-Microbe Interact.,* **26,** 398-406.

Vasse, J., Frey, P. and Trigalet, A. (1995) Microscopic studies of intercellular infection and protoxylem invasion of tomato roots by Pseudomonas solanacearum. *Mol. Plant-Microb. Interact.,* **8,** 241-251.

Wairuri, C. K., van der Waals, J. E., van Schalkwyk, A. and Theron, J. (2012) Ralstonia solanacearum needs Flp pili for virulence on potato. *Mol. Plant-Microbe Interact.,* **25,** 546-556.

Wang, J. F., Ho, F. I., Truong, H. T. H., Huang, S. M., Balatero, C. H., Dittapongpitch, V.*, et al.* (2013a) Identification of major QTLs associated with stable resistance of tomato cultivar 'Hawaii 7996' to *Ralstonia solanacearum*. *Euphytica,* **190,** 241-252.

Wang, J. F., Olivier, J., Thoquet, P., Mangin, B., Sauviac, L. and Grimsley, N. H. (2000) Resistance of tomato line Hawaii7996 to Ralstonia solanacearum Pss4 in Taiwan is controlled mainly by a major strain-specific locus. *Mol. Plant-Microb. Interact.,* **13,** 6-13.

Wang, K., Dang, F., Liu, Z., Wang, X., Eulgem, T., Lai, Y.*, et al.* (2013b) CaWRKY58, encoding a groupe I WRKY transcription factor of Capsicum annuum, negatively regulates resistance to Ralstonia solanacearuù infection. *Mol. Plant Pathol.,* **14,** 131-144.

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

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M.*, et al.* (2009a) Ralstonia solanacearum Strains from Martinique (French West Indies) Exhibiting a New Pathogenic Potential (vol 73, pg 6790, 2007). *Appl. Environ. Microbiol.,* **75,** 558-558.

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D. and Prior, P. (2009b) Epidemiological evidence for the emergence of a new pathogenic variant of Ralstonia solanacearum in Martinique (French West Indies). *Plant Pathol.,* **58,** 853-861.

Wicker, E., Lefeuvre, P., de Cambiaire, J. C., Lemaire, C., Poussier, S. and Prior, P. (2012) Contrasting recombination patterns and demographic histories of the plant pathogen Ralstonia solanacearum inferred from MLSA. *ISME J.,* **6,** 961-974.

Xu, J., Zheng, H. J., Liu, L., Pan, Z. C., Prior, P., Tang, B.*, et al.* (2011) Complete Genome Sequence of the Plant Pathogen Ralstonia solanacearum Strain Po82. *J. Bacteriol.,* **193,** 4261-4262.

Yao, J. and Allen, C. (2006) Chemotaxis is required for virulence and competitive fitness of the bacterial wilt pathogen Ralstonia solanacearum. *J. Bacteriol.,* **188,** 3697-3708.

Yao, J. and Allen, C. (2007) The plant pathogen Ralstonia solanacearum needs aerotaxis for normal Biofilm formation and interactions with its tomato host. *J. Bacteriol.,* **189,** 6415-6424.

Yoshimochi, T., Hikichi, Y., Kiba, A. and Ohnishi, K. (2009a) The global virulence regulator PhcA negatively controls the Ralstonia solanacearum hrp regulatory cascade by repressing expression of the PrhIR signalling proteins. *J. Bacteriol.,* **1,** 3424-3428.

Yoshimochi, T., Zhang, Y., Kiba, A., Hikichi, Y. and Ohnishi, K. (2009b) Expression of hrpG and activation of response regulator HrpG are controlled by distinct signal cascades in Ralstonia solanacearum. *J.Gen. Plant Pathol.,* **75,** 196-204.

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

Zolobowska, L. and Van Gijsegem, F. (2006) Induction of lateral root structure formation on petunia roots: A novel effect of GMI1000 Ralstonia solanacearum infection impaired in Hrp mutants. *Mol. Plant-Microb. Interact.,* **19,** 597-606.

