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ABSTRACT

For the development of pathogen-informed breeding strategies, identifying the microbial genes involved in interactions with the plant is a critical step. To identify Type III effector (T3E) repertoires associated to virulence of the bacterial wilt (BW) pathogen *Ralstonia solanacearum* on Solanaceous crops, we used an original association genetics approach combining DNA microarray data and pathogenicity data on resistant eggplant, pepper and tomato accessions. From this first screen, twenty-five Type III effectors were further full-length PCR-amplified within a 35-strain field collection, to assess their distribution and allelic diversity. Six T3E repertoire groups were identified, within which 11 representative strains were chosen to challenge the BW-resistant eggplants “Dingras multiple Purple” and “AG91-25”, and the tomato Hawaii 7996. The virulence or avirulence phenotypes could not be explained by specific T3E repertoires, but rather by individual T3E genes. We identified seven highly avirulence-associated genes, among which *ripP2*, primarily referenced as conferring avirulence to *Arabidopsis thaliana*. Interestingly, no T3E was associated to avirulence to both eggplants. Highly virulence-associated genes were also identified: *ripA5_2*, *ripU,* and *ripV2*. This study should be regarded as a first step towards investigating both avirulence or virulence function of the highlighted genes, but also their evolutionary dynamics in natural *R. solanacearum* populations.

INTRODUCTION

Effective and durable control of plant pathogens is a critical challenge for global food security. Once a pathogen has established in an area, the use of genetically resistant plants is one of the most effective control approaches, particularly against bacterial pathogens for which chemical control and agricultural practices remain ineffective or unpractical solutions (Strange and Scott 2005). Now the central challenge of this approach is the identification of broad-spectrum and durable resistance genes/QTLs. Resistance durability was first measured retrospectively (see for example van den Bosch and Gilligan (2003)), but recent studies demonstrated that this trait may be inferred from the resistance level and spectrum of the plant R genes/QTLs and their genetic background (Palloix et al. 2009, Quenouille et al. 2014). Evolutionary potential of the pathogen (McDonald and Linde 2002), and specifically evolutionary forces at work on the pathogen genes involved in virulence are also important traits for resistance durability. Leach's seminal paper proposed that durability of plant R genes could be inferred from the pathogen fitness penalty induced by adaptation to this gene, including the loss of the cognate avirulence gene (Leach et al. 2001).

In the model currently describing the evolution of plant-pathogen microbes interactions (Jones and Dangl 2006), pathogens secrete effectors that suppress or subvert the resistance responses triggered by microbe-associated molecular patterns (MAMPs). This dichotomy between MAMP-Triggered immunity (MTI) and Effector-Triggered Immunity (ETI) has been recently questioned by Cook et al. (2015), who proposed to consider the plant innate immunity as "a system that evolves to detect invasion". Plant-pathogenic bacteria possess a large repertoire of secreted effectors, where the Type III Secretion System (T3SS) plays a central role in virulence (Kenny and Valdivia 2009). Numerous functional genetics assays have tried to decipher the respective role of many individual effectors (for a review, see for example

(Chang et al. 2014, Feng and Zhou 2012)), but the association of individual effectors to an avirulence or virulence phenotype remains problematic due to functional redundancy (Cunnac et al. 2011). Genome-wide association mapping may be a promising alternative approach, allowing to associate not only individual genes but gene repertoires to a phenotype (Falush and Bowden 2006). We applied such an approach on the soilborne Beta-Proteobacterium *Ralstonia solanacearum*, a vascular plant pathogen whose host range is particularly huge (more than 54 botanical families). It induces bacterial wilt (BW) on Solanaceae but also more than fifty other families (among which Zingiberaceae, Fabaceae, Myrtaceae, …), causing heavy losses throughout the tropical and subtropical belt in Asia, Africa, and America. Breeding efforts for resistance to this disease have been hindered for years by the lack of complete resistance genitors, the strong interactions between resistance and local strains, as well as by the huge genomic and phenotypic plasticity of the pathogen. Among proteobacterial plant pathogens, *R. solanacearum* possesses a very large repertoire of genes contributing to virulence, including genes involved in aero- and chimio-tactism (Yao and Allen 2006, 2007), reactive oxygen species (ROS) detoxification (Flores-Cruz and Allen 2011), multidrug efflux pumps (Brown et al. 2007), Tat secretome (Gonzalez et al. 2007), but the secreted proteins (effectors) distributed by the T3SS constitute the key virulence factors (Poueymiro and Genin 2009). Whereas most proteobacterial plant pathogens possess 20-30 Type III effectors (or T3E), *R. solanacearum* meta-repertoire gathers 94 families (orthologous groups) (Peeters et al. 2013), and individual strains usually carry 60-75 effectors (Deslandes and Genin 2014). Genes governing specific plant-*R.solanacearum* interactions have been identified in the model plants *Arabidopsis thaliana* and *Medicago truncatula* (for a review, see Deslandes and Genin (2014)). The most documented example is the acetyltransferase popP2 (Deslandes et al. 2003), recently renamed ripP2 (Peeters et al. 2013), whose interaction with the *A.thaliana* gene RRS1-R (Deslandes et al. 1998, Deslandes et al. 2002) and the cysteine protease RD19 (Bernoux et al. 2008) triggers plant immunity, making it the first avirulence gene described in *R. solanacearum*. It was recently demonstrated that RRS1-R forms a receptor complex with RPS4 (conferring resistance to *Pseudomonas syringae*) that detect the WRKY-targeting effectors, and convert the lysine-acetylase activity of RipP2 to immunity (Le Roux et al. 2015, Sarris et al. 2015). Other effectors have been identified, such as the cysteine protease ripP1 that elicits a Hypersensitive Response (HR) on some Petunia (Poueymiro et al. 2009, Poueymiro and Genin 2009) and tobacco species, and RipG7 which is required for pathogenicity on *Medicago truncatula* (Angot et al. 2006). Whereas, bacterial genetic factors that are critical for virulence and avirulence to cultivated species remain largely unknown. The Zinc-dependent protease rip36 (Nahar et al. 2014), renamed ripAX2 (Peeters et al. 2013), induces a HR on *Solanum torvum*, a wild relative of eggplant. RipA2 contributes to pathogenicity to tomato, whereas ripA5 elicits a HR-like on some tobacco species (Sole et al. 2012). The ripG2, ripG3, ripG6 and ripG7 effector proteins collectively contribute to pathogenicity to tomato and Arabidopsis (Remigi et al. 2011).

By challenging reference resistance genitors of tomato, eggplant and pepper (CoreTEP) to a worldwide collection of *R. solanacearum* strains (CoreRS2), Lebeau et al. (2011) identified several cases of "incompatible interactions", phenotyped as "no wilt and no colonization", that may be indicative of gene-for-gene interactions. One of the accessions involved in such incompatible interactions was the eggplant AG91-25, which carries the *ERs1* resistance gene (Lebeau et al. 2013). The coreRS2 strains were hybridized on a DNA microarray (Guidot et al. 2009, Remenant et al. 2010), in order to get access to their gene content. The combined analysis of such genotypic and phenotypic data was carried out in order to identify phenotype-associated genes.

In this study we thus aimed to identify, by a top-down approach *sensu* Falush and Bowden (2006), the *R. solanacearum* T3E gene repertoires associated with avirulence or virulence

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phenotypes on resistant eggplant, pepper, and tomato cultivars. The specific questions we addressed were: (i) what is the T3E distribution variability in a large strain collection ?; (ii) is the virulence on Solanaceae explained by T3E repertoires, individual T3E presence/absence, or individual T3E allelic differences ?; (iii) are the avirulence /virulence effectors involved in interactions with several cultivars, or is there a cultivar-specificity (Lewis et al. 2014) ?

MATERIALS AND METHODS

Bacterial strains

Two *Ralstonia solanacearum* strain collections were used for this study. A first set of 12 international strains called coreRS2 was selected from a global reference collection, based on their aggressiveness on tomato, eggplant and pepper susceptible reference accessions (Lebeau et al. 2011); this was used from the first step of this study. Two additional strains belonging to the highly harmful “emerging ecotype” (Wicker et al. 2007) were added to this set but were studied only from the second step of our study. The second set gathered 35 “environmental” strains collected on different diseased hosts throughout Reunion Island (Table 1). Bacterial strains were all stored in Cryobank beads at -80°C. They were grown first on Nutrient Broth overnight at 28°C, then streaked (50 µL) on Kelman’s triphenyl tetrazolium chloride (TZC) agar medium (Kelman 1954) supplemented with 1% yeast extract, and sub-cultured two days at 28°C. Bacterial DNA was extracted from fresh cultures (~1.0 to 2.0 x 10^9 cells), using the DNeasy Blood and Tissue kit (QIAGEN) following the manufacturer’s instructions for Gram-negative bacteria; DNA solutions were then quantified with a NanoDrop ND-8000 spectrophotometer (NanoDrop technologies Inc., Wilmington, DE, USA), adjusted to 10 ng.μL^{-1}, and stored at -20°C until use.

Plant accessions

Tomato, eggplant, and pepper accessions were chosen within the core-TEP according to their bacterial wilt (BW)-resistance level and spectrum (Lebeau et al. 2011) (Table 2). For tomato, L390 (coded T10 throughout the article) is highly susceptible (Lebeau et al. 2011, Truong et al. 2008, Wang et al. 1998). The accession Hawaii7996 (encoded T5) displays the highest resistance level, and controls the broadest spectrum of strains (Lebeau et al. 2011); it is also the best known BW-resistant tomato accession, with several mapped quantitative trait loci

(QTLs) (Carmeille et al. 2006, Wang et al. 2013, Wang et al. 2000). For eggplant, the susceptible accession chosen was MM738 (encoded E8 by Lebeau et al.(2011) and in the following), given its high susceptibility (Lebeau et al. 2011) and its status of susceptible parent of the mapping population MM738xAG91-25 (Lebeau et al. 2013). The resistant accessions chosen display complete incompatible interactions (i.e. no wilt and no stem colonization) with at least one *R. solanacearum* strain of the coreRS2 : (i) Dingras multiple Purple, referenced as MM853 in INRA germplasm collection (E1 in this study), displays both a high resistance level and a broad resistance spectrum; (ii) SM6, referenced as MM643 (E2 in this study), is completely resistant to phylotype IIB "emerging strains"(Deberdt et al. 2014, Lebeau et al. 2011) but also IIA and III strains (detailed in table 3); (iii) Ceylan, referenced as MM152 (E3 in this study), is completely resistant to phylotype I, IIA and III strains (table 3); (iv) Surya, referenced as MM1811 (E4 in this study), is resistant to IIA and III strains; and (v) AG91-25, referenced as MM960 (E6 in this study), is totally resistant to strain CFBP6942 (encoded RUN0145) and is poorly colonized by CFBP7032 (RUN0150) and PSS366 (RUN0155), whereas susceptible to virulent strains (N'Guessan et al. 2012). AG91-25 also carries the dominant gene *Ers1*, the first BW-resistance gene identified in a crop (Lebeau et al. 2013). For pepper, resistant accessions were (i) P687 (P2 in this study), resistant to phylotype I strains, (ii) CA8, referenced as PM1580 (P6 in this study), resistant to phylotype IIA strains, (iii) Perennial, referenced as PM659 (P8 in this study), resistant to phylotype III strains (Table 3).

Comparative Genomic Hybridization (CGH) data

The DNA microarray used in these experiments, generated by C. Boucher and collaborators (INRA-CNRS, Toulouse, France), consisted of 6516 65-mer and 70-mer oligonucleotides representative of the genes identified within the *R. solanacearum* genomes GMI1000 (phylotype I), IPO1609 (phylotype IIB /clade 5/sequevar 1) and MOLK2 (phylotype IIB /clade 5 /sequevar 3), as previously described (Remenant et al. 2010, Wicker et al. 2012). Each gene was represented by a single oligonucleotide, except for 117 Type III effector (T3E) or effector-like genes, which were represented by two to six oligonucleotides to distinguish allelic forms of a given gene. Each oligonucleotide was spotted twice on a microarray. DNA extraction and labelling, microarray hybridization, hybridization analyses and gene detection threshold are detailed in Guidot et al.(2007) and Remenant et al.(2010). Effector data sets were complete for 10 coreRS2 strains; however in the case of PSS4 (RUN0157) and PSS366 (RUN0155), only 65 effectors out of 117 were correctly identified. In downstream analyses, these two strains were thus analyzed apart from the others.

Identification of genes associated with effector probes

The correspondence between original probe names and Rip T3E new nomenclature (Peeters et al. 2013) was established by using the "RalstoT3E" website hosted in LIPM Toulouse (https://iant.toulouse.inra.fr/bacteria/annotation/cgi/ralso_effectome/ralso_effectome.cgi).

Genotype/phenotype association Workflow

The whole process, articulated in three main steps, is summarized on Figure 1.

STEP 1: CGH screening of gene repertoires for association with virulence

In the literature, genes that were experimentally demonstrated as involved in *R. solanacearum* virulence are genes encoding Type III effectors (T3E) and the T3SS (Poueymiro and Genin 2009), genes involved in bacterial motility (Tans-Kersten et al. 2004, Tans Kersten et al. 2001), aero-and chemotaxis (Yao and Allen 2006, 2007), transcription regulation, toxin resistance (Brown et al. 2007, Gonzalez et al. 2007), and genes encoding extracellular enzymes secreted through the Type II Secretion System (Denny 2006). In this work, we focused on the distribution of Type III effectors or T3E-like coding sequences within the 12 strains of the first collection, and on their association with virulence phenotypes. Distribution patterns of the other genes were investigated in a preliminary study but no correlation with virulence phenotypes was found.

We considered 117 genes present on the 7K microarray, including 79 T3E *sensu* Poueymiro and Genin (2009) and 10 putative T3Es, six genes (*hpaB, hrpZ, hrpY, hrpX, hrpW, hrpV*) coding for the T3SS, and 22 coding sequences that shared homologous domains with T3E from different bacterial genera, or that contained homeobox domain. Each gene or coding sequence was represented by 2 to 5 probes.

Association of Type III effectors with virulence or avirulence on Solanaceae.- To identify genes associated with virulence or avirulence, we considered published phenotyping data obtained on the core-collections of BW-resistant tomato, eggplant and pepper (coreTEP) (Lebeau 2010, Lebeau et al. 2011). We particularly focused on plant accessions displaying incompatible interactions (defined on this pathosystem as “zero wilting AND zero colonization”) with at least one *R. solanacearum* strain, i.e. (i) eggplant lines Dingras Multiple Purple (MM853), SM6 (MM643), Ceylan (MM152), Surya (MM1811), AG91-25 (MM960), respectively encoded here as E1, E2, E3, E4, E6, (ii) pepper lines PM687, CA8 (PM1580) and Perennial (PM659), respectively encoded P2, P6 and P8, and (iii) tomato Hawaii7996 (encoded T5) (Lebeau et al. 2011) (Table 3).Thus, we distinguished a “avirulent strains” pool and a “virulent strains” pool for each accession considered, and compared their gene content as estimated from CGH data. All probes present in avirulent pool and absent in virulent pool were assigned to the “putative avirulence” gene probes, whereas all probes absent in avirulent pool and present in virulent pool were assigned as “putative virulence” gene probes. We then assessed the association of each T3E gene with avirulence/virulence by considering the frequency of its respective probes within “avirulent strains” and “virulent strains” pools.

STEP 2: PCR genotyping on selected Type III effector genes

The CGH data screening allowed to retain a set of 25 genes, associated to virulence or avirulence. The distribution of these 25 genes was PCR–checked within the two strain collections described above. These genes consisted of two harpin genes, *popW* (Li et al. 2010) and *hrpZ* (Lin et al. 2010), and 23 putative or validated Type III effectors (Peeters et al. 2013).

PCR primer design- Since effector gene sequences are available for only a few strains belonging to our collections, we aligned determined orthologous effector sequences of GMI1000 (phylotype I), CMR15 (Phylotype III), PSI07 (Phylotype IV), IPO1609, Molk2 (both in phylotype IIB) and CFBP2957 (phylotype IIA). Orthologous gene families were defined from the T3E sequences found within the complete genome sequences harbored in the Mage Web interface (http:/genoscope.cns.fr/microscope/mage) of the MicroScope platform. On this platform, search for orthologous genes was performed by applying the following parameters: (i) gene identity above 80%; (ii) ratios of alignment lengths computed for each comparison using the BLAST software (minLrap and maxLrap) above 90%. We also used the “Ralsto-T3E” website (https://iant.toulouse.inra.fr/bacteria/annotation/site/prj/T3Ev2/) to assign each coding sequence to a *rip* family following the nomenclature proposed in the reference paper of Peeters et al. (2013).

Based on these alignments, we identified conserved zones and designed the primers that would enable to amplify putative T3E in all the strains studied. When possible, the primers were designed to amplify entire genes; for gene sizes exceeding 1 kb, primers were designed to amplify several gene fragments. Primer design was performed using the Primer 3 software (http://frodo.wi.mit.edu/primer3/input.htm) following the parameters: annealing temperature around 60°C and primer size between 18 and 25 nucleotides. All PCR primer sequences and PCR conditions are detailed in the Table S6.

Gene amplification and visualization - For each strain, putative effectors were PCR amplified on 20 ng sample DNA template. PCR reactions (total volume of 25µL) consisted of 1U of Red Goldstar Taq DNA polymerase, 25 pmol of each primer, 1X PCR buffer, 1,5 mM $MgCl_2$, 0.2 mM of each dNTPs and 1X Q-solution. The reaction was cycled in Eppendorf Mastercycler Gradient or Applied Biosystems "GenAmp PCR System 9700" thermocyclers with a first denaturation step at 96°C for 5 min at followed by 30 cycles of 30 s at 95°C, 60 s at 56 °C, and 60 s per kb at 72°C, and a final elongation step of 10 min at 72°C. All PCR products were resolved on a 2% agarose gel and visualized with UV light after ethidium bromide staining (5 µg.mL^{-1}); fragment sizes were estimated as compared with a 100 bp DNA ladder (New England BioLabs).

STEP 3: Pathogenicity tests (phenotyping) with representative strains

Tomato (susceptible T10, and resistant T5) and eggplant (susceptible E8, resistant E1 and E6) seeds were sown in a greenhouse respectively 4 and 3 weeks before inoculation and were transplanted one week later into FLORADUR potting mix (9x9 cm pots). Once the stage "three to four fully expanded leaves" was reached, plants were transferred (at least two days before inoculation) into a high quarantine security level (NS3) growth chamber (Rotoplan), to cope with inoculation of exotic strains (notably from the "emerging ecotype" [phylotype IIB/clade4/sequevar 4NPB]). Climatic parameters were set at 85% relative humidity, with a photoperiod of 12h, and a thermoperiod of 30°C day / 24°C night (± 2°C). Bacterial suspensions of selected strains were prepared in Tris Buffer (10^8 cells.mL^{-1}), and inoculated (5 mL per plant) as previously described (N'Guessan et al. 2012) after root scarification. Each strain was inoculated on 15 plants from each accession. The experiment was repeated once. Bacterial wilt incidence and severity were monitored every 2 to 3 days for 28 days, by using a 0 to 4 scale which conveys the percentage of leaves wilted (N'Guessan et al. 2012). Each plant that scored 3 and 4 was considered wilted. Plants showing no symptoms at the end of the experiment were harvested and sampled for latent infections as previously described (Deberdt et al. 2014, Lebeau et al. 2011, N'Guessan et al. 2012). The percentage of wilted plants and the colonization index (Prior et al. 1996) were thus accessed.

Data analysis

All statistical analyses were done using the R software, version 3.1.3 (R 2013).

***Typologies of T3E gene repertoires (STEP 2)* -** For each strain, the expected PCR results were (i) presence or absence of the T3E, as estimated by PCR amplification success or failure, and (ii) the size of the amplified fragment. For each putative gene, class 1 corresponded to a fragment amplification of the expected size, class 2 referred to no amplification, and classes 3 to 6 corresponded to the different alleles (band size) obtained after amplification. Each class, named "allele score" further down in the paper, was considered a qualitative factor within each variable (T3E gene or Coding Sequence (CDS)). The strains were then clustered on the basis of each allele score for all 25 genes amplified, using an agglomerative hierarchical clustering (Maechler et al. 2015) with the Euclidean distance and considering the "Ward" method. We identified the variables (genes) best describing each cluster (named "Type III effector Repertoire Group" [TRG] further down) by using a Chi-squared test (Husson et al. 2009, Husson et al. 2015).

Comparison of wilting and colonization rates across strains (STEP 3) - The effect of strain and plant accession on wilting and colonization incidence, and Area Under the Disease Progression Curve (AUDPC), was assessed using a generalized linear model considering respectively binomial data (wilted vs. non-wilted) with Chi-square test (*P*=0.05), and an analysis of variance with Fisher-Snedecor test (*P*=0.05). Incidence and AUDPC means were compared within each plant accession, using a pairwise comparison test based on the Tukey's methods at level 0.05 (Hothorn et al. 2008).

Typologies of virulence phenotypes (STEP 3) - The combination of final wilting incidence and colonization index scores allowed to calculate reference phenotypes using the *"k-nearest neighbor"* algorithm (Venables and Ripley 2002) as previously described (Lebeau et al. 2011, N'Guessan et al. 2012). Virulence phenotypes ranged from 1 (highly resistant) to 5 (highly susceptible). The 0 class used in this study corresponded to "No wilt and no colonization". Each representative strain was thus assigned to a virulence phenotype on the different plant accessions. The virulence phenotypes were considered as ordered factors ranked in ascending order from 0 to 5. A phenotype clustering was constructed by using an agglomerative hierarchical clustering as described above, considering each accession separately.

A principal component analysis based on gene presence/absence patterns and the phenotype on the different resistant accessions (E1, E6, T5) was performed using the *ade4* package (Dray and Dufour 2007).

RESULTS

Type III Effector (T3E) distribution within the Core-RS2 as estimated by CGH

We first aimed to assess the distribution of 117 genes coding for proteins secreted by, or constitutive of the structure of, the Type III secretion system, within the reference 12-strain Core-RS2 collection. Among the 117 genes considered, 91 were T3E (78) or putative T3E (13), as summarized on Table S1. Considering the 10 strains with complete validated hybridization results (i.e. apart from PSS366 (RUN0155) and PSS04 (RUN0157)), the distribution of the 91 T3E genes was compared (Table S1) across the CoreRS2 strains. It is important to note that we considered the gene present in a strain if this strain was positive for at least one probe of this gene. We also compared this CGH-based repertoire to the repertoire of 26 genomes available on the "Ralsto-T3E" website (Table S3). Twenty T3E genes *sensu* Peeters et al.(2013) were absent, or were not detected in our conditions, from this CoreRs2 collection:*ripAF2, ripAG, ripAI* (RSp0838), *ripAZ2, ripBA*_fragment2 (RSc0228), *ripBB, ripBC, ripBD, ripBF, ripBG, ripC2*_Fragment 1 and 2 (RSp0593 and 0592, respectively), *ripF2, ripG8, ripH4, ripK, ripO2, ripP3*_fragment 1 and 2 (RSc3444 and 3443, respectively), *ripS6, ripS7, ripS8* (RSc3447). Except *ripAI* which was found a core-effector (Ailloud et al. 2015, Clarke et al. 2015, Peeters et al. 2013) (Table S3), most of these "lacking effectors" were either phylotype IV-specific (as detailed below), or found within phylotype IIB strains.

Type III effector repertoires ranged from 61 (CMR32) to 79 (GMI1000). Although there was no clear relationship between phylotype and repertoire richness, phylotype III strains seemed to contain fewer T3Es (61 to 67) than the other phylotypes : 74 to 78 in phylotype I, 73 to 77 in phylotype IIA, 74-75 in phylotype IIB (Table S2). Forty-one T3E genes and three putative T3Es were common to all ten strains, among which four *ripA* (*ripA2-5*), four *ripG* (*ripG2-5*), three *ripH* (*ripH1-3*), four *ripS* (*ripS1-4*). Conversely, *ripA5_2* was the least shared T3E (20% strains), as well as *ripT* and *ripG1* (30% strains) (Table S2). To check for the effector prevalence evenness within our collection, and detect eventual phylotype-specific effectors, we compared the distribution of the 91 T3E genes within each phylogenetic group (phylotype I: 3 strains; phylotype II: 4 strains; phylotype III: 3 strains). Effector distributions within phylotypes were not significantly different from expected (Chi-square test, *P*-value= 0.999 to 1), indicating that phylotype had no detectable influence on effector distribution. Considering the distribution of individual T3E across phylotypes, only *ripA5_2* (PTO1391) and *ripC1* had a distribution different from expected (Chi-square test, P-value= 0.038 for both), the first one appearing specific to phylotype II while the latter was found absent from phylotype III strains.

Considering the 12 strains (including PSS366 and PSS4), only 65 T3Es gave unambiguous scorable results. From the comparison of T3E distribution across phylotypes (phylotype I containing five strains this time), the conclusions were highly similar (no apparent phylotype-specific T3E composition), except that (i) *ripG1* looked significantly overrepresented in phylotype I, and (ii) *ripBH* was overrepresented in phylotype II (Chi-square test, *P-value*=0.044 and 0.020 respectively).

The effector prevalence evenness was also assessed on the 26 *R.solanacearum* genomes harboured on the “RalstoT3E” website (Table S3). It is important to note that this dataset included phylotype I genomes (four, including GMI1000), numerous phylotype II genomes (four IIA including CFBP2957, and 14 IIB including CFBP6783), phylotype IV genomes (3), but contained one single phylotype III genome (CMR15). Type III effector repertoires ranged 68 to 75 for phylotype I, 54 to 67 for phylotype IIA, 54 to 70 for phylotype IIB, 59 for phylotype III, 46 to 62 for phylotype IV. Effector distributions within phylotypes were significantly different from expected for phylotype IV and phylotype I (Chi-square test, *P*-value= 0.014 and 0.029 respectively). Considering the distribution of individual T3E across phylotypes, 15 effectors had a distribution different from expected. Most of them were significantly specific to phylotype IV (*ripAF2, ripAG, ripAK, ripAZ2, ripBF, ripH4, ripO2*; Chi-square P-values ranging between $5.88\text{x}10^{-6}$ and 0.029), or to both phylotypes IV and I (*ripT*, P=0.004), or specific to phylotype I (*ripA1, ripAH, ripBA, ripS6, ripS8, ripTAL*; P-values ranging from $3.25 \text{ x } 10^{-4}$ to 0.025). *RipG8* was specific to phylotype III ($P=1.831 \text{ x}10^{-6}$). We confirmed that *ripG1* was significantly overrepresented within, phylotype I whereas absence within phylotype II genomes (P=0.004); ripBH was found both in phylotype II and phylotype IV genomes, and its distribution ws thus not different from expected (P=0.171).

Analysis of effector repertoires of the core-RS2 reveals the association of some T3Es to specific virulence phenotypes

Comparing the presence of each probe within each couple of “avirulent” and “virulent” strain panels (Table 3), we chose to rely on the ratio “present probes/total probes per gene” to infer a degree of association to a particular phenotype. As an example, *ripP1* was 100% associated with avirulence to the eggplant “Surya” (Table 4) because all three *ripP1* probes were present in all avirulent strains whereas absent in all virulent strains.

Association with avirulence

Sixty genes were associated to avirulence, mainly to pepper P8 (35 genes), then eggplants E6 (28 genes), E2 (11 genes), E1 (10 genes), E3 (4 genes) and E4 (2 genes) (Table S4). One single gene, *ripAX2*, was associated to avirulence to tomato T5 (Table 4, Table S4). Interestingly, 20 genes were associated with avirulence to two accessions (mostly one eggplant and pepper P8), and five genes were associated with avirulence to three cultivars: *ripA4* and *ripN* on E1-E6-P8; *ripAS* on E3-E6- P8; *ripD* on E2-E6-P8; *ripG5* on E1-P6-P8 (Table S4).

We focused on the 31 genes (one harpin, four putative T3Es, 26 T3Es among which *ripAP* was in two fragments) whose (i) at least 50% probes (corresponding to 0.5 in Table 4 and Table S4) were associated with avirulence to at least one accession, and/or (ii) were associated with avirulence to E1 or E6 even with less than 50% probes (Table 4), and/or (iii) were related to fitness on one or several hosts in a previous study (Macho et al. 2010). Highest association scores (from 1 downto 0.67) were observed with avirulence to E6 (*ripAP-ripE1, ripP2, ripAJ-ripAU-ripAZ1-ripP1*), P8 (RSp0216, RSp0218-*ripAS*, *ripG3*), E4 (*ripP1*, *ripAZ1*), T5 (*ripAX2*). Some effectors were associated with avirulence to more than one accession. Indeed, avirulence to both E1 and P8 was associated with four effectors (*hrpZ*, *ripG4*, *ripS5*, RSc1723); avirulence to E3 and P8 with two (Rsp0216, RSp0218), like E4 and E6 (*ripAZ1* and *ripP1*); avirulence to E6 and P8 was associated with *ripG3* (Table 4). Three genes were even associated to avirulence on three accessions: *ripAS* (E3-E6-P8), *ripN* (E1-E6-P8), *ripG5* (E1-P6-P8) (Table S4). Effectors associated to avirulence to E1 were unexpectedly few, and mildly associated (one probe/gene): *hrpZ, ripG4, ripG5, ripN, ripS5,* RSc1723. Only *ripN* was associated with avirulence to both E1 and E6.

Association with virulence

Seventy-three genes (including 51 T3Es and 7 putative T3Es) were associated to virulence (Table S5), mainly on pepper P8 (58 genes) and P2 (22 genes), then eggplants E2 (17 genes), E3 (11 genes), tomato T5 (9 genes), eggplants E1 (7 genes), E4 (6 genes), E6 (5 genes), and pepper P6 (4 genes). Focusing on the 37 genes whose at least 50% probes (score of 0.5 in Table 5) were associated with virulence to at least one accession, highest association scores (1 downto 0.67) were observed on P8 (18 genes and one *ripM* fragment) and P2 (8 genes); virulence on eggplants was associated with five (E2), three (E3), two (E1, E6) or the gene RSp0213 (E4). Some genes were associated to virulence on several accessions: BA02498 on

E1-E3-P2-P6-P8; PTO7001 on E1-E3-E6-P2-P8-T5; PTO3558 on E1-E3-E6-P2-P8-T5; *ripA5_2* on E3-E6-P2-P8-T5; *ripE2* on E1-E3-P2-P6-P8.

Association with both avirulence and virulence

Thirty-seven genes were both associated to avirulence and virulence, but in most cases high association scores (over 50%) were with one unique phenotype, avirulence or virulence (Tables S4 and S5). Some interesting cases were observed on high association scores, several effectors being associated to avirulence to eggplant and virulence to pepper. Hence, *ripP1* was associated to avirulence to both eggplants E4 and E6, and to virulence to pepper P8; *ripAZ1*, avirulent to E4 and virulent to P8. Conversely *ripAF1* was associated to avirulence to P8 and virulence to both E2 and E4; RSp0213 was associated to avirulence to P8 and to virulence to E4 and P2.

From this first screening, the effectors associated with interaction (avirulence or virulence) to eggplants E6 and E1 and tomato T5, were retained for further experiments. . Twenty five T3Es and "effector-like" were thus selected (Table 6), among which 16 avirulence-associated and nine virulence-associated genes.

The avirulence/virulence-associated effectors repertoire can be described in six distribution patterns, some of which being phylotype-specific

The 25 T3Es associated with interaction to eggplants and tomato were selected based on CGH results, targeting specific short regions of the gene. To assess their actual presence and size within the two *R. solanacearum* strain collections (n=48), all these genes were PCR-amplified using consensus primers. From this PCR screening, it appeared that T3Es were (i) successfully amplified giving the expected gene size, (ii) not amplified despite two or three independent replications, (iii) successfully amplified but giving a gene size different from the expected one. These different “states” of each effector within each strain were used to build a typology of the T3E repertoires.

Each strain was thus described by a pattern of 25 variables (gene) that may have up to six different levels: 1= amplified at the expected size; 2=not amplified, 3 to 6= amplified at sizes different from the expected one – the correspondences between alleles and amplicon sizes are detailed in Table S7. The screening of the 25 T3Es in the two *R. solanacearum* strain collections thus led to the identification of 6 main groups of strains sharing similar T3E repertoire patterns, thus named “Type III effector Repertoire Groups” (TRG) (Table 7). The *catdes* function (Husson et al. 2009) allowed identifying the variables’ levels best describing each TRG (Table S8). These TRG seem to be phylotype specific, except for TRG3 and TRG6. The TRG1 gathered strains of phylotype IIB, and was best characterized by BA02498 presence [allele 1], the absence of *ripG3*, *ripS5* and *ripP2* (*P*-values ranging from 2.31×10^{-4} to 9.54×10^{-3}). TRG2 gathered phylotype IIA strains, and best characterized by BA07003-allele 3 , the presence of *ripAS* [allele 1] (*P*-values ranging from 2.66×10^{-3} to 2.63×10^{-2}). TRG3 was dominated by phylotype IIB strains (n=25), and was best characterized by *RipG3*-allele 4, the presence of PTO1265, *ripV2*, BA07003 [allele 1], the absence of *RipN, ripAS* (*P*-values ranging from 9.86 x 10-13 to 3.31×10^{-8}). TRG4 contained 80% of phylotype III strains and was best characterized by *RipG3*-allele 3, *ripW*-allele 4, and *ripAU*-allele 1and the absence of *ripU*, PTO3558, BA07003 (*P*-values ranging from 2.38×10^{-8} to 7.96×10^{-6}). TRG5 only contained phylotype I strains, and was best described by the presence of *ripG3* [allele 1], *ripW*-allele 3, *ripG4, ripAZ1, ripS5* and the absence of BA07003 (*P*-values ranging from = 3.50×10^{-6} to 2.45×10^{-4}) . TRG6, containing only RUN0930 (phylotype I) and RUN0657 (phylotype III), was characterized by *ripG3*-allele 5 and PTO1265-allele 3 (*P*= 0.042 for both).

The TRG representatives display high variability in virulence and aggressiveness on eggplants and tomato

To test the hypothesis that the T3E repertoire may explain the virulence phenotype, we selected 11 strains representative of the six TRG to challenge the resistant tomato and eggplant accessions. Considering both T3E content and phylogenetic position (phylotype/clade/sequevar), we thus chose (i) RUN0017 (IIB /clade 4 /sequevar 4NPB) to represent TRG1, (ii) RUN0058 (IIA /clade 2 /sequevar 39) for TRG2, (iii) RUN0059, RUN0147, RUN0928 and RUN0941 (all in IIB /clade 5 /sequevar 1) for TRG3, (iv) RUN0145 and RUN0523 (both in phylotype III /clade 6 /sequevar 29 and 19 respectively) for TRG4, (v) RUN0054 (the reference GMI1000) and RUN0157 (both in phylotype I /clade 1 /sequevars 18 and 15 respectively) for TRG5; and (vi) RUN0657 (III /clade6 /sequevar 19) for TRG6.

The strains were inoculated in two sets at different dates, with RUN0017 inoculated on both dates as a control. Because the wilting incidence, colonization index and AUDPC were found not significantly different across the two dates (P=0.143, 0.082, 0.615, respectively) we pooled the two sets in one. After 28 days of incubation, the control susceptible accessions were significantly more diseased than the resistant accessions (detailed results are shown on the Figure S1). However some strains induced few symptoms on the susceptible controls. RUN0523 (TRG4) induced less than 20% wilt and colonization on T10 and E8, and was thus regarded a poorly aggressive strain on eggplant and tomato. RUN0058 (TRG2) induced a low wilting incidence but more than 60% colonization to T10. All the other strains induced more than 70% wilt and 80% colonization. On eggplant E8 the strains RUN0017 (TRG1), RUN0059 and RUN0928 (TRG3), RUN0523 (TRG4), RUN0657 (TRG6) caused less than

20% wilt and colonization; highest aggressiveness (more than 80% wilt and colonization) was observed for RUN0157 (TRG5), RUN0147 and RUN0941 (TRG3). The resistant eggplant E1 showed the highest resistance level since only RUN0147 induced more than 20% wilt on it. The accession E6 was susceptible (30% wilt and 60% colonization or above) to the TRG3 strains RUN0147, RUN0928, RUN0941, and to the TGR5 strain RUN0157. The resistant tomato T5 was most highly affected (80% wilt and colonization or above) by the TRG3 strains RUN0147, RUN0941, RUN0928, and by RUN0017 (TRG1). Incompatible interactions (no wilt and no colonization) were observed on the couples (i) E1x RUN0523 and E1 x RUN0058, (ii) E6 x RUN0054, E6 x RUN0523, E6 x RUN0145, E6 x RUN0059.

Thehe T3E repertoire is not globally descriptive of the virulence phenotype

The hierarchical ascending classification of the 11 strains representative of the 6 TRG built based on their T3E repertoire did not clearly match with their virulence phenotypes on eggplant and tomato (Figure 2). More precisely, strains RUN0058 (TRG2) and RUN0017 (TRG1), despite having very similar T3E repertoires, highly differed in virulence on the five cultivars. Similarly RUN0147 and RUN0059 belong to the same TRG3 but the former is highly virulent on E6 and T5 whereas the latter is avirulent on E6 and poorly aggressive on T5. The strains RUN0157 and RUN0054, though gathered in the same TRG5, greatly differ in virulence on the resistant accession E6 (Figure 2). From these results we concluded that the entire T3E repertoire does not determine the phenotype of a strain.

Some T3Es are individually highly associated with avirulence or virulence

We performed a principal component analysis based on the presence or absence of genes, regardless of their allelic state. Projecting the phenotypic classes (0 to 5) (Figure S1) and the gene presence on the same factorial plan, we considered that genes projected in the same zone as extreme phenotypes (avirulence, coded as 0; virulence; coded as 3 for E1, or 4 and 5 for E6 and T5) were associated to this phenotype. On the eggplant E1, the two axes opposed avirulent strains (bottom- left) to virulent strains (top-right) (Figure 3A). *RipA5_2* (PTO1391) was associated to virulence, whereas *ripAS* (RSp1384), and more secondarily *ripN* (RSp1130), were associated to avirulence. On the eggplant E6 (Figure 3B), avirulent strains were in the top-left quarter of the plan, whereas virulent strains were in the top-right and bottom-left quarters. PTO3558 and *ripU* (PTO3560), more secondarily *ripA5_2* (PTO1391) were associated to virulence, while *ripP2* (RSc0868), *ripAU* (RSp1460), *ripG3* (RSp0028), and more secondarily *ripAX2* (RSp0572) and *ripP1* (RSc0826), were associated to avirulence. On the tomato T5 (Figure 3C), the phenotypes were mainly separated along the first axis, from quasi-avirulent strains on the left to virulent strains on the right. *RipV2* (PTO1326), BA07003, PTO3558 and *ripU* (PTO3560) were associated to virulence whereas *RipP1* (RSc0826), *RipN* (RSp1130), *ripAX2* (RSp0572), and more secondarily *ripP2* (RSc0868), *ripG3* (RSp0028), *RipAU* (RSp1460) and *ripG5* (RSc1801) were associated to avirulence.