Zou, L. F., Wang, X. P., Xiang, Y., Zhang, B., Li, Y. R., Xiao, Y. L.*, et al.* (2006) Elucidation of the hrp clusters of Xanthomonas oryzae pv. oryzicola that control the hypersensitive response in

nonhost tobacco and pathogenicity in susceptible host rice. *Appl. Environ. Microbiol.,* **72,** 6212-6224.

Figure legends

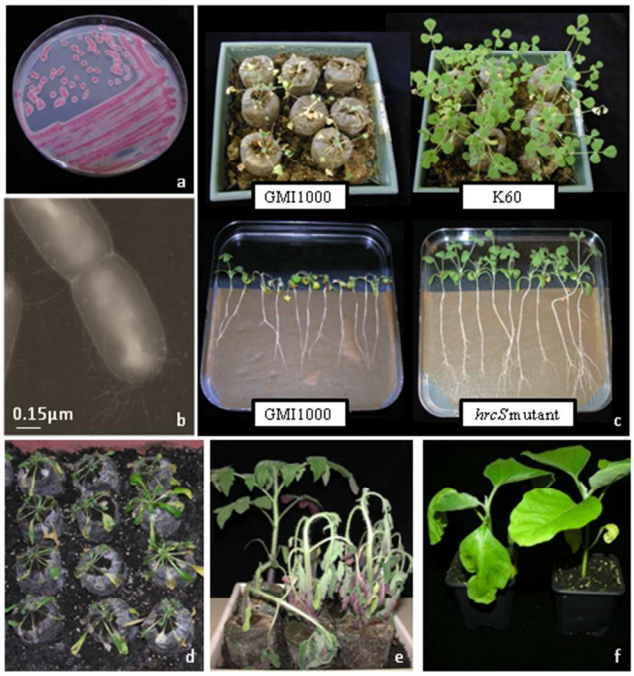
Fig. 1 Macro- and microscopic views of *R. solanacearum* and illustration of symptoms associated on plant bioassays.- (a) *R. solanacearum* growing on complete BG medium (Boucher *et al.*, 1985). The pink color of the colonies is due to the presence of triphenyl tetrazolium chloride in the medium. (b) Electron microscopy image of *R. solanacearum* rod-shaped cells under division displaying pili 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.

Formatted: Normal, Line spacing: single

Formatted: Space After: 10 pt, Line spacing: Multiple 1.15 li

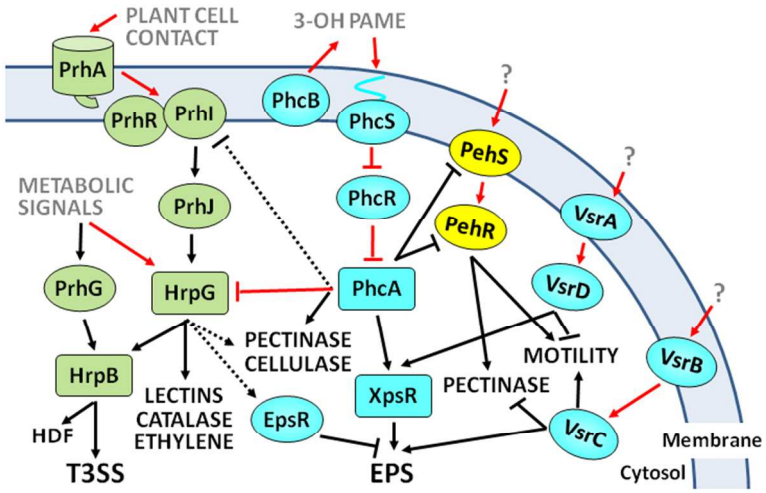
Fig. 2. Major pathways controlling expression of *Ralstonia solanacearum* virulence genes.

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.



190x254mm (96 x 96 DPI)

Figure 2



254x190mm (96 x 96 DPI)