Then, we followed a stringent approach, hypothesizing that the phenotype could be explained by the presence or absence of key T3Es. For each resistant cultivar, we identified T3Es called (i) virulence effectors that were present in virulent strains and absent in avirulent strains, and (ii) avirulent effectors that were absent in virulent strains and present in avirulent strains. We thus identified the following effectors as highly associated with strain phenotypes (Table 8): *ripA5_2* was associated to virulence on both eggplant E1 and tomato T5; *ripE2*, but also PTO1265 and PTO7001 were associated to virulence on the eggplant E1; PTO3558 and *ripU* associated to virulence to both E6 and T5; BA7003 was associated to virulence to tomato T5. *ripAS* was associated to avirulence to E1, *ripP2* and *ripAX2* to avirulence on E6; *ripP1, ripP2, ripAX2, ripN,* and *ripS5* were associated to avirulence to T5.

DISCUSSION

Although bacterial wilt has been a major biotic stress in solanaceous crops throughout the world, knowledge remains scarce about the plant genes allowing hosts to resist to *R. solanacearum* attacks, and about the bacterial genes most contributing to adaptation to new host or to a resistant accession. To prime the development of durable resistance sources, given the huge genomic and phenotypic plasticity of the pathogen, it is now critical to favor pathogen-informed resistance breeding. Such a strategy requires identifying the bacterial genes involved in virulence to hosts and selected for that function, as well as those counter-selected based on detection in resistant hosts, for further exploring their diversity and evolutionary dynamics in natural populations. Studies on *R. solanacearum*-plant interactions have been focused for long on the model species *Arabidopsis thaliana* (Deslandes et al. 1998, Deslandes et al. 2003, Digonnet et al. 2012) and *Medicago truncatula* (Ben et al. 2013, Turner et al. 2009, Vailleau et al. 2007), but transposition of these findings to crops has been problematic, some critical mechanistic differences having been identified between pathogenesis to model species in one hand and to crops in the other hand (Lin et al. 2008, Remigi et al. 2011). Deciphering plant-microbe interactions on crop species is thus essential.

Our three-step association genetics approach constituted a first published snapshot of the Type III effector diversity existing in natural plant-associated *R. solanacearum* populations, and allowed to identify avirulence and virulence candidate genes. Because population structure can cause bias in the association to phenotype (Falush and Bowden 2006), we checked by Chi-square test that there was no correlation between the phylotype and the effector presence.

A first snapshot of the T3E distribution in a large natural collection

This study gives a different view of the core-effectome within the *Ralstonia solanacearum* Species Complex (RSSC (Genin and Denny 2012)). The coreRS2 core-effectome contains 40

T3Es, which does not completely match the Core-effectome identified by Peeters et al. (2013). Hence, the Peeters's core-effectors *ripAO*, *ripE1, ripG7* were present in 90% coreRS2 strains; the core *ripAC* was present in 80% coreRS2 strains, and the core *ripAA* was even more variable, being absent from the phylotype III strains (except CMR15) and from two phylotype I strains, PSS366 and PSS358 (Table S2). The core *ripC1* and *ripU* were found also variable in our study, but we observed a discrepancy between our results and Peeters' results on CMR15 (Table S3). This absence on our dataset may be explained by technical artifacts. Recent genomics studies focusing on phylotype II genomes identified core-effectomes of different composition, including either 14 T3Es (Ailloud et al. 2015), or 31 T3E (Clarke et al. 2015); our own analysis on publicly available genomes identified 11 core-effectors on a quite phylogenetically unbalanced dataset (Table S3). Additional genomic sequences, especially within phylotype III, are needed to approach the actual composition of the RSSC core-effectome.

The T3E repertoires from the Reunion collection should be regarded partial since 25 T3Es only were considered, but they yet give interesting insights on the prevalence and diversity of these effectors. Effector distribution could indeed be described in six patterns (TRG) based on effector presence but also alleles. The most polymorphic effector was *ripG3*, which alleles were major descriptors of the different TRGs: allele 1 for TRG5, allele 3 for TRG4, allele 4 for TRG3, allele 5 for TRG6 (table 7). Such diversity may be a signature of the diversifying selection evoked for this gene in the reference paper describing the GALA family evolution (Remigi et al. 2011).

Some effectors were present throughout the collection, like *ripA2, ripA4, ripW* (Pattern 5 in Table 7). *ripA2* and *ripA4* were monomorphic, but it is important to note that these were partial sequences (3065 bp over the 3381 bp of $\textit{ripA2}_{GMI1000}$, and the 960 bp region of the 3990 bp-$\textit{ripA4}_{GMI1000}$). Whereas ripA2 (formerly AWR2) was primarily characterized as contributing to virulence (Sole et al. 2012), it could be successfully amplified in the T5-avirulent strains. This difference could be due to sequence polymorphism, or functional redundancy; it also may indicate that ripA2 plays a dual role within tomato, supporting the Invasion Model (Cook et al. 2015). Whereas, *ripW* (formerly called *popW*) gave three different alleles: one being widespread in the collection (1202 bp with IIB primers and 1119 pb with GMI1000 primers), another (allele 4: 1202 bp with IIB primers only) being specific to TRG 4, composed of phylotype III strains, and a third one (allele 3: 1119 pb with GMI1000-primers only) specifically found in the TRG5 (phylotype I strains) (Tables S7 and S8). We did not find correlation between these alleles and an avirulence phenotype. *ripW* was previously characterized as a two-domain protein (Li et al. 2010), whose harpin activity (detected on tobacco) resides in its N-terminal domain (first 159 residues), and which doesn't significantly contribute to virulence on tomato. The analysis of a 20-strain collection, covering six countries but whose phylotypes were not determined, allowed the identification of six alleles ranging from 1131 to 1155 bp. The range of variation that we observed was thus higher, and it may be interesting to determine the critical residues involved in the harpin activity on tobacco and solanaceous.

An approach with rich outputs, but with some limitations

Although most of the associations detected in STEP 3 were consistent with those found in STEP 1, some distortions should be evoked and discussed. Indeed, *ripAP*, *ripE1*,and *ripAJ*, though associated with avirulence to E6 from STEP1 (Table 5), were not retained in the downstream analyses. *RipAP* is present in two fragments in the GMI1000 genome, and experiments established that it is not secreted by the T3SS (A.C Cazalé and N. Peeters, personal communication 2014). Moreover, the associations found in STEP 1 were not always confirmed in STEP 3 (Table 9). Main discrepancies concerned avirulence to Hawaii 7996 (T5): several associations found on full-length gene analysis (for *ripAU*, *ripG3, ripN, ripP1* and *ripP2*, *ripG5,* and *ripS5*) were not primarily detected from probe data analysis. Avirulence to eggplant detected in STEP 1 was mostly confirmed on STEP 3, with some exceptions. The E6- avirulence of *RipAX2* was found only in STEP3, whereas this of *ripN* was not confirmed in STEP 3; *ripAS*, primarily associated to avirulence on E3 and E6, was found avirulent to E1 only from STEP 3. Regarding virulence effectors, the virulence of *ripE2* on E1, *ripA5_2* on E6, and *ripV2* on T5, were confirmed, whereas *ripU*, virulent to T5 from the STEP1, was found virulent to T5 but also E6 in the STEP3. It is thus strongly recommended to combine both approaches, and to favor full-length gene analysis for the choice of candidate genes.

Avirulence and virulence phenotypes are not explained by repertoires, but rather by individual effectors which constitute promising candidates interacting with eggplant and tomato

Strains of identical phylotype and TRG displayed very different virulence phenotypes (see RUN0054 and RUN0157 as an example), whereas other strains displaying identical phenotypes were in different TRG (see RUN0157 (TRG5) and RUN0941 (TRG3)). The "repertoire-for-repertoire" hypothesis, stated to explain the *Xanthomonas* host specificity (Hajri et al. 2009), does not match the *Ralstonia solanacearum* situation.

Collectively, the final principal component analysis and the stringent analysis have allowed to define a short list of avirulence- and virulence-associated effectors to be further investigated.

Among avirulence effectors, *ripP2* is one of the best candidates, associated to both eggplant E6 and tomato T5. Then follow *ripAU* and *ripG3*, as well as *ripP1* and *ripAX2* (also for avirulence to E6 and T5). Then we identify *ripN* (avirulence to E1 and T5), *ripAS* (avirulence to E1 only). Since *ripG5* is only associated to avirulence to T5, and because it is expressed in operon with *ripG4*, we chose not to keep it in our short list.

Among virulence effectors, most promising candidates are PTO3558 and *ripU* (virulence to both E6 and T5), then *ripA5_2* (virulence to both E6, E1, and T5), then *ripV2* and BA07003 (virulence to T5). It will be critical first to check the expression and secretion of PTO3558 and BA07003 by the T3SS. This short list of effectors is now nominated for further experiments that may validate their avirulence or virulence function.

ripP2 (former *popP2*) has been the first described *R solanacearum* avirulence gene to *Arabidopsis thaliana* Nd1 (Deslandes et al. 2003), whose functions have been most thoroughly investigated (see the review of Deslandes and Genin (2014), as well as (Le Roux et al. 2015, Sarris et al. 2015)). Amplifying the 8-989 bp region (over 1464 bp in total), we identified two alleles: 982 bp (allele 1) being found within almost all phylotype III strains and two phylotype I strains (RUN0054 and RUN0215), and 950bp (allele 3) found in most phylotype IIA and IIB strains, three phylotype III strains (RUN0039, RUN0145, RUN0133) and two phylotype I strains (RUN0155, RUN0471). To our knowledge, this is the first published paper describing *ripP2* diversity. Whether these *ripP2* alleles keep their enzymatic functions and their ability to be detected within plant (their “invasion pattern” *sensu* (Cook et al. 2015)) remains to be tested (Tasset et al. 2010). It is now tempting to speculate that the interaction Eggplant AG91-25 / *ripP2* may follow the Arabidopsis *RRS1-R*/*ripP2* model (Bernoux et al. 2008, Deslandes et al. 1998, Deslandes et al. 2002 , Deslandes et al. 2003, Le Roux et al. 2015, Sarris et al. 2015). According to this model, the AG91-25 major resistance gene *ERs1* (Lebeau et al. 2013) could be homologous of RRS1-R, or coupled to a RPS4 homologue. However, preliminary BLAST search of RRS1-R on the eggplant sequence genome (Hirakawa et al. 2014) returned no hit (S. Salgon and C. Sauvage, personal communication 2015). Recently, ripP2 was demonstrate to specifically interact with a new eggplant resistance protein, RE-BW (Xiao et al. 2014); it remains to be determined whether this gene co-localizes with *ERs1*.

ripAX2, also called *rip36* (Peeters et al. 2013, Poueymiro and Genin 2009), codes for the other demonstrated avirulence effector, eliciting HR on *Solanum torvum* (Nahar et al. 2014), a wild relative of eggplant. This paper gives first insights on the diversity on this poorly known effector, whose prevalence looks highest in phyloypes III and I strains.

ripP1 (former *popP1*), whose protein was demonstrated as an avirulence factor on Petunia (Lavie et al. 2002) and HR-elicitor on *Nicotiana glutinosa* (Poueymiro et al. 2009), was only detected in phylotypes I and III, giving a single allele of 1088 bp (over the 1104 bp total gene), and its presence was quite variable, even within phylotype I strains (absent in two over six strains). This variability was also reported in a Japanese phylotype I 22 strain-collection, but *popP1* presence was not correlated with HR on tobacco (Liu et al. 2009). Its homolog in *Xanthomonas perforans*, XopJ4, was also reported as avirulence protein recognized by the *Solanum penelli RXopJ4* gene (Sharlach et al. 2013).

ripA5_2 (former AWR5-2), in the other hand, was not reported as a virulence effector in the literature. AWR5 was indeed recognized by *Arabidopsis* and induced an HR on *Nicotiana tabacum* (Sole et al. 2012). It remains to assess the actual expression and the respective roles of the two *ripA5* paralogs in plant-pathogen interactions.

Potential candidate effectors in interaction with other species

Because the resistance levels observed were highest on eggplant, we focused our quest on this species. But scientists should also use these results to further investigate the pepper-*R. solanacearum* molecular interactions. One should focus on the role, distribution, diversity and evolution of the 2 harpins *hrpZ* and *ripW* and the 10 effectors associated to avirulence to both eggplant and the pepper Perennial (P8). Among these are two members of the *ripA* family (former AWR family) *ripA2* and *ripA4* (Sole et al. 2012), three members for the *ripG* family (former GALA family), *ripG3*, *ripG4*, *ripG5*, whose functions remain unknown on pepper. Furthermore, some cases of eggplant-pepper differential phenotypes may deserve further investigation. *ripP1* and *ripAZ1* were associated to avirulence to eggplant “Surya”(E4) and virulence to pepper “Perennial”(P8). Interestingly, this host –specific differential function has been reported for the *ripP1* homolog in *Xanthomonas euvesicatoria*, *XopJ* (formerly AvrXv4), whose protein displays avirulence function on tomato (Astua-Monge et al. 2000) but virulence function on pepper, reducing the salicylic acid accumulation (Ustun et al. 2013). Conversely *ripAF1* was associated with virulence to eggplants “SM6” and “Surya” and avirulent to pepper “Perennial”. Again, these findings may support the view of Type III effectors as dual interactors, contributing to virulence or overall bacterial fitness within a species or a species cultivar, and specifically recognized by other cultivars to trigger defense responses (Cook et al. 2015).

Future research

This study should be regarded as a first step towards decomposing the molecular bases of solanaceaous-*R. solanacearum* interactions. As previously stated (Kirzinger and Stavrinides 2012), “changes in host specificity can range from the smallest to the largest genetic change”, including SNPs, residue change, intragenic or total gene insertion/deletions, gene repertoire, up to genomic island. In this study, we considered only the repertoire and gene scales. Future research is now needed at the sequence level to identify regions or residues that may be critical for detection by the plant (previously named “avirulence”) or for its virulence function. The alleles of the effector short-list that we identified will be now monitored in natural *R. solanacearum* populations, to assess their distribution, diversity, and the type of selection they are subjected to, to complete the Clarke’s inventory of conserved effectors (Clarke et al. 2015).

In future research, it will be important also to validate the “avirulence function” of these candidates by knock-out and “gain-of-function” experiments (thus following the Falush-Bowden approach (Falush and Bowden 2006)), and using complemental functional screens that will help elucidate the actual mechanism of their recognition by plants (in which organ, at which pathogenesis stage). Once such a validation is completed, future research should also focus on the plant targets of these bacterial proteins. Finally, a large field of research is open for deciphering the interaction networks (synergies, antagonisms) involving Type III effectors and the architecture of Solanaceae innate immunity.

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Captions to Figures

Figure 1. Genotype-phenotype association workflow. Data priming this study were generated by Lebeau et al. (Lebeau 2010, Lebeau et al. 2011) and Guidot et al. (2009). T3E: Type III Effector, HAC: Hierarchical Ascending Classification, TRG: Type III effector Repertoire Group. In Step 3, the eggplant accessions were either resistant (R) or susceptible (S).

Figure 2. T3E genotypes do not match virulence phenotypes. Hierarchical ascending classification of *R. solanacearum* strains (named by their RUN number and phylotype) based on their T3E repertoire, and correspondence with phenotypes[a] observed on resistant (R) and susceptible (S) eggplants and tomatoes. TRG, Type III effector Repertoire Group.

[a] The phenotype score was defined by the combination of final wilting incidence and colonization index, and calculated following Lebeau et al.(2011), where 0 = complete resistance (no wilt and no colonization), 1=highly resistant, 2= moderately resistant, 3= partially resistant, 4=moderately susceptible, and 5= highly susceptible.

Figure 3. Distribution of the T3E genes on the factorial plan of the two mostly informative axes of the Principal Component Analysis performed on gene presence-absence data and phenotypic data collected from pathogenicity tests on Dingras multiple Purple (E1) (**A**), AG91-25 (E6) (**B**), Hawaii7996 (T5) (**C**). T3E genes associated to avirulence were shaded in blue, while those associated to virulence were shaded in red.

Figure S1. Virulence of the 11 *R.solanacearum* strains on the eggplants E1, E6 (resistant) and E8 (susceptible) and tomatoes T10 (susceptible) and T5 (resistant), as determined by the colonization index (left), final wilting rate (middle), and AUDPC (right). Strains, named after their RUN number (abscissa), are representative of the TRG 1 (orange), TGR2 (dark blue), TRG3 (red), TRG4 (green), TRG5 (yellow), TRG6 (dark turquoise). Values marked with similar letters within each barplot are not significantly different from each other (Tukey test, threshold = 0.05).

Table 1. Characteristics of *Ralstonia solanacearum* strains belonging to the world collection core-RS2 and to the "Reunion Island" collection

Strain	RUN number	Host	Origin	Phylotype	Sequevar
Core-RS2 (Lebeau et al. 2011)					
GMI1000	54	*Solanum lycopersicum*	French Guiana	I	18
PSS366	155	*Solanum lycopersicum*	Taiwan	I	15
PSS004	157	*Solanum lycopersicum*	Taiwan	I	15
PSS358	159	*Solanum lycopersicum*	Taiwan	I	15
CFBP7058	215	*Solanum scabrum*	Cameroon	I	13
CFBP2957	36	*Solanum lycopersicum*	Martinique	IIA	36
CFBP7032	150	*Solanum lycopersicum*	Cameroon	IIA	41
CFBP6783	17	*Heliconia rostrata*	Martinique	IIB	4NPB
CFBP7029	147	*Solanum lycopersicum*	Cameroon	IIB	1
CFBP3059	39	*Solanum melongena*	Burkina Faso	III	23
CFBP6941	133	*Solanum lycopersicum*	Cameroon	III	29
CFBP6942	145	*Solanum scabrum*	Cameroon	III	29
« Emerging ecotype » strains					
CFBP6784	16	*Anthurium andreanum*	Martinique	IIB	4NPB
ANT80	18	*Anthurium andreanum*	Martinique	IIB	4NPB
Collection from Reunion Island					
JT519	471	*Pelargonium*	-	I	31
JT523	608	*Solanum tuberosum*	-	I	31
JQ1044	930	*Pelargonium*	Trois Bassins	I	NA
JQ1143	58	*Solanum tuberosum*	Bois Court	IIA	39
JT510	59	*Solanum tuberosum*	Notre Dame de la Paix	IIB	1
JT516	160	*Solanum tuberosum*	-	IIB	1
JS529	476	*Solanum tuberosum*	-	IIB	1
CFBP4801	623	*Solanum lycopersicum*	-	IIB	1
LNPV28.23	654	*Solanum tuberosum*	-	IIB	1
JT511	681	*Solanum tuberosum*	-	IIB	1
JT514	697	*Solanum tuberosum*	-	IIB	1
JQ1006	843	*Solanum tuberosum*	Notre Dame de la	IIB	1

Strain	RUN number	Host	Origin	Phylotype	Sequevar
			Paix		
JQ1073	845	*Solanum lycopersicum*	Saint Pierre	IIB	1
JQ1023	848	*Solanum lycopersicum*	Mont Vert	IIB	1
JQ1078	880	*Solanum lycopersicum*	Saint Pierre	IIB	1
JQ1107	889	*Solanum tuberosum*	Bois Court	IIB	1
JQ1006	919	*Solanum tuberosum*	Notre Dame de la Paix	IIB	1
JQ1007	921	*Solanum tuberosum*	Grand Tampon	IIB	1
JQ1009	923	*Solanum tuberosum*	Piton Maho	IIB	1
JQ1017	924	*Solanum tuberosum*	Plaine des Cafres	IIB	1
JQ1018	925	*Solanum tuberosum*	Piton Hyacinthe	IIB	1
JQ1019	926	*Solanum tuberosum*	Notre Dame de la Paix	IIB	1
JQ1023	928	*Solanum lycopersicum*	Mont Vert	IIB	1
JQ1051	931	*Solanum lycopersicum*	Grand Anse	IIB	1
JQ1078	934	*Solanum lycopersicum*	Saint Pierre	IIB	1
JQ1101	936	*Solanum lycopersicum*	Grand Tampon	IIB	1
JQ1131	941	*Solanum tuberosum*	Piton Hyacinthe	IIB	1
CFBP2148	944	*Solanum tuberosum*	-	IIB	1
JT525	60	*Pelargonium*	-	III	19
JT528	61	*Solanum tuberosum*	-	III	19
NCPPB1029	77	*Pelargonium*	-	III	19
CFBP4963	523	*Solanum tuberosum*	-	III	19
CFBP2146	657	*Pelargonium*	-	III	19
CFBP4964	693	*Pelargonium*	Trois Bassins	III	19
JQ1092	913	*Solanum tuberosum*	Tan Rouge	III	NA

Lebeau, A., Daunay, M. C., Frary, A., Palloix, A., Wang, J. F., Dintinger, J., Chiroleu, F., Wicker, E., and Prior, P. 2011. Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. Phytopathology 101(1):154-165.

Table 2, Pensec, *Phytopathology*

Table 2. Genetic resources in tomato, eggplant, pepper, selected in the core-TEP collection (Lebeau et al. 2011)

Code	Accession	Species	Inoculated in STEP 3
Tomato			
T5	Hawaii 7996	*Solanum lycopersicum*	YES
T10	L390	*S. lycopersicum var. cerasiforme*	YES
Eggplant			
E1	Dingras multiple Purple, MM853[a]	*S. melongena*	YES
E2	SM6, MM643	*S. melongena*	NO
E3	Ceylan, MM152	*S. melongena*	NO
E4	Surya, MM1811, EG203	*S. melongena*	NO
E6	AG91-25, MM960	*S. melongena*	YES
E8	MM738	*S. melongena*	YES
Pepper			
P2	PM687	*Capsicum annuum*	NO
P6	PBC631A, CA8, PM1580	*C.annuum*	NO
P8	Perennial, PM659	*C.annuum*	NO

[a] MM and PM numbers correspond to accession codes in the INRA Germplasm Collection.

Table 3, Pensec, *Phytopathology*

Table 3. Summary of the avirulent/virulent *R. solanacearum* strain pools, as defined by their virulence phenotypes on eggplant (E code), tomato (T code), and pepper (P code) accessions. Avirulence was defined as complete absence of wilt and colonization on the plant accession. Virulent strains were able to colonize and wilt accessions, inducing a phenotype 4 or 5 *sensu* Lebeau et al. (2011). *R. solanacearum* strains belong to phylotype I (□), IIA (○), IIB (●), or III (■).

Accession (code)	AVIRULENT (phenotype 0)	VIRULENT (phenotypes 4-5)
Eggplant		
Dingras (E1)	CMR32 ■ CFBP2957 ○ GMI1000 □	CFBP6783 ●
SM6 (E2)	CMR32 ■ CFBP2957 ○ CFBP6783 ●	PSS4 □ PSS366 □
Ceylan (E3)	CMR32 ■ CMR15 ■ CFBP2957 ○ CMR39 ○ PSS358 □	CFBP6783 ●
Surya (E4)	CMR32 ■ CFBP2957 ○	CMR15 ■
AG91-25 (E6)	CMR32 ■ CMR39 ○	CFBP3059 ■ CMR15 ■ CFBP6783 ● CMR34 ●
Tomato		
Hawaii7996 (T5)	CMR32 ■ CMR39 ○	CMR15 ■ CMR34 ● CFBP6783 ●
Pepper		
PM687 (P2)	PSS358 □	CFBP3059 ■ CFBP6783 ● CMR39 ■
CA8 (P6)	CFBP2957 ○ CMR39 ○	CFBP6783 ●
Perennial (P8)	CMR15 ■	CFBP6783 ●

Table 4. Type III effectors (T3E) and T3E-like genes associated with avirulence on eggplant, pepper and tomato accessions, as determined by the ratio present probes/total probes per gene in each "avirulent strains" panel. This selection gathers genes whose (i) at least 50% probes (corresponding to 0.5 score) are associated to avirulence to at least one plant accession, and/or (ii) were associated to avirulence on E1 and E6, and/or (iii) significantly contributed to *in planta* fitness (Macho et al. 2010). Genes selected for STEP 2 analysis are written in bold.

Gene Code	Rip Name	Former /other name, description	No probes /gene	EGGPLANT					PEPPER	TOMATO	Related to fitness on[a]
				Dingras (E1)	SM6 (E2)	Ceylan (E3)	Surya (E4)	AG91-25 (E6)	Perennial (P8)	Hawaii7996 (T5)	
RSc1723	_	**putative T3E**	**4**	0.25					0.5		
RSc2897	_	putative T3E	4		0.25			0.5			
RSp0216	_	putative T3E	4			0.5			1		
RSp0218	_	Putative T3E	4			0.5			0.75		
RSp0854	_	**hrpZ**	**2**	0.5					0.5		
RSp0099	**ripA2**	**AWR2**	**4**	0.25					0.25		
RSp0847	**ripA4**	**AWR4**	**4**	0.25				0.25	0.25		
RSp0822	ripAF1	HopF1-like	4					0.25	0.5		EGGPLANT, BEAN
RSc2101	ripAJ	-	3					0.67			
RSp1218	ripAP-fragment1	-	1					1			
RSp1215	ripAP-fragment2	-	3					1			
RSp1384	**ripAS**	**-**	**4**			0.25		0.5	0.75		
RSp1460	**ripAU**	**-**	**3**					0.67	0.33		
RSp0572	**ripAX2**	**HopH1-like**	**4**							0.75	
RSp1022	ripAY	-	3					0.33	0.33		EGGPLANT

Table 4, Pensec, *Phytopathology*

Gene Code	Rip Name	Former /other name, description	No probes /gene	EGGPLANT					PEPPER	TOMATO	Related to fitness on[a]
				Dingras (E1)	SM6 (E2)	Ceylan (E3)	Surya (E4)	AG91-25 (E6)	Perennial (P8)	Hawaii7996 (T5)	
RSp1582	**ripAZ1**	**-**	**3**				**0.67**	**0.67**			
RSp0304	ripD	HopD1-like	3		0.33			0.33	0.33		TOMATO, EGGPLANT, BEAN
RSc3369	ripE1	-	4					**1**			
RSp0028	**ripG3**	**GALA3**	**5**					**0.5**	**0.6**		
RSc1800	**ripG4**	**GALA4**	**4**	**0.25**					**0.25**		
RSc1801	**ripG5**	**GALA5**	**4**	**0.25**					**0.25**		
RSc1357	ripG7	-	4		**0.5**						
RSp0215	ripH2	HLK2	4						**0.5**		
RSp0160	ripH3	HLK3	4					**0.5**	0.25		
RSp1130	**ripN**	**-**	**3**	**0.33**				**0.33**	**0.33**		
RSc0826	**ripP1**	**PopP1**	**3**				**1**	**0.67**			
RSc0868	**ripP2**	**PopP2**	**4**					**0.75**			TOMATO, EGGPLANT, BEAN
RSc1839	ripS4	-	5					0.2			EGGPLANT
RSp0296	**ripS5**	**SKWP5**	**4**	**0.25**					**0.5**		
RSc1815	ripTAL	AvrBs3-like	5						0.2		EGGPLANT
RSc2775	**ripW**	**-**	**4**	**0.25**					**0.25**		
RSp0877	ripX	popA	2					**0.5**			

Table 5, Pensec, *Phytopathology*

Table 5. Type III effectors (T3E) and T3E-like genes associated with virulence on eggplant, pepper and tomato accessions, as determined by the ratio present probes/total probes per gene in each "virulent strains" panel. This selection gathers genes whose at least 50% probes (score 0.5) are associated to virulence on at least one plant accession. Genes selected for STEP 2 analysis are represented in bold.

Gene or probe	Rip Name	Former /other name, description	No probes /gene	EGGPLANT					PEPPER			TOMATO
				Dingras (E1)	SMA6 (E2)	Ceylan (E3)	Surya (E4)	AG91-25 (E6)	PM687 (P2)	CA8 (P6)	Perennial (P8)	Hawaii7996 (T5)
BA02498	**-**	**CPUF, AvrPtoB-like domain**	**3**	**1**		**1**			**1**	**1**	**1**	
BA07003	**-**	***pcaD2***	**2**	**0.5**		**0.5**			**0.5**	**0.5**	**1**	
PT00619	-	putative glycosyltransferase	2								0.5	
PT01265	**-**	**CPUF**	**3**	**0.33**	**0.33**	**0.33**		**0.33**	**0.67**		**0.33**	**0.33**
PT03045	-	CPUF (TPR domain)	2								1	
PT03558	**-**	**CPUF (RRSL_04659)**	**3**	**0.33**		**0.33**		**0.33**	**0.33**		**0.67**	**0.33**
PT04098	-	Putative T3E (RALIP_4318)	3								1	
PT04281	-	CPUF	3								1	

Table 5, Pensec, *Phytopathology*

Gene or probe	Rip Name	Former /other name, description	No probes /gene	EGGPLANT					PEPPER			TOMATO
				Dingras (E1)	SMA6 (E2)	Ceylan (E3)	Surya (E4)	AG91-25 (E6)	PM687 (P2)	CA8 (P6)	Perennial (P8)	Hawaii7996 (T5)
PT04284	-	putative T3E (RALIP_4533)	2								1	
PT07000	-	putative T3E RALIP_1709)	3						0.67			
PT07001	**-**	**putative T3E (ripM fragment)**	**2**	**1**		**1**		**1**	**1**		**1**	**1**
RSc2131	-	PUF	3		0.67				0.33		0.33	
RSc3174	-	putative T3E	4						1			
RSp0213	-	putative T3E	2				1		1			
RSc2139	ripA1	AWR1	4		0.75							
PT01391	**ripA5_2**	**AWR5-2 (RALIP_1563)**	**3**			**1**		**1**	**1**		**1**	**1**
RSc0321	ripAE	-	4								0.75	
RSp0822	ripAF1	HopF1-like	4		0.5		0.5					
RSc0895	ripAH	-	3		0.67							
RSc2359	ripAK	-	4		0.5							
RSp1582	ripAZ1	-	3								0.67	
PT04502	ripBH	RALIP_4767	3								1	

Table 5, Pensec, *Phytopathology*

Gene or probe	Rip Name	Former /other name, description	No probes /gene	EGGPLANT					PEPPER			TOMATO
				Dingras (E1)	SMA6 (E2)	Ceylan (E3)	Surya (E4)	AG91-25 (E6)	PM687 (P2)	CA8 (P6)	Perennial (P8)	Hawaii7996 (T5)
PT04434	ripBI	RALIP_4696	3								1	
RSp1239	ripC1	-	4		0.25				0.25		0.75	
PT04834	**ripE2**	**RALIP_0863**	**4**	**0.5**		**0.5**			**0.75**	**0.5**	**0.5**	
RSp0914	ripG1	GALA1	3		0.67				0.33			
RSc1800	ripG4	GALA4	4								0.5	0.25
RSc1356	ripG6	GALA6	4								0.5	
RSc2132	ripJ	-	3								1	
RSc0826	ripP1	PopP1	3								1	
BA00250	ripS4	RCFBP_11536	3								0.67	
RSc1839	ripS4	RSc1839	5								0.4	
RSc3212	ripT	-	2						0.5			
RSc1815	ripTAL	-	5		0.6						0.2	
PT03560	**ripU**	**RRSL_04660**	**2**								**1**	**0.5**
RSp1212	ripU	-	5						0.8			
PT01326	**ripV2**	**RALIP_1493**	**3**								**1**	**0.33**

Table 6, Pensec, *Phytopathology*

Table 6. Type III effectors and "effector-like" genes selected on basis of their association to phenotype (avirulence or virulence) to eggplants E1 and E6 and tomato T5 following STEP 1

Gene Code	Rip Name /other name	EGGPLANT					PEPPER			TOMATO
		E1	E2	E3	E4	E6	P2	P6	P8	T5
BA02498	-	V	-	V	-	-	V	V	V	-
BA07003	-	V	-	V	-	-	V	V	V	-
PT01265	-	V	V	V	-	V	V	-	V	V
PT03558	-	V	-	V	-	V	V	-	V	V
PT07001	-	V	-	V	-	V	V	-	V	V
RSc1723	-	A[a]	-	-	-	-	-	-	A	-
RSp0854	*hrpZ*	A	-	-	-	-	-	-	A	-
RSp0099	*ripA2*	A	-	-	-	-	-	-	A	-
RSp0847	*ripA4*	A	-	-	-	A	-	-	A	-
PT01391	*ripA5_2*	-	-	V	-	V	V	-	V	V
RSp1384	*ripAS*	A	-	A	-	A	-	-	A	-
RSp1460	*ripAU*	-	-	-	-	A	-	-	A	-
RSp0572	*ripAX2*	-	-	-	-	-	-	-	-	A
RSp1582	*ripAZ1*	-	-	-	A	A	-	-	-	-
PT04834	*ripE2*	V	-	V	-	-	V	V	V	-
RSp0028	*ripG3*	-	-	-	-	A	-	-	A	-
RSc1800	*ripG4*	A	-	-	-	-	-	-	A	V
RSc1801	*ripG5*	A	-	-	-	-	-	-	A	-
RSp1130	*ripN*	A	-	-	-	A	-	-	A	-
RSc0826	*ripP1*	-	-	-	A	A	-	-	V	V
RSc0868	*ripP2*	-	-	-	-	A	-	-	-	-
RSp0296	*ripS5*	A	-	-	-	-	-	-	A	-
PT03560	*ripU*	-	-	-	-	-	-	-	V	V
PT01326	*ripV2*	-	-	-	-	-	-	-	V	V
RSc2775	*ripW*	A	-	-	-	-	-	-	A	-

[a] Phenotypes: A, avirulence; V, virulence

Table 7, Pensec, *Phytopathology*

Table 7. Distribution of the 25 T3E and "T3E-like" genes selected among *Ralstonia solanacearum* strains from CoreS2 and Reunion Island, as determined by PCR amplifications. Genes were either amplified at the expected size (allele 1), or not amplified (allele 2). In this latter case, absence of amplification may be interpreted as absence of the gene, or high divergence in the region targeted by the PCR primers. Genes were also amplified at a different size than expected (alleles 3, 4, 5). Gene typology led to identify six T3E repertoire groups (TRG) within which were chosen representative strains (written in bold) to be inoculated on eggplant and tomato.

			Gene Name, Rip Name																								
			-	ripP1	_	ripAX2	ripG4	ripN	ripAZ1	ripAS	ripG5	ripAU	ripE2	ripP2	ripS5	hrpZ	ripG3	-	-	ripU	ripA5_2	-	ripV2	-	ripW	ripA2	ripA4
RUN #	Phylotype	TRG[a]	BA2498	RSc0826	RSc1723	RSp0572	RSc1800	RSp1130	RSp1582	RSp1384	RSc1801	RSp1460	PTO4834	RSc0868	RSp0296	RSp0854	RSp0028	BA7003	PTO3558	PTO3560	PTO1391	PTO1265	PTO1326	PTO7001	RSc2775	RSp0099	RSp0847
16	IIB	1	1	2	2	2	2	2	2	2	2	2	1	2	2	3	2	1	1	1	1	2	2	2	1	1	1
17	**IIB**		1	2	2	2	2	2	2	2	2	2	1	2	2	3	2	1	1	1	1	2	2	2	1	1	1
18	IIB		1	2	2	2	2	2	2	2	2	2	1	2	2	3	2	1	1	1	1	2	2	2	1	1	1
36	IIA	2	2	2	2	2	2	1	2	1	2	3	3	3	2	1	2	3	1	1	2	2	1	2	1	1	1
58	**IIA**		2	2	2	2	2	2	2	1	2	2	2	3	3	3	2	1	1	1	2	2	1	2	1	1	1
150	IIA		1	2	2	1	2	2	2	1	2	2	3	3	2	1	3	3	1	1	1	2	1	2	1	1	1
59	**IIB**	3	2	2	2	2	2	2	2	2	2	2	2	3	3	3	4	1	1	1	1	1	1	1	1	1	1
147	**IIB**		2	2	2	2	2	2	2	2	2	2	3	2	2	3	4	1	1	1	1	1	1	1	1	1	1

Table 7, Pensec, *Phytopathology*

			Gene Name, Rip Name																									
			-	ripP1	-	ripAX2	ripG4	ripN	ripAZ1	ripAS	ripG5	ripAU	ripE2	ripP2	ripS5	hrpZ	ripG3	-	-	ripU	ripA5_2	-	ripV2	-	ripW	ripA2	ripA4
RUN #	Phylotype	TRG[a]	BA2498	RSc0826	RSc1723	RSp0572	RSc1800	RSp1130	RSp1582	RSp1384	RSc1801	RSp1460	PTO4834	RSc0868	RSp0296	RSp0854	RSp0028	BA7003	PTO3558	PTO3560	PTO1391	PTO1265	PTO1326	PTO7001	RSc2775	RSp0099	RSp0847
160	IIB		2	2	2	1	2	2	2	2	2	2	3	3	2	3	4	1	1	1	1	1	1	1	1	1	1
476	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1
623	IIB		2	2	2	2	2	2	2	2	2	2	2	2	3	3	4	1	1	1	2	1	1	1	1	1	1
654	IIB		2	2	2	2	2	2	2	2	2	2	3	2	3	3	4	1	1	1	1	1	1	1	1	1	1
681	IIB		2	2	1	1	2	2	2	2	2	2	2	3	3	3	4	1	1	1	2	1	1	1	1	1	1
697	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1
843	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	2	1	1	1
845	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1
848	IIB		2	2	2	2	2	2	2	2	2	2	3	2	3	3	4	1	1	1	1	1	1	1	1	1	1
880	IIB		2	2	2	2	2	2	2	2	2	2	3	2	3	3	4	1	1	1	1	1	1	1	1	1	1
889	IIB		2	2	1	1	2	2	2	2	2	2	2	2	3	3	4	1	1	1	2	1	1	1	1	1	1
913	III		2	2	2	2	2	2	2	2	2	2	3	2	3	3	4	1	1	1	1	1	1	1	1	1	1
919	IIB		2	2	2	2	2	2	2	2	2	2	3	1	3	1	4	1	1	1	1	1	1	1	1	1	1
921	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1
923	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1
924	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1
925	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1
926	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	1	1	1	1

Table 7, Pensec, *Phytopathology*

Gene Name, Rip Name

RUN #	Phylotype	TRG[a]	BA2498 -	RSc0826 ripP1	RSc1723 _	RSp0572 ripAX2	RSc1800 ripG4	RSp1130 ripN	RSp1582 ripAZ1	RSp1384 ripAS	RSc1801 ripG5	RSp1460 ripAU	PTO4834 ripE2	RSc0868 ripP2	RSp0296 ripS5	RSp0854 hrpZ	RSp0028 ripG3	BA7003 -	PTO3558 -	PTO3560 ripU	PTO1391 ripA5_2	PTO1265 -	PTO1326 ripV2	PTO7001 -	RSc2775 ripW	RSp0099 ripA2	RSp0847 ripA4
928	**IIB**		2	2	2	2	2	2	2	2	2	2	3	3	2	3	4	1	1	1	1	1	1	1	1	1	1
931	IIB		2	2	2	2	2	2	2	2	2	2	3	3	2	3	4	1	1	1	1	1	1	2	1	1	1
934	IIB		2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	1	1	1	1	1	1	2	1	1	1
936	IIB		2	2	2	2	2	2	2	2	2	2	3	3	2	3	4	1	1	1	1	1	1	2	1	1	1
941	**IIB**		2	2	1	1	2	2	2	2	2	2	2	3	3	3	4	1	1	1	2	1	1	1	1	1	1
944	IIB		2	1	1	1	2	2	2	2	2	2	1	3	3	3	4	1	1	1	2	1	1	1	1	1	1
39	III		2	1	1	2	3	1	1	2	2	1	1	3	1	1	3	2	2	2	2	2	2	2	1	1	1
145	**III**		2	1	1	1	2	1	2	2	1	1	1	3	1	1	3	2	2	2	2	2	2	2	4	1	1
133	III		2	2	1	1	1	1	2	1	2	1	2	3	1	1	3	2	2	2	2	2	2	2	4	1	1
60	III	4	2	2	1	1	1	1	1	1	1	1	2	1	1	1	3	2	2	2	2	2	2	2	4	1	1
61	III		2	2	1	1	1	1	1	1	1	1	2	1	1	1	3	2	2	2	2	2	2	2	4	1	1
77	III		2	2	1	1	1	1	1	1	1	1	2	1	1	1	3	2	2	2	2	2	2	2	4	1	1
523	**III**		2	2	1	1	2	1	1	1	1	1	2	1	1	1	3	2	2	2	2	2	2	2	4	1	1
693	III		2	2	2	2	1	1	1	1	1	4	3	1	1	1	3	2	2	2	2	2	2	2	4	1	1
54	**I**		2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	2	2	2	2	2	2	2	3	1	1
215	I	5	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	2	1	1	2	2	2	2	3	1	1
155	I		2	1	1	1	1	1	1	2	2	2	1	3	1	1	1	2	1	1	2	2	2	2	3	1	1

Table 7, Pensec, *Phytopathology*

RUN #	Phylotype	TRG[a]	BA2498 -	RSc0826 ripP1	RSc1723 -	RSp0572 ripAX2	RSc1800 ripG4	RSp1130 ripN	RSp1582 ripAZ1	RSp1384 ripAS	RSc1801 ripG5	RSp1460 ripAU	PTO4834 ripE2	RSc0868 ripP2	RSp0296 ripS5	RSp0854 hrpZ	RSp0028 ripG3	BA7003 -	PTO3558 -	PTO3560 ripU	PTO1391 ripA5_2	PTO1265 -	PTO1326 ripV2	PTO7001 -	RSc2775 ripW	RSp0099 ripA2	RSp0847 ripA4
157	**I**		2	2	1	2	1	1	1	3	2	2	2	2	1	1	1	2	1	1	2	2	2	2	3	1	1
471	I		2	1	1	1	1	1	1	1	1	4	3	3	1	1	1	2	1	1	2	2	2	1	1	1	1
608	I		2	2	1	1	1	1	1	1	1	1	1	3	1	1	1	2	2	1	2	2	2	2	1	1	1
657	**III**	6	2	2	2	2	1	1	1	1	1	4	3	1	1	1	4	1	1	1	1	3	2	1	1	1	1
930	I		2	2	2	2	1	1	1	1	2	2	3	3	1	1	5	1	1	1	1	1	2	2	1	1	1
			Pattern 1[b]										Pattern 2				Pattern 3	Pattern 4							Pattern 5		

[a] Type III effector Repertoire Group, determined using the ascending hierarchical classification (function *agnes*, package *cluster*) with strains as individuals and genes as variables.

[b] Pattern, determined using the ascending hierarchical classification (function *agnes*, package *cluster*) with genes as individuals and strains as variables.

Table 8. T3Es highly associated to strain phenotypes on the three resistant accessions

Plant accession		Strain (RUN #)	Associated T3Es[a]
Dingras multiple Purple (E1)	Virulent strains	147 941 17	ripA5_2 (PTO1391) PTO1265 PTO7001 ripE2 (PTO4834)
	Avirulent strains	523 58	**ripAS (RSp1384)**[b]
AG91-25 (E6)	Virulent strains	941 157 147	PTO3558 ripU (PTO3560)
	Avirulent strains	523 145 59 54	**ripP2 (RSc0868)** ripAX2 (RSp0572)
Hawaii7996 (T5)	Virulent strains	941 147 17	**PTO3558**[c] **ripU (PTO3560)** BA7003 ripA5_2 (PTO1391)
	Quasi-avirulent strains[d]	145 54	**ripP1 (RSc0826)** **ripAX2 (RSp0572)** **ripP2 (RSc0868)** ripN (RSp1130) ripS5 (RSp0296)

[a] The T3Es associated to virulence were absent in all avirulent strains and present in all but one virulent strains, whereas those associated to avirulence were absent in all virulent strains and present in all but one avirulent strains.

[b] The avirulence T3Es marked in bold were absent in all virulent strains and present in all avirulent strains.

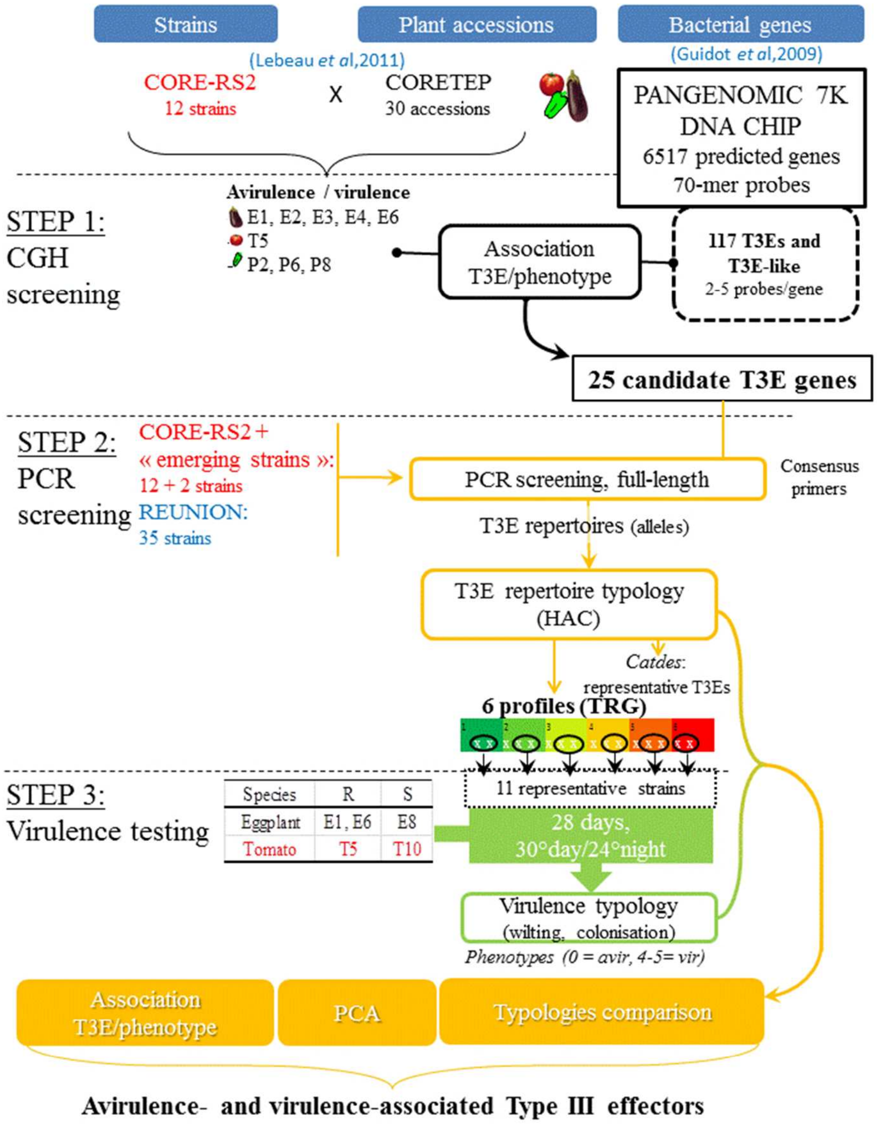
[c] The virulence T3Es markeed in bold were present in all virulent strains and absent in all avirulent strains.

[d] The quasi-avirulent strains induced no wilt, but were able to colonize Hawaii7996, corresponding a virulence phenotype of 1 instead of 0.

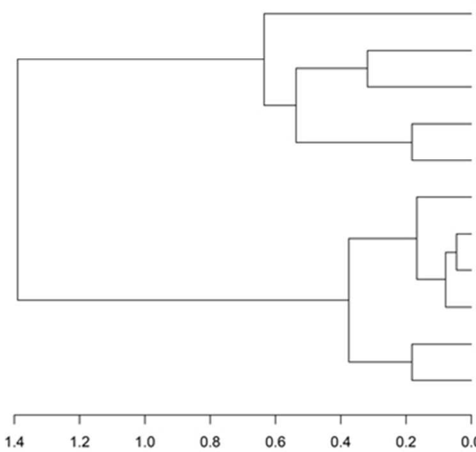
Table 9. Correspondence between phenotype-genotype associations inferred from CGH data (STEP 1) and from full-length PCR data (STEP 3) for the 25 Type III effector and "effector-like" genes. Phenotype-CGH associations were summarized in the Table 6, whereas phenotype-PCR data associations were summarized in Table 8 and Figure 3. Genes marked in bold were identified from full-length PCR, or from both approaches.

Gene code	Rip name /other name	Dingras (E1)		AG91-25 (E6)		Hawaii7996 (T5)	
		CGH	PCR	CGH	PCR	CGH	PCR
BA02498	-	V[a]	-	-	-	-	-
BA07003	**-**	V	-	-	-	-	V
PT01265	-	V	V	V	-	-	-
PT03558	**-**	V	-	-	V	-	V
PT07001	-	V	V	V	-	-	-
RSc1723	-	A	-	-	-	-	-
RSp0854	HrpZ	A	-	-	-	-	-
RSp0099	ripA2	A	-	-	-	-	-
RSp0847	ripA4	A	-	A	-	-	-
PT01391	**ripA5_2**	-	V	V	V	-	V
RSp1384	**RipAS**	-	A	A	-	-	-
RSp1460	**RipAU**	-	-	A	A	-	A
RSp0572	**ripAX2**	-	-	-	A	A	A
RSp1582	ripAZ1	-	-	A	-	-	-
PT04834	ripE2	V	V	V	-	-	-
RSp0028	**ripG3**	-	-	A	A	-	A
RSc1800	ripG4	A	-	-	-	-	-
RSc1801	**ripG5**	A	-	-	-	-	A
RSp1130	**RipN**	A	A	A	-	-	A
RSc0826	**ripP1**	-	-	A	A	-	A
RSc0868	**ripP2**	-	-	A	A	-	A
RSp0296	ripS5	A	-	-	-	-	A
PT03560	**RipU**	-	-	-	V	V	V
PT01326	**ripV2**	-	-	-	-	V	V
RSc2775	RipW	A	-	-	-	-	-

[a] A: associated to avirulence; V: associated to virulence.



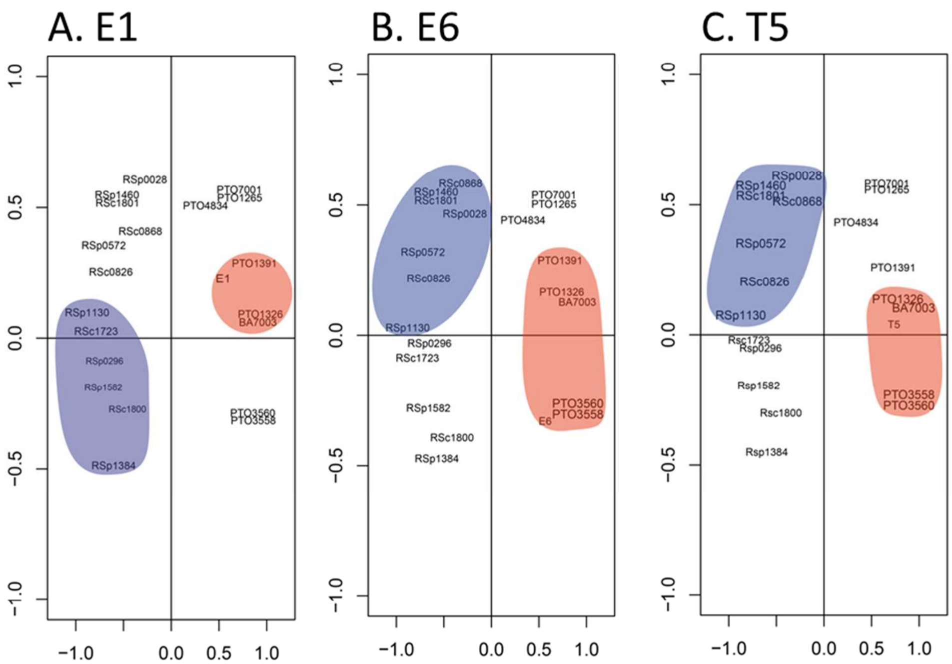
Genotype-phenotype association workflow. Data priming this study were generated by Lebeau et al. (Lebeau 2010, Lebeau et al. 2011) and Guidot et al. (2009). T3E: Type III Effector, HAC: Hierarchical Ascending Classification, TRG: Type III effector Repertoire Group. In Step 3, the eggplant accessions were either resistant (R) or susceptible (S).
254x338mm (72 x 72 DPI)



Strain	TRG	Phenotype on: Eggplant R E1	Eggplant R E6	Eggplant S E8	Tomato R T5	Tomato S T10
657 III	6	1	1	0	2	5
157 I	5	1	4	5	2	5
54 I	5	1	0	4	1	5
523 III	4	0	0	1	2	2
145 III	4	1	0	3	1	4
941 IIB	3	2	4	5	5	5
928 IIB	3	1	3	2	3	4
59 IIB	3	1	0	2	2	4
147 IIB	3	3	4	5	5	5
58 IIA	2	0	1	4	2	2
17 IIB	1	2	2	2	4	5

T3E genotypes do not match virulence phenotypes. Hierarchical ascending classification of R. solanacearum strains (named by their RUN number and phylotype) based on their T3E repertoire, and correspondence with phenotypesa observed on resistant (R) and susceptible (S) eggplants and tomatoes. TRG, Type III effector Repertoire Group.

a The phenotype score was defined by the combination of final wilting incidence and colonization index, and calculated following Lebeau et al.(2011), where 0 = complete resistance (no wilt and no colonization), 1=highly resistant, 2= moderately resistant, 3= partially resistant, 4=moderately susceptible, and 5= highly susceptible.



Distribution of the T3E genes on the factorial plan of the two mostly informative axes of the Principal Component Analysis performed on gene presence-absence data and phenotypic data collected from pathogenicity tests on Dingras multiple Purple (E1) (A), AG91-25 (E6) (B), Hawaii7996 (T5) (C). T3E genes associated to avirulence were shaded in blue, while those associated to virulence were shaded in red.

Table S1. Type III effector genes *putative T3Es,* and T3E-related genes of *Ralstonia solanacearum*, named by Probe codes and Rip family name, and their distribution frequency (calculated as number of probes/total gene probes) within CoreRS2 strains.

Code	Rip Name	Description[a]	Category	No probes /gene	Phylotype I					Phylotype IIA		Phylotype IIB		Phylotype III		
					GMI1000 RUN0054	PSS366 RUN0155	PSS4 RUN0157	PSS358 RUN0159	CMR134 RUN0215	CFBP2957 RUN0036	CMR39 RUN0150	CFBP6783 RUN0017	CMR34 RUN0147	CFBP3059 RUN0039	CMR15 RUN0133	CMR32 RUN0145
BA00250	ripS4	_	T3E	3	0.33	NA	NA	0.33	0.33	1.00	1.00	1.00	0.33	0.33	0.33	0.33
BA02498	-	CPUF	other	3	0.00	NA	NA	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
BA02930	ripAL	_	T3E	3	0.00	NA	NA	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00
BA07003	-	*pcaD2* (b-keto adipate enol lactone hydrolase protein	other	2	0.00	0.00	0.00	0.00	0.00	0.50	0.50	1.00	0.50	0.00	0.00	0.00
PTO0619	-	putative glycosyltransferase	other	2	0.00	NA	NA	0.00	0.00	0.50	0.50	0.50	0.50	0.50	0.00	0.00
PTO1265	-	CPUF	other	3	0.00	0.33	0.33	0.33	0.33	0.00	0.00	0.33	1.00	0.33	0.00	0.00
PTO1326	ripV2	_	T3E	3	0.00	NA	NA	0.00	0.00	1.00	0.67	1.00	1.00	0.00	0.00	0.00
PTO1391	ripA5_2	_	T3E	3	0.00	NA	NA	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00
PTO1808	-	putative T3E	putative T3E	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00
PTO3045	-	CPUF, TPR domain	other	2	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
PTO3558	-	CPUF, exopolysaccharide phosphotransferase domain	other	3	0.00	NA	NA	0.00	0.00	0.33	0.33	0.67	0.67	0.00	0.00	0.00
PTO3560	ripU	_	T3E	2	0.00	NA	NA	0.00	0.00	1.00	0.50	1.00	1.00	0.00	0.00	0.00
PTO4098	-	putative T3E	putative T3E	3	0.00	NA	NA	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
PTO4281	-	CPUF, papd-like transmembrane protein domain	other	3	0.00	NA	NA	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
PTO4284	-	putative T3E	putative T3E	2	0.00	NA	NA	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
PTO4397	-	CPUF	other	3	0.00	0.00	0.00	0.00	0.00	1.00	0.67	0.33	1.00	0.00	0.00	0.00
PTO4434	ripBI	_	T3E	3	0.00	NA	NA	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
PTO4502	ripBH	_	T3E	3	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
PTO4834	ripE2	_	T3E	4	0.00	0.25	0.00	0.25	0.00	0.25	0.25	0.75	0.50	0.25	0.25	0.25
PTO7000	-	LRR domain	putative T3E	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00
PTO7001	-	putative T3E (fragment)	putative T3E	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00
PTO7002	-	intergene	other	1	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00
RSc0041	ripI	_	T3E	4	1.00	NA	NA	1.00	1.00	1.00	1.00	0.75	1.00	0.50	0.50	0.50
RSc0227	ripBA_fragment1	_	T3E	3	1.00	NA	NA	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RSc0245	ripB	_	T3E	4	1.00	1.00	1.00	1.00	1.00	0.75	0.75	0.75	0.75	0.75	0.75	1.00
RSc0257	ripY	_	T3E	3	1.00	0.33	1.00	0.00	0.67	1.00	1.00	1.00	0.67	0.33	1.00	1.00
RSc0321	ripAE	_	T3E	4	0.75	0.75	0.75	0.75	0.75	0.75	0.75	1.00	0.75	0.50	0.25	0.25
RSc0608	ripAA	_	T3E	5	0.80	0.00	0.20	0.00	0.80	1.00	0.60	0.40	1.00	0.00	0.20	0.00
RSc0826	ripP1	_	T3E	3	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
RSc0868	ripP2	_	T3E	4	1.00	1.00	0.00	1.00	1.00	0.25	0.00	0.50	0.00	0.00	0.25	0.00
RSc0895	ripAH	_	T3E	3	1.00	1.00	0.67	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
RSc1349	ripV1	_	T3E	5	1.00	NA	NA	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.60	0.60
RSc1356	ripG6	_	T3E	4	1.00	0.75	0.75	0.75	0.50	1.00	0.75	1.00	0.75	0.00	0.50	0.75
RSc1357	ripG7	_	T3E	4	1.00	NA	NA	0.50	0.00	0.50	0.50	0.75	0.75	0.25	0.50	1.00
RSc1386	ripH1	_	T3E	4	1.00	NA	NA	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.50	0.50
RSc1475	ripM	_	T3E	3	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
RSc1723	-	putative	putative T3E	4	0.50	NA	NA	0.50	0.50	0.75	0.25	0.50	0.50	0.50	0.50	0.50
RSc1800	ripG4	_	T3E	4	0.50	0.50	0.50	0.50	0.50	1.00	0.50	0.75	0.50	0.25	0.50	0.50
RSc1801	ripG5	_	T3E	4	0.75	0.75	0.75	0.75	0.75	1.00	0.75	0.75	1.00	0.75	0.75	0.75
RSc1815	ripTAL	_	T3E	5	1.00	1.00	1.00	1.00	1.00	0.00	0.80	0.20	0.00	0.00	0.20	0.20
RSc1839	ripS4	_	T3E	5	0.80	0.80	0.80	0.80	0.80	1.00	1.00	1.00	0.80	0.60	0.60	0.40
RSc2101	ripAJ	_	T3E	3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.33	1.00	0.33
RSc2131	-	PUF	other	3	1.00	0.67	1.00	0.67	1.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00
RSc2132	ripJ	_	T3E	3	1.00	0.67	0.33	0.67	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00

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Code	Rip Name	Description[a]	Category	No probes /gene	Phylotype I					Phylotype IIA		Phylotype IIB		Phylotype III		
					GMI1000 RUN0054	PSS366 RUN0155	PSS4 RUN0157	PSS358 RUN0159	CMR134 RUN0215	CFBP2957 RUN0036	CMR39 RUN0150	CFBP6783 RUN0017	CMR34 RUN0147	CFBP3059 RUN0039	CMR15 RUN0133	CMR32 RUN0145
RSc2139	ripA1	_	T3E	4	1.00	1.00	1.00	1.00	1.00	0.25	0.00	0.00	0.00	0.25	0.25	0.00
RSc2291	-	putative transglycosylase	other	5	0.80	0.60	0.80	0.60	0.60	0.60	0.20	0.60	0.40	0.80	0.80	0.60
RSc2359	ripAK	_	T3E	4	1.00	1.00	1.00	1.00	1.00	0.25	0.00	0.00	0.00	0.00	0.00	0.25
RSc2775	ripW	_	T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.75	1.00	0.50	0.75	0.75
RSc2897	-	putative	putative T3E	4	1.00	NA	NA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50
RSc3155	-	putative hydrolase protein	other	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSc3174	-	putative T3E	putative T3E	4	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RSc3212	ripT	_	T3E	2	1.00	NA	NA	0.00	1.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00
RSc3241	-	putative n-terminal part of a truncated yopp/avrrvx-related protein	other	4	1.00	NA	NA	1.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00
RSc3272	ripAM	_	T3E	4	0.75	NA	NA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75
RSc3290	ripAX1	_	T3E	3	1.00	0.33	1.00	0.67	0.33	0.00	0.33	0.00	0.33	0.33	0.00	0.00
RSc3369	ripE1	_	T3E	4	1.00	NA	NA	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00	0.00
RSc3401	ripS1	_	T3E	4	1.00	0.50	1.00	0.25	0.25	1.00	1.00	1.00	1.00	0.75	0.75	0.50
RSp0028	ripG3	_	T3E	5	0.60	0.60	0.60	0.60	0.60	0.40	0.60	0.40	0.60	0.40	0.60	0.60
RSp0099	ripA2	_	T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.75	1.00	1.00	1.00	1.00
RSp0160	ripH3	_	T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.75	0.75	1.00	1.00	0.25
RSp0193	ripL	_	T3E	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	0.50
RSp0213	-	putative T3E	putative T3E	2	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RSp0215	ripH2	_	T3E	4	1.00	1.00	1.00	0.75	0.75	0.25	0.25	0.50	0.75	0.75	1.00	0.75
RSp0216	-	putative type III effector protein (Serine/threonine-protein kinase)	putative T3E	4	1.00	NA	NA	0.25	1.00	1.00	0.75	0.00	0.00	1.00	1.00	0.00
RSp0218	-	putative T3E	putative T3E	4	1.00	NA	NA	0.50	1.00	0.50	0.50	0.00	0.00	0.75	0.75	0.25
RSp0296	ripS5	_	T3E	4	1.00	1.00	1.00	1.00	1.00	0.25	0.00	0.50	0.50	1.00	1.00	0.75
RSp0304	ripD	_	T3E	3	1.00	NA	NA	1.00	1.00	0.67	0.33	0.67	0.67	0.67	1.00	0.33
RSp0323	ripO1	_	T3E	5	1.00	0.80	0.80	0.80	0.40	0.80	1.00	0.80	0.80	0.40	0.80	0.40
RSp0527	-	conserved exported protein of unknown function	other	4	1.00	NA	NA	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00
RSp0572	ripAX2	_	T3E	4	1.00	1.00	0.00	1.00	1.00	0.00	1.00	0.25	0.25	0.00	0.00	1.00
RSp0672	ripG2	_	T3E	3	1.00	1.00	1.00	1.00	0.33	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0731	ripTPS	_	T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00
RSp0732	ripAV	_	T3E	4	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00	1.00	1.00	1.00
RSp0822	ripAF1	_	T3E	4	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.50	0.25	0.25	1.00	0.00
RSp0837	-	conserved hypothetical protein	other	4	1.00	1.00	1.00	1.00	1.00	0.75	0.75	0.75	0.75	1.00	1.00	1.00
RSp0839	-	CPUF	other	4	0.50	0.25	0.50	0.25	0.25	0.75	0.75	0.75	0.75	0.75	0.50	0.50
RSp0842	-	putative leucine-rich-repeat type III effector protein (popC-like)	putative T3E	4	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0845	ripAN	_	T3E	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0846	ripA3	_	T3E	3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0847	ripA4	_	T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.75	0.75	1.00	0.75
RSp0853	-	hpaB	SSTT	3	1.00	NA	NA	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00
RSp0854	-	hrpZ	SSTT	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50	1.00	1.00	1.00
RSp0855	-	hrpY	SSTT	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0856	-	hrpX	SSTT	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0857	-	hrpW	SSTT	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0858	-	hrpV	SSTT	2	1.00	NA	NA	1.00	1.00	1.00	0.50	1.00	1.00	0.50	0.50	0.50
RSp0875	ripAC	_	T3E	3	0.67	0.67	0.67	0.67	0.67	0.33	0.33	0.33	0.33	0.67	0.00	0.00
RSp0876	ripAB	_	T3E	4	1.00	0.75	0.75	1.00	1.00	0.75	0.50	1.00	0.75	0.75	0.75	0.75
RSp0877	ripX	_	T3E	2	0.50	0.50	1.00	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	0.50
RSp0879	ripAO	_	T3E	2	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.00	0.50	0.50	0.50	0.50
RSp0882	-	putative T3E	putative T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp0885	ripAQ	_	T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	0.75	1.00	1.00

Code	Rip Name	Description[a]	Category	No probes /gene	Phylotype I					Phylotype IIA		Phylotype IIB		Phylotype III		
					GMI1000	PSS366	PSS4	PSS358	CMR134	CFBP2957	CMR39	CFBP6783	CMR34	CFBP3059	CMR15	CMR32
					RUN0054	RUN0155	RUN0157	RUN0159	RUN0215	RUN0036	RUN0150	RUN0017	RUN0147	RUN0039	RUN0133	RUN0145
RSp0914	ripG1	_	T3E	3	1.00	0.67	1.00	0.67	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
RSp0930	ripS3	_	T3E	3	1.00	1.00	1.00	1.00	1.00	0.67	0.67	0.67	1.00	1.00	0.67	0.67
RSp1022	ripAY	_	T3E	3	0.67	NA	NA	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.33
RSp1024	ripA5	_	T3E	4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RSp1031	ripZ	_	T3E	4	1.00	NA	NA	0.75	1.00	0.75	0.75	0.75	0.75	0.75	0.75	0.75
RSp1130	ripN	_	T3E	3	1.00	1.00	0.67	1.00	1.00	0.67	0.67	0.67	0.67	0.67	1.00	0.67
RSp1212	ripU	_	T3E	5	0.60	0.00	0.00	0.00	0.00	0.20	0.40	0.00	0.40	0.00	0.00	0.00
RSp1215	ripAP_fragment2	_	T3E	3	1.00	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	0.33	1.00	0.00
RSp1218	ripAP_fragment1	_	T3E	1	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00
RSp1236	ripAR	_	T3E	4	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
RSp1239	ripC1	_	T3E	4	0.75	0.75	0.75	0.50	0.75	0.75	0.75	0.75	1.00	0.00	0.00	0.00
RSp1277	ripQ	_	T3E	3	1.00	1.00	1.00	1.00	1.00	0.67	0.67	0.67	0.33	0.00	0.33	0.00
RSp1281	ripR	_	T3E	4	1.00	1.00	1.00	0.75	1.00	1.00	0.75	1.00	1.00	0.75	0.75	0.75
RSp1374	ripS2	_	T3E	3	1.00	NA	NA	1.00	1.00	0.67	0.67	0.67	0.67	0.67	0.67	0.67
RSp1384	ripAS	_	T3E	4	0.75	0.25	0.75	0.25	0.75	0.50	0.50	0.25	0.25	0.25	0.75	0.50
RSp1388	ripAT	_	T3E	4	0.75	0.25	0.75	0.25	0.50	0.50	0.50	0.50	0.50	0.00	0.25	0.25
RSp1460	ripAU	_	T3E	3	0.67	NA	NA	0.67	0.67	0.67	0.33	0.33	0.00	0.67	0.67	0.67
RSp1461	-	conserved exported protein of unknown function	other	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00
RSp1462	-	putative outer membrane efflux protein	other	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00
RSp1465	-	putative macrolide export ATP-binding/permease protein macB	other	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00
RSp1475	ripAW	_	T3E	3	1.00	1.00	1.00	1.00	1.00	0.67	0.33	0.67	0.33	1.00	1.00	1.00
RSp1529	-	Gibberellin 3-beta-dioxygenase (Ethylene-forming enzyme) (EFE)	other	2	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.50	1.00	1.00	1.00
RSp1555	ripF1_1	_	T3E	4	0.75	NA	NA	0.50	0.50	1.00	1.00	1.00	1.00	0.25	0.75	0.75
RSp1582	ripAZ1	_	T3E	3	1.00	1.00	1.00	1.00	1.00	0.67	0.33	0.67	0.00	1.00	0.00	1.00
RSp1601	ripAD	_	T3E	3	1.00	1.00	1.00	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	0.67

[a] Description of the gene product, from automatic and expert annotation of genomic sequences in MAGE. PUF: Protein of unknown function; CPUF: Conserved Protein of unknown function; T3E: Type III Effector; T3SS: Type III Secretion System.

Table S2. Type III and putative type III effector genes *of Ralstonia solanacearum* ranked by decreasing frequencies within CoreRS2 strains. Their presence in core-effectomes described so far are listed.

Rip Name, or description	CORE (Peeters et al. 2014)	CORE (Ailloud et al. 2015)	CORE IIB/1-R3Bv2 (Clarke et al., 2015)	CORE (Data RalstoT3E, 26 genomes, August 2015)	Phylotype I					Phylotype IIA		Phylotype IIB		Phylotype III			Frequency (percentage of presence, over 10 strains)	
					RUN0054 GMI1000	RUN0155 PSS366[a]	RUN0157 PSS4[a]	RUN0159 PSS358	RUN0215 CMR134	RUN0036 CFBP2957	RUN0150 CMR39	RUN0017 CFBP6783	RUN0147 CMR34	RUN0039 CFBP3059	RUN0133 CMR15	RUN0145 CMR32		
RSc2897, *putative*					1	1	1	1	1	1	1	1	1	1	1	1	100	CORE-EFFECTOME
RSp0842, *putative T3E (popC-* like)					1	1	1	1	1	1	1	1	1	1	1	1	100	
RSp0882, *putative*					1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripA2	x				1	1	1	1	1	1	1	1	1	1	1	1	100	
ripA3					1	1	1	1	1	1	1	1	1	1	1	1	100	
ripA4	x				1	1	1	1	1	1	1	1	1	1	1	1	100	
ripA5	x				1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAB	x	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAD	x		x	x	1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAE			x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAJ	x	x			1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAM	x	x		x	1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripAN	x	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAQ	x				1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAR	x		x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAS					1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAV					1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAW					1	1	1	1	1	1	1	1	1	1	1	1	100	
ripAY	x	x	x	x	1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripB	x	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	100	
ripD	x				1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripF1_1	x	x	x		1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripG2			x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripG3			x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripG4			x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripG5	x	x	x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripH1	x		x		1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripH2	x		x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripH3	x				1	1	1	1	1	1	1	1	1	1	1	1	100	
ripI			x		1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripM	x				1	1	1	1	1	1	1	1	1	1	1	1	100	
ripN			x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripO1					1	1	1	1	1	1	1	1	1	1	1	1	100	
ripR	x	x		x	1	1	1	1	1	1	1	1	1	1	1	1	100	
ripS1			x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripS2					1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripS3	x				1	1	1	1	1	1	1	1	1	1	1	1	100	
ripS4					1	1	1	1	1	1	1	1	1	1	1	1	100	
ripS4					1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripTPS					1	1	1	1	1	1	1	1	1	1	1	1	100	
ripV1			x		1	NA	NA	1	1	1	1	1	1	1	1	1	100	
ripW	x	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	100	
ripX			x		1	1	1	1	1	1	1	1	1	1	1	1	100	
ripZ					1	NA	NA	1	1	1	1	1	1	1	1	1	100	
RSp0218, *putative T3E*					1	NA	NA	1	1	1	1	0	0	1	1	1	80	PRESENCE IN ≥ 80 % STRAINS
ripAC	x	x	x	x	1	1	1	1	1	1	1	1	1	1	0	0	80	
ripAF1					1	1	1	1	1	1	1	1	1	1	1	0	90	
ripAL					0	NA	NA	1	1	1	1	1	1	1	1	1	90	
ripAO	x			x	1	1	1	1	1	1	1	0	1	1	1	1	90	
ripAP_fragment2			x		1	1	1	1	1	1	1	1	1	1	1	0	90	
ripAT					1	1	1	1	1	1	1	1	1	0	1	1	90	
ripAU					1	NA	NA	1	1	1	1	1	0	1	1	1	90	
ripAZ1					1	1	1	1	1	1	1	1	0	1	0	1	80	
ripE1	x		x		1	NA	NA	1	1	1	1	1	1	1	1	0	90	
ripE2					0	1	0	1	0	1	1	1	1	1	1	1	80	
ripG7	x		x		1	NA	NA	1	0	1	1	1	1	1	1	1	90	
ripL					1	1	1	1	1	1	1	1	1	0	1	1	90	
ripQ					1	1	1	1	1	1	1	1	1	0	1	0	80	
ripS5					1	1	1	1	1	1	0	1	1	1	1	1	90	
ripG6			x		1	1	1	1	1	1	1	1	1	0	1	1	90	
ripY					1	1	1	0	1	1	1	1	1	1	1	1	90	
RSp0216, *putative*					1	NA	NA	1	1	1	1	0	0	1	1	0	70	
PTO4098*, putative*					0	NA	NA	0	0	1	1	1	1	0	0	0	40	
PTO4284, *putative*					0	NA	NA	0	0	1	1	1	1	0	0	0	40	
ripA1					1	1	1	1	1	1	0	0	0	1	1	0	60	
ripAA	x				1	0	1	0	1	1	1	1	1	0	1	0	70	

Rip Name, or description	CORE (Peeters et al. 2014)	CORE (Ailloud et al. 2015)	CORE IIB/1-R3Bv2 (Clarke et al., 2015)	CORE (Data RalstoT3E, 26 genomes, August 2015)	Phylotype I					Phylotype IIA		Phylotype IIB		Phylotype III			Frequency (percentage of presence, over 10 strains)	
					RUN0054 GMI1000	RUN0155 PSS366[a]	RUN0157 PSS4[a]	RUN0159 PSS358	RUN0215 CMR134	RUN0036 CFBP2957	RUN0150 CMR39	RUN0017 CFBP6783	RUN0147 CMR34	RUN0039 CFBP3059	RUN0133 CMR15	RUN0145 CMR32		
ripP1					1	1	0	1	1	1	0	1	0	1	0	1	70	VARIABLE EFFECTORS
ripAK					1	1	1	1	1	1	0	0	0	0	0	1	50	
ripAP_fragment1					1	1	1	1	1	1	0	1	1	0	1	0	70	
ripAX1					1	1	1	1	1	0	1	0	1	1	0	0	60	
ripAX2					1	1	0	1	1	0	1	1	1	0	0	1	70	
ripBA_fragment1					1	NA	NA	1	1	0	0	0	0	0	0	0	30	
ripBH					0	0	0	0	0	1	1	1	1	0	0	0	40	
ripBI			x		0	NA	NA	0	0	1	1	1	1	0	0	0	40	
ripC1	x		x		1	1	1	1	1	1	1	1	1	0	0	0	70	
ripG1					1	1	1	1	1	0	0	0	0	0	0	0	30	
ripJ					1	1	1	1	1	1	1	1	1	0	0	0	70	
ripP2					1	1	0	1	1	1	0	1	0	0	1	0	60	
ripAH					1	1	1	1	1	0	0	0	0	1	0	0	40	
ripT					1	NA	NA	0	1	1	0	0	0	0	0	0	30	
ripTAL					1	1	1	1	1	0	1	1	0	0	1	1	70	
ripU	x		x		1	0	0	0	0	1	1	0	1	0	0	0	40	
ripV2			x		0	NA	NA	0	0	1	1	1	1	0	0	0	40	
ripA5_2	x				0	NA	NA	0	0	0	0	1	1	0	0	0	20	≤ 20% STRAINS
RSp0213, *putative*					1	0	1	0	0	0	0	0	0	0	1	0	20	
PTO1808, *putative*					0	0	0	0	0	0	0	1	1	0	0	0	20	
PTO7001, *putative* (*ripM* fragment)					0	0	0	0	0	0	0	1	1	0	0	0	20	
RSc3174, *putative*					1	0	0	0	0	0	0	0	0	0	0	0	10	
PTO7000, *putative*					0	0	0	0	0	0	0	0	1	0	0	0	10	
TOTAL genes					78	56[a]	54[a]	74	75	77	73	75	74	62	66	60		

[a] On these two strains, 25 genes could not be considered as present or absent.

b Within this collection, several Type III effectors were absent or not detected: *ripAI* (core-effecteor *sensu* Peeters *et al* 2013 and Ailloud *et al* 2015), *ripBA, ripC2, ripF1_2, ripP3, ripS8.*

Table S3. Type III effector genes of *Ralstonia solanacearum* ranked by decreasing frequencies within 26 complete genomes available on the "RalstoT3E" website. strains. We summarized their presence in the different core-effectomes described so far. The complete repertoire of each genome is summarized in the table's bottom.

Family	Description	CORE			Phylotype I				Phylotype III	Phylotype IV			Phylotype IIA				Phylotype IIB														Frequency (percentage of presence, over 26 genomes)	
		(Peeters et al. 2013)	(Ailloud et al. 2015)	IIB/1, R3Bv2 (Clarke et al. 2015)	161	244	FQY_4	GMI1000	CMR15	BDBR229	Psi07	R24	B50	CFBP2957	Grenada91	K60	23_10BR	CFBP1416	CFBP6783	CIP417	IBSBF1503	IPO1609	Molk2	NCPPB_282	P673	Po82	POPS2	UW163	UW179	UW551		
RipAB	(PopB),NLS_harboring_protein	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	C C
RipAC	(PopC),LRR_domain	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipAD	NA	x		x	1	1	1	1	1	f	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipAI	NA	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipAM	NA	x	x		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipAN	NA	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	f	1	1	1	100.00	
RipAO	NA	x			1	1	1	1	1	f	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	100.00	
RipAY	NA	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipB	Inosine-uridine_nucleoside_N-ribohydrolase	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipR	NA	x	x		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipW	Harpin_with_pectate_lyase_domain	x	x	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100.00	
RipA2	AWR2	x			1	1	1	1	1	1	1	1	1	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	8 IV
RipA3	AWR3				1	1	1	1	1	f	1	1	1	1	1	1	1	1	1	1	1	f	f	1	1	1	f	1	1	0	96.15	
RipA4	AWR4	x			1	1	1	1	1	f	1	1	1	1	1	1	1	1	1	1	1	f	1	p	1	f	1	1	1	f	96.15	
RipAE	Putative_acetyltransferase			x	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	
RipAJ	NA	x	x		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	p	1	1	1	96.15	
RipC1	HAD-like_phosphatase	x		x	1	1	1	1	1	1	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	
RipD	NA	x			1	1	1	1	1	1	1	1	1	1	1	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	
RipE1	NA	x	x	x	1	1	1	1	1	1	d	1	1	1	1	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	
RipF1	(PopF1)_T3SS_translocator	x	x	x	p	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	
RipG5	F-box_LRR_protein_GALA5	x	x	x	1	1	1	1	1	1	1	1	1	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	
RipG7	F-box_LRR_protein_GALA7	x		x	1	1	1	1	1	f	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	p	1	1	1	96.15	
RipH2	HLK2	x		x	1	1	1	1	1	d	d	p	1	1	1	1	1	1	d	1	d	1	1	1	d	d	1	d	d	1	96.15	
RipU	NA	x		x	1	1	1	1	1	f	1	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.15	
RipAQ	NA	x			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	p	f	d	p	1	92.31	
RipAT	NA				1	1	1	p	1	f	1	1	1	1	1	1	1	1	1	1	1	1	1	p	1	1	1	1	1	1	92.31	
RipG3	F-box_LRR_protein_GALA3			x	1	1	1	1	1	0	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	92.31	
RipG4	F-box_LRR_protein_GALA4			x	1	1	1	1	1	p	0	1	1	1	1	1	1	1	1	1	1	f	1	1	1	1	1	1	1	1	92.31	
RipG6	F-box_LRR_protein_GALA6			x	1	1	1	1	1	1	1	p	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	92.31	
RipH1	HLK1	x		x	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	p	1	1	1	92.31	
RipS3	SKWP3	x			1	1	1	1	1	1	1	0	p	1	1	1	1	1	1	1	1	f	1	1	1	1	1	1	1	f	92.31	
RipX	(Pod),Harpin	x		x	1	1	1	1	1	p	1	1	1	1	0	1	1	1	1	1	1	1	f	1	1	1	1	1	1	1	92.31	
RipAR	Ubiquitin_ligase_domain	x		x	1	1	p	1	1	1	1	1	p	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88.46	
RipG2	F-box_LRR_protein_GALA2			x	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	d	1	1	1	1	1	1	1	1	1	88.46	
RipTPS	Trehalose -phosphate_synthase				d	d	d	1	1	0	1	p	1	1	d	1	d	d	d	d	d	0	1	d	d	f	d	d	d	1	88.46	
RipV1	Ubiquitin_ligase_domain			x	1	1	0	1	1	f	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	p	1	1	1	88.46	
RipA5	AWR5	x			1	1	1	1	f	f	1	1	1	1	1	1	1	d	d	d	d	d	d	p	d	d	p	p	p	d	84.62	
RipAP	Ankyrin_Repeats			x	1	1	1	p	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	f	1	1	1	84.62	
RipAU	NA				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1	0	84.62	
RipN	Nudix_hydrolase			x	1	1	1	1	1	p	1	0	0	1	1	1	p	1	1	1	1	1	1	1	1	1	1	1	1	1	84.62	
RipZ	NA	x			1	1	1	1	1	1	1	1	0	1	p	1	1	p	1	p	1	1	1	1	1	1	f	1	1	f	84.62	
RipAA	(AvrA)	x			1	1	1	1	1	f	1	1	1	1	1	p	1	0	0	1	0	1	1	f	1	1	p	1	1	1	80.77	
RipI	NA			x	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	d	f	p	f	1	p	1	1	P	80.77	
RipM	NA	x			1	1	1	1	1	1	1	0	1	1	1	1	1	1	p	1	p	f	1	1	f	f	1	p	p	1	80.77	
RipAL	Lidse_domain				1	1	1	0	f	f	1	p	1	1	0	1	1	1	1	0	p	0	1	1	1	1	1	1	1	1	76.92	R A
RipAS	NA				1	1	1	1	1	p	p	1	0	1	1	p	1	1	1	1	p	1	1	p	1	1	1	1	1	1	76.92	
RipAV	NA				1	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0	1	p	1	1	1	1	1	1	76.92	
RipE2	NA				0	1	0	0	f	1	p	0	p	1	1	1	1	1	1	1	1	1	f	1	1	1	1	1	1	1	76.92	
RipH3	HLK3	x			1	1	1	1	1	f	1	1	p	1	1	p	1	1	p	p	p	1	1	1	1	1	1	p	1	f	76.92	
RipO1	NA				1	p	1	1	1	0	0	0	1	1	1	1	p	1	1	1	1	1	1	1	1	1	p	1	1	1	76.92	
RipY	Ankyrin_Repeats				1	1	1	1	1	1	1	0	1	1	1		0	0	1	0	1	1	p	1	1	1	1	1	1	1	76.92	
RipF2	T3SS_translocator			x	0	0	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	f	1	1	1	1	1	1	1	1	73.08	
RipBH	NA				0	0	0	0	0	1	1	0	1	1	1	0	1	1	1	1	1	1	f	1	1	1	p	1	1	1	69.23	
RipS5	SKWP5				1	1	1	1	1	1	1	p	d	p	1	1	1	1	p	p	1	p	f	1	1	f	1	p	p	0	69.23	
RipV2	Ubiquitin_ligase_domain			x	0	0	1	0	0	f	1	1	1	1	1	1	1	p	1	p	1	1	f	1	1	f	1	p	p	1	69.23	
RipQ	NA				1	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	p	1	p	1	1	p	1	p	p	65.38	
RipAZ1	NA				1	1	1	1	p	1	1	f	1	1	1	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0	61.54	
RipBI	NA			x	0	0	0	0	0	0	0	0	p	1	1	p	1	1	1	1	1	1	f	1	1	1	1	1	1	1	61.54	
RipL	Pentatricopeptide_Repeats				1	1	1	1	1	0	f	1	1	0	0	1	1	0	1	0	1	0	0	0	1	1	0	1	1	0	61.54	
RipAW	Ubiquitin_ligase_domain				1	1	1	1	1	0	1	0	0	1	1	1	1	0	1	0	1	0	0	0	1	1	p	0	1	0	57.69	
RipS7	SKWP7				0	0	0	0	0	1	1	1	0	1	1	0	p	p	1	1	1	f	1	1	1	1	1	p	p	1	57.69	
RipS1	SKWP1			x	1	0	0	1	1	0	0	0	p	1	p	1	1	p	1	p	p	1	1	1	1	1	1	p	2	1	53.85	
RipS2	SKWP2				1	1	1	1	1	0	1	0	p	1	0	1	p	p	p	p	p	1	f	1	1	1	1	p	p	0	53.85	
RipAF1	Putative_ADP-ribosyltransferase				1	1	1	1	1	0	0	0	1	1	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	50.00	
RipAX1	NA				p	1	d	1	0	1	0	0	p	0	1	0	0	1	0	1	0	1	f	f	0	1	1	0	0	1	50.00	
RipJ	Putative_acetyltransferase				1	1	1	1	0	0	0	0	0	1	0	1	1	0	1	1	0	0	1	p	p	1	p	d	1	p	50.00	
RipP2	(PopP2),Acetyltransferase				1	1	1	1	1	0	0	0	0	0	1	p	1	0	1	0	1	0	1	0	1	1	p	1	1	0	50.00	
RipS4	SKWP4				1	1	1	1	1	0	1	1	p	1	0	1	p	p	p	p	1	0	f	0	1	1	0	p	p	0	50.00	
RipC2	HAD-like_phosphatase				0	1	P	P	p	0	0	p	0	1	1	1	1	0	1	0	1	0	0	0	1	0	1	1	1	0	42.31	
RipBC	YopJ_acetyltransferase_domain_&_Ankyrin_Repeats				0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	1	0	0	0	1	1	f	1	1	0	38.46	
RipBB	Ankyrin_repeats				0	1	0	0	0	f	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	1	0	0	0	34.62	
RipBD	NA				0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0	30.77	
RipBG	Ubiquitin_ligase_domain				0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	1	0	0	1	1	0	30.77	
RipAX2	NA				1	p	p	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	f	p	0	1	1	0	0	26.92	
RipTAL	Transcription_Activator-Like_protein				1	1	1	1	p	1	1	0	0	p	p	0	0	p	0	p	0	0	d	0	0	0	0	0	0	0	26.92	
RipG1	F-box_LRR_protein_GALA1				1	1	p	1	1	d	d	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	23.08	
RipP1	(PopP1),putative_acetyltransferase				0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	23.08	
RipS6	SKWP6				1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	23.08	
RipAG	NA				1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.23	0 2
RipA1	AWR1				1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.38	

RipAH	NA	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.38
RipK	YopJ_acetyltransferase_domain	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.38
RipS8	SKWP8	1	1	1	p	0	0	1	0	0	0	0	0	0	p	0	0	0	0	0	0	0	0	0	0	0	0	15.38
RipT	Cysteine_protease	0	1	p	1	0	p	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.38
RipAK	NA	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.54
RipBF	NA	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.54
RipH4	HLK4	0	0	0	0	0	1	1	f	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.54
RipBA	NA	1	1	p	p	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.69
RipP3	(PopP3)	0	1	0	p	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	7.69
RipAF2	Putative_ADP-ribosyltransferase	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.85
RipAZ2	NA	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.85
RipG8	F-box_LRR_protein_GALA8	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.85
RipO2	NA	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.85
RipBE	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
# Protein (1)[a]		67	74	66	68	58	35	58	44	54	67	57	54	59	56	64	55	61	46	52	51	65	62	46	57	59	50	
# Frameshift (f)		0	0	0	0	3	14	1	2	0	0	0	0	0	0	0	0	0	7	11	3	2	5	6	0	0	4	
# Gene in duplications (d)		1	1	2	1	1	2	3	0	1	0	1	0	1	2	3	2	4	2	2	1	3	2	2	4	2	1	
# Present genes *sensu* Peeters et al. 2013 (= 1+f+d). Min and Max values		68	75	68	69	62	51	62	46	55	67	58	54	60	58	67	57	65	55	65	55	70	69	54	61	61	55	
# Absence (0)		24	17	20	19	29	38	30	40	29	25	31	34	27	28	22	29	23	37	28	31	22	24	27	23	22	36	
# Pseudogene (p)		2	2	5	6	3	5	2	8	10	2	5	5	7	8	5	8	6	2	1	8	2	1	13	9	9	3	
# Absent genes *sensu* Peeters et al. 2013 (= 0+p)		26	19	25	25	32	43	32	48	39	27	36	39	34	36	27	37	29	39	29	39	24	25	40	32	31	39	

[a] Definitions of frameshifted sequences, pseudogene sequences were followed from Peeters et al. 2013. To estimate the Type III Effector evenness, we considered "Protein", "Frameshift", and "Duplicate"-scored genes as functional, following Peeters et al.,2013. Pseudogene sequences were considered non-functional, and thus pooled with the "Absent" category.

Table S4.Type III Effector and "effector-like" genes associated with avirulence of *Ralstonia solanacearum* to eggplant, pepper, tomato (the score is calculated as number of probes/total gene probes).

Gene code	Rip Name	Former /other Name, description	No Probes /gene	EGGPLANT					PEPPER[a]		TOMATO	Related to FITNESS on[b]
				Dingras (E1)	SM6 (E2)	Ceylan (E3)	Surya (E4)	AG91-25 (E6)	CA8 (P6)	Perennial (P8)	Hawaii 7996 (T5)	
RSc1723	-	putative T3E	4	0.25						0.50		
RSc2291	-	Putative transglycosylase	5	0.20				0.20		0.40		
RSc2897	-	putative T3E	4		0.25			0.50				
RSp0213	-	putative T3E	2							1.00		
RSp0216	-	putative T3E (Ser/Threonin domain)	4			0.50				1.00		
RSp0218	-	Putative T3E	4			0.50				0.75		
RSp0527	-	CPUF	4		0.25							
RSp0837	-	Conserved hypothetical protein	4							0.25		
RSp0839	-	CPUF	4		0.25							
RSp0853	-	hpaB	3						0.33	0.33		
RSp0854	-	hrpZ	2	0.50						0.50		
RSp1529	-	Gibberellin 3-beta-dioxygenase (Ethylene-forming enzyme) (EFE)	2							0.50		EGGPLANT
RSc2139	*ripA1*	AWR1	4							0.25		
RSp0099	*ripA2*	AWR2 (RipA)	4	0.25						0.25		
RSp0847	*ripA4*	AWR4	4	0.25				0.25		0.25		
RSc0608	*ripAA*	AvrA	5			0.20		0.20				
RSp1601	*ripAD*		3					0.33		0.33		
RSp0822	*ripAF1*	HopF1-like	4					0.25		0.50		EGGPLANT, BEAN
RSc2101	*ripAJ*		3					0.67				
RALMO_1580	*ripAL*		3		0.33							
RSc3272	*ripAM*		4		0.25			0.25				
RSp0879	*ripAO*		2							0.50		
RSp1218	*ripAP-fragment1*		1					1.00				
RSp1215	*ripAP-fragment2*		3					1.00				
RSp1236	*ripAR*		4							0.25		
RSp1384	*ripAS*		4			0.25		0.50		0.75		
RSp1388	*ripAT*		4		0.25							
RSp1460	*ripAU*		3					0.67		0.33		
RSp1475	*ripAW*		3							0.33		
RSp0572	*ripAX2*	HopH1-like	4								0.75	
RSp1022	*ripAY*		3					0.33		0.33		EGGPLANT
RSp1582	*ripAZ1*		3				0.67	0.67				
RSc0245	*ripB*		4							0.25		
RSp0304	*ripD*	HopD1-like	3		0.33			0.33		0.33		TOMATO, EGGPLANT, BEAN
RSc3369	*ripE1*		4					1.00				
RSp1555	*ripF1-1*		4		0.25							
RSp0028	*ripG3*	GALA3	5					0.50		0.60		
RSc1800	*ripG4*	GALA4	4	0.25						0.25		
RSc1801	*ripG5*	GALA5	4	0.25					0.25	0.25		
RSc1357	*ripG7*		4		0.50							
RSc1386	*ripH1*	HLK1	4		0.25							
RSp0215	*ripH2*	HLK2	4							0.50		
RSp0160	*ripH3*	HLK3	4					0.50		0.25		
RSc0041	*ripI*		4		0.25							
RSp0193	*ripL*		2					0.50				
RSp1130	*ripN*		3	0.33				0.33		0.33		
RSp0323	*ripO1*	HopG1-like	5					0.40		0.20		
RSc0826	*ripP1*	PopP1	3				1.00	0.67				
RSc0868	*ripP2*	PopP2	4					0.75				TOMATO, EGGPLANT, BEAN
RSp1277	*ripQ*		3					0.33				
RSc3401	*ripS1*	SKWP1	4					0.25				
RSp1374	*ripS2*	SKWP2	3							0.33		
RSp0930	*ripS3*	SKWP3	3							0.33		
RSc1839	*ripS4*		5					0.20				EGGPLANT
RSp0296	*ripS5*	SKWP5	4	0.25						0.50		
RSc1815	*ripTAL*	AvrBs3-like	5							0.20		EGGPLANT
RSc1349	*ripV1*		5					0.20				
RSc2775	*ripW*	popW	4	0.25						0.25		
RSp0877	*ripX*	popA	2					0.50				
RSp1031	*ripZ*		4		0.25					0.25		

[a] No Type III Effector was associated to avirulence to pepper PM687 (P2).

[b] Determined on infection competitiveness bioassays challenging the T3E-defective strain and the wild-type strain (Macho *et al* 2010).

Table S5. Type III Effector or "effector-like" genes associated with virulence of *Ralstonia solanacearum* to eggplant, pepper, tomato, as determined by probe frequency

Gene /probe code	Rip Name	Representative gene member	Description	# Probes /gene	Eggplant					Pepper			Tomato
					Dingras (E1)	SM6 (E2)	Ceylan (E3)	Surya (E4)	AG91-25 (E6)	PM687 (P2)	CA8 (P6)	Perennial (P8)	Hawaii7996 (T5)
BA02498	-	NA	C.P.U.F., AvrPtoB-like domain	3	1.00		1.00			1.00	1.00	1.00	
BA07003	-	*pcaD2*	b-ketoadipate enol lactone hydrolase protein	2	0.50		0.50			0.50	0.50	1.00	
PT00619	-	NA	putative glycosyltransferase	2								0.50	
PT01265	-	NA	C.P.U.F.	3	0.33	0.33	0.33		0.33	0.67		0.33	0.33
PT01808	-	RALIP_3273	putative T3E	3	0.33		0.33		0.33	0.33		0.33	0.33
PT03045	-	NA	C.P.U.F. (TPR domain)	2								1.00	
PT03558	-	RRSL_04659	C.P.U.F., exopolysaccharide phosphotransferase domain	3	0.33		0.33		0.33	0.33		0.67	0.33
PT04098	-	RALIP_4318	Putative T3E	3								1.00	
PT04281	-	NA	C.P.U.F. (papd-like transmembrane protein domain)	3								1.00	
PT04284	-	RALIP_4533	putative T3E	2								1.00	
PT04397	-	RALIP_4651	C.P.U.F.	3								0.33	
PT07000	-	RALIP_1709	putative T3E, LRR domain	3						0.67			
PT07001	-	NA	putative T3E (fragment)	2	1.00		1.00		1.00	1.00		1.00	1.00
RSc1723	-	RSc1723	putative T3E	4								0.50	0.25
RSc2131	-	RSc2131	P.U.F.	3		0.67				0.33		0.33	
RSc2291	-	RSc2291	putative transglycosylase	5			0.20			0.20	0.20	0.20	
RSc3174	-	RSc3174	putative T3E	4						1.00			
RSc3241	-	RSc3241	putative T3E, fragment	4			0.25						
RSp0213	-	RSp0213	putative T3E	2				1.00		1.00			
RSp0218	-	RSp0218	putative T3E	4		0.25							
RSp0839	-	RSp0839	C.P.U.F.	4						0.25		0.25	
RSp0858	-	RSp0858	hrpV	2								0.50	
RSc2139	ripA1	RSc2139	AWR1	4		0.75							
PT01391	ripA5_2	RALIP_1563	AWR5-2	3			1.00		1.00	1.00		1.00	1.00
RSc0608	ripAA	RSc0608	AvrA	5								0.20	
RSp0876	ripAB	RSp0876	popB	4								0.25	
RSp0875	ripAC	RSp0875	popC	3		0.33						0.33	
RSc0321	ripAE	RSc0321		4								0.75	
RSp0822	ripAF1	RSp0822	HopF1-like	4		0.50		0.50					
RSc0895	ripAH	RSc0895		3		0.67							
RSc2359	ripAK	RSc2359		4		0.50							
	ripAP_fragment2	RSp1215		3				0.33					
RSp1236	ripAR	RSp1236		4								0.25	
RSp1384	ripAS	RSp1384		4						0.25		0.25	
RSp1388	ripAT	RSp1388		4		0.25				0.25		0.25	
RSc3290	ripAX1	RSc3290		3		0.33	0.33			0.33			
RSp0572	ripAX2	RSp0572	HopH1-like	4								0.25	
RSp1022	ripAY	RSp1022		3								0.33	
RSp1582	ripAZ1	RSp1582		3								0.67	
RSc0245	ripB	RSc0245		4								0.25	
RSc0227	ripBA_fragment1	RSc0227		3		0.33							
PT04502	ripBH	RALIP_4767	OspD_family	3								1.00	
PT04434	ripBI	RALIP_4696	XopX_family	3								1.00	
RSp1239	ripC1	RSp1239		4		0.25				0.25		0.75	
RSp0304	ripD	RSp0304	HopD1-like	3		0.33		0.33					
PT04834	ripE2	RALIP_0863		4	0.50		0.50			0.75	0.50	0.50	
RSp1555	ripF1_1	RSp1555		4								0.25	
RSp0914	ripG1	RSp0914	GALA1	3		0.67				0.33			
RSp0028	ripG3	RSp0028	GALA3	5								0.40	
RSc1800	ripG4	RSc1800	GALA4	4								0.50	0.25
RSc1801	ripG5	RSc1801		4								0.25	
RSc1356	ripG6	RSc1356	GALA6	4								0.50	
RSc1357	ripG7	RSc1357		4						0.25		0.25	
RSc1386	ripH1	RSc1386	HLK1	4		0.25						0.25	
RSc0041	ripI	RSc0041		4								0.25	
RSc2132	ripJ	RSc2132		3								1.00	
RSp0323	ripO1	RSp0323	HopG1-like	5								0.20	
RSc0826	ripP1	RSc0826	PopP1_(YopJ_family)	3								1.00	
RSc0868	ripP2	RSc0868	PopP2_(YopJ_family)	4				0.25				0.25	
RSp1277	ripQ	RSp1277		3								0.33	
RSp1281	ripR	RSp1281		4								0.25	
RSc3401	ripS1	RSc3401	SKWP1	4								0.25	
RSp1374	ripS2	RSp1374	SKWP2	3								0.33	
RSp0930	ripS3	RSp0930	SKWP3	3								0.33	
BA00250	ripS4	RCFBP_11536	SKWP4	3								0.67	
RSc1839	ripS4	RSc1839		5								0.40	

RSp0296	ripS5	RSp0296	SKWP5	4		0.25		0.25					
RSc3212	ripT	RSc3212	YopT_family	2						0.50			
RSc1815	ripTAL	RSc1815	AvrBs3 family	5		0.60						0.20	
PT03560	ripU	RRSL_04660		2								1.00	0.50
RSp1212	ripU	RSp1212		5						0.80			
RSc1349	ripV1	RSc1349		5								0.20	
PT01326	ripV2	RALIP_1493	SspHI_family	3								1.00	0.33
RSc2775	ripW	RSc2775	popW	4								0.25	
RSp1031	ripZ	RSp1031		4								0.25	
TOTAL genes associated					7	17	11	6	5	22	4	58	9

Table S6. PCR primers used for Type III effector amplification

Gene name	Rip Name /Gene Name	Primer name	Sequence 5'-3'	Expected size (bp)	Annealing T°	PCR cycles
BA2498	–	BA2498_5F BA2498_468R	CGGAACGAGACCCTGCGGAAA GGGATGTTTGGGATTGCTGACGAGA	464	56	30
BA7003	pcaD2	BA7003_142F BA7003_1087R	GCGACGAGGTCTGGAGCGAA CCGCTTGCTGGACGGGTG	946	56	30
PTO1265	–	PTO1265A_26F PTO1265A_2338R	TCGATCAAGCCGGGCAAAGCA CGTTGGCCTTCAGGGTCTCCA	2313	55	30
		PTO1265C_1874F PTO1265C_3974R	GAGCGAGCCGAGACGAAGGT GACCCGAGGAACCCGAGGAG	2081	56	30
PTO1326	ripV2	PTO1326_4F PTO1326_2072R	CCAACCTCGCCCATTTCCACCAG CGCAGACCCGCGCATTGGA	2069	56	30
PTO1391	ripA5-2	AWR6_RALIP_466F AWR6_RALIP_2047R	GCCCGTCCGTCCTATCCCATTC ACACCGTTTCCTTGCCATCCACC	1582	56	30
PTO3558	–	PTO3558_24F PTO3558_1090R	GCAATGGGCCGACCACACCAA ATGAGGATGTGGCTCTCCGGCTC	1067	57	30
PTO3560	ripU	PTO3560_4F PTO3560_2072R	CCAACCTCGCCCATTTCCACCAG CGCAGACCCGCGCATTGGA	2069	56	30
PTO4834	ripE2	PTO4834_RALIP_15F PTO4834_RALIP_1166R	GGCGCTGAATCTCTCGTATCACGG TCGCGCCGGGCTTCTCTTT	1152	56	30
PTO7001	Putative ripM	PTO7001_RALMO_9F PTO7001_RALMO_540R	GGCGTGGGAGGTCGGTCA ACGCGACGACAAGACAGGAGG	532	56	25
RSc0826	ripP1	popP1_19F popP1_1106R	GCATTGGGCGTCAGTCAACCG CACGACTCCAGGGCATGTCGAA	1088	55	30
RSc0868	ripP2	PopP2_8F PopP2_989R	ATCCTTTGCCGGGGCGCA TTGCGTTTGACGAGATGGCGGG	982	56	30
RSc1723	–	RSc1723_55F RSc1723_304R	GCCACATTCGAGGATGCCGATGATT TTTTCTTTGGGGCGCTGTCGATTG	250	55	30
RSc1800	ripG4	RSc1800_126F RSc1800_1176R	GACCATCACGCACCGGGACA GGCCTCCAGTGCCRGCAT	1051	56	25
RSc1801	ripG5	RSc1801_75F RSc1801_1478R	CGGCTCGTCGCTCCTGCAA GACGACAGCGTGCGGTTGG	1404	56	30
RSc2775	ripW	RSc2775IIB1_2F RSc2775IIB1_1203R	TGCTACGCGCCTCATCCGAG GGCCTTGTAGCTCACCTTGTTGGT	1202	56	30
		RSc2775univ_76F RSc2775univ_1194R	GATCGCCCGAGTGACCATTTCCA GCTCACCTTGTTGGTGCCCG	1119	56	30
RSp0028	ripG3	GALA3_CMR_566F GALA3_CMR_1609R	ACCTGATCGGACTGCCTGCC GGGATTGGCGGAGATTGAGCGT	1044	56	30
		G3_117F G3_545R	GACAGTGATCGCCCATCG GGGTTGTCGGCCAGGTAG	425	57	30
RSp0099	ripA2	RSp0099_176F RSp0099_3240R	GACCCGCCGCCATCAACG GGTGTAGCCGTGCGTGGTGA	3065	56	25
RSp0296	ripS5	RSp0296_10F RSp0296_2209R	AATCGCACCCACCGCAACCT CGCTCAGGGCGTTGCTCAC	2200	56	30
		RSp0296_2184F RSp0296_4533R	GACAAGCGTGAGCAACGCCC CTTCCAGCGCGACAGCACC	2350[a]	56	30
		RSp0296_4513F RSp0296_6854R	AAGGTGCTGTCGCGCTGGAA TCCCGGACTTTCTCGTAATCCCTGT	2298	55	30
RSp0572	ripAX2	RSp0572_60F RSp0572_527R	CGAAGCTGACCGTTATGCGGG CCTGCCTCGCTGGTTTCGTTG	468	55	30
RSp0847[b]	ripA4	RSp0847_3015F RSp0847_3974R	GGCCGAGCAGGAGTTCAAGGT GCCTCGCTGGTGCCGTACA	960	56	30
RSp0854	hrpZ	RSp0854_11F RSp0854_221R	GCGGCTCYCTCCGTYYCCC AGCAGRTCCTTGGCSGCCTT	211	55	30
		RSp0854univ_38F RSp0854univ_222R	YGRYRGAYCCGAVCGSCAT GAGCAGRTCCTTGGCSGCCTT	185	55	30
RSp1130	ripN	RSp1130_123F RSp1130_1353R	CTCGGACGTGACCAGCAACCT CGTCTCCCCGGCCTTCAACT	1231	56	30
RSp1384	ripAS	RSp1384_6F RSp1384_2412R	AGTCAATCCACCCGCTTCGCC CAGCTCCGTTTGCAGTTGCCC	2407	56	25
		RSp1460_6F	GCTCACACGCACTCCACCC	812	56	30

RSp1460	ripAU	RSp1460_817R	GCGGCGCTTTCCGATGCT	812	56	30
		RSp1460_274F	CAGCCCGTGCGGACCAAG	544	56	30
		RSp1460_817R	GCGGCGCTTTCCGATGCT			
RSp1582	ripAZ1	RSp1582_23F	ACAAGGACTATGGGGAAGACGACGC	541	56	30
		RSp1582_563R	TCGCGCAAGGCATCGAGCAAG			

[a] Cases of aspecific amplifications

[b] Primers 179F/3049R gave many aspecific amplifications and were thus not retained.

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1 **Table S7**. Correspondance between amplicon sizes obtained and effector allele numbers.

	Gene name	Rip Name /other Name	Region amplified	Expected size (bp) : ALLELE 1	ALLELE 3	ALLELE 4	ALLELE 5	Gene total size (nt) in the genome of origin	Genome of origin
4	BA2498	AvrPtoB-like	5-468	464	NA	NA	NA	528	MOLK2
5	BA7003		142-1087	946	1100	NA	NA	2472	IPO1609
6	PTO1265		26-2338	2313	2313	NA	NA	4158	IPO1609
7			1874-3974	2081	0	NA	NA		
8	PTO1326	ripV2	4-2072	2069	NA	NA	NA	2088	IPO1609
9	PTO1391	ripA5_2	466-2047	1582	NA	NA	NA	3636	IPO1609
10	PTO3558		24-1090	1067	NA	NA	NA	1098	IPO1609
11	PTO3560	ripU	2-859	858	NA	NA	NA	882	IPO1609
12	PTO4834	ripE2[a]	15-1166	1152	1152	NA	NA	1197	IPO1609
13			323-1166	0	843	NA	NA		
14	PTO7001	ripM (fragment)	9-540	532	NA	NA	NA	1287	MOLK2
15	RSc0826	RipP1	19-1106	1088	NA	NA	NA	1104	GMI1000
16	RSc0868	RipP2	8-989	982	950	NA	NA	1464	GMI1000
17	RSc1723	putative T3E	55-304	250	NA	NA	NA	444	GMI1000
18	RSc1800	RipG4	126-1176	1051	650	NA	NA	1386	GMI1000
19	RSc1801	RipG5	75-1478	1404	NA	NA	NA	1614	GMI1000
20	RSc2775	RipW-2B1	2-1203	1202	0	1202	NA	1152	IPO1609
21		RipW	76-1194	1119	1119	0	NA	1140	GMI1000
22	RSp0028	RipG3[b]	566-1609	1044	1044	450	450	1824	CMR15
23			117-544	425	0	0	425		
24	RSp0099	RipA2	176-3240	3065	NA	NA	NA	3381	GMI1000
25	RSp0296	RipS5	10-2209	2200	0	NA	NA	7014	GMI1000
26			2184-4533	2350	1700	NA	NA		
27			4513-6854	2298	2298	NA	NA		
28	RSp0572	RipAX2	60-527	468	NA	NA	NA	654	GMI1000
29	RSp0847	RipA4	3015-3974	960	NA	NA	NA	3990	GMI1000
30	RSp0854	hrpZ	11-221	211	0	NA	NA	228	GMI1000
31			(univ) 38-222	185	185	NA	NA		
32	RSp1130	ripN	123-1353	1231	NA	NA	NA	1422	GMI1000
33	RSp1384	ripAS	6-2412	2407	2023	NA	NA	2634	GMI1000
34	RSp1460	ripAU	8-817	812	0	812	NA	822	GMI1000
35			214-817	544	544	0	NA		
36	RSp1582	ripAZ1	23-563	541	NA	NA	NA	852	GMI1000

38 [a] The IPO1609 ortholog (RALIP_0863) size was 1197 nt, whereas the 3 other orthologs were 285 nt-shortened in 5'.

39 [b] The CMR15 ortholog (CMR15v5_mp0361) was1824 nt, whereas the GMI1000 ortholog was 256-shortened in 5'.

Table S8. Effector alleles best describing the 6 Type III Effector Repertoire Groups (TRG), as determined by the function *catdes* . Each TRG is considered a class, and each T3E allele a modality. Cla/Mod: proportion of strains carrying the allele considered belonging to the TRG considered. Mod/Cla: proportion of strains of the TRG considered carrying the allele considered. Global: Global frequence of the allele considered. v.test: value-test corresponding to the quantile of the normal distribution associated to the critical p-value. Positive sign indicates a overrepresentation of the allele in the TRG, whereas a negative sign indicates the underrepresentation.

TRG`1`

Gene levels	Cla/Mod	Mod/Cla	Global	p.value	v.test
BA2498=1	75	100	8.333333	0.0002313	3.682159
RipG3.RSp0028.=2	60	100	10.416667	0.0005782	3.441654
ripE2.PTO4834.=1	37.5	100	16.666667	0.0032377	2.944216
RipS5.RSp0296.=2	30	100	20.833333	0.006938	2.699805
RipP2.RSc0868.=2	27.27273	100	22.916667	0.0095398	2.592079
BA2498=2	0	0	91.666667	0.0002313	-3.682159

TRG`2`

Gene levels	Cla/Mod	Mod/Cla	Global	p.value	v.test
phylotype=IIA	100	100	6.25	5.78E-05	4.021548
BA7003=3	100	66.66667	4.166667	2.66E-03	3.004569
RipG3.RSp0028.=2	40	66.66667	10.416667	2.60E-02	2.225949
RipAS.RSp1384.=1	20	100	31.25	2.63E-02	2.221655
RipAS.RSp1384.=2	0	0	66.666667	3.24E-02	-2.13972

TRG`3`

Gene levels	Cla/Mod	Mod/Cla	Global	p.value	v.test
RipG3.RSp0028.=4	96.296296	100	56.25	9.86E-13	7.132459
PTO1265=1	96.296296	100	56.25	9.86E-13	7.132459
ripV2.PTO1326.=1	89.655172	100	60.416667	1.33E-10	6.423213
phylotype=IIB	89.285714	96.153846	58.333333	2.42E-09	5.966761
RipN.RSp1130.=2	83.870968	100	64.583333	6.20E-09	5.811154
RSp0854=3	86.206897	96.153846	60.416667	1.67E-08	5.642651
RipAS.RSp1384.=2	81.25	100	66.666667	3.31E-08	5.524248
BA7003=1	81.25	100	66.666667	3.31E-08	5.524248
RipS5.RSp0296.=3	95.454545	80.769231	45.833333	5.39E-08	5.437823
PTO7001=1	91.666667	84.615385	50	1.11E-07	5.308289
RipAZ1.RSp1582.=2	76.470588	100	70.833333	6.63E-07	4.971905
RipG4.RSc1800.=2	76.470588	100	70.833333	6.63E-07	4.971905
RipAU.RSp1460.=2	72.222222	100	75	9.28E-06	4.433263
RipW.RSc2775.=1	70.27027	100	77.083333	3.12E-05	4.164377
PTO3558=1	68.421053	100	79.166667	9.89E-05	3.893361
RipA5_2.PTO1391.=1	77.777778	80.769231	56.25	2.57E-04	3.655194

RipG5.RSc1801.=2	66.666667	100	81.25	2.97E-04	3.618257
ripU.PTO3560.=1	66.666667	100	81.25	2.97E-04	3.618257
ripE2.PTO4834.=3	76.923077	76.923077	54.166667	7.62E-04	3.366219
RSc1723=2	70.967742	84.615385	64.583333	2.17E-03	3.065804
RipAX2.RSp0572.=2	67.741935	80.769231	64.583333	1.39E-02	2.458687
RipP1.RSc0826.=2	60.97561	96.153846	85.416667	3.10E-02	2.15733
BA2498=2	59.090909	100	91.666667	3.76E-02	2.079256
RipW.RSc2775.=3	0	0	8.333333	3.76E-02	-2.079256
BA2498=1	0	0	8.333333	3.76E-02	-2.079256
RipP1.RSc0826.=1	14.285714	3.846154	14.583333	3.10E-02	-2.15733
RipG3.RSp0028.=2	0	0	10.416667	1.54E-02	-2.423322
RipG3.RSp0028.=1	0	0	10.416667	1.54E-02	-2.423322
RipAX2.RSp0572.=1	29.411765	19.230769	35.416667	1.39E-02	-2.458687
ripE2.PTO4834.=1	12.5	3.846154	16.666667	1.34E-02	-2.471743
RipP2.RSc0868.=1	11.111111	3.846154	18.75	5.55E-03	-2.773215
RipW.RSc2775.=4	0	0	14.583333	2.32E-03	-3.046366
phylotype=I	0	0	14.583333	2.32E-03	-3.046366
phylotype=III	10	3.846154	20.833333	2.18E-03	-3.06523
RSc1723=1	23.529412	15.384615	35.416667	2.17E-03	-3.065804
RipAU.RSp1460.=1	0	0	16.666667	8.47E-04	-3.336825
RipG3.RSp0028.=3	0	0	18.75	2.97E-04	-3.618257
RipG5.RSc1801.=1	0	0	18.75	2.97E-04	-3.618257
ripU.PTO3560.=2	0	0	18.75	2.97E-04	-3.618257
RipA5_2.PTO1391.=2	23.809524	19.230769	43.75	2.57E-04	-3.655194
PTO3558=2	0	0	20.833333	9.89E-05	-3.893361
RipG4.RSc1800.=1	0	0	27.083333	2.58E-06	-4.701841
RipAZ1.RSp1582.=1	0	0	29.166667	6.63E-07	-4.971905
BA7003=2	0	0	29.166667	6.63E-07	-4.971905
RipAS.RSp1384.=1	0	0	31.25	1.56E-07	-5.245338
RipS5.RSp0296.=1	0	0	31.25	1.56E-07	-5.245338
PTO7001=2	16.666667	15.384615	50	1.11E-07	-5.308289
RSp0854=1	5.263158	3.846154	39.583333	1.67E-08	-5.642651
RipN.RSp1130.=1	0	0	35.416667	6.20E-09	-5.811154
ripV2.PTO1326.=2	0	0	39.583333	1.33E-10	-6.423213
PTO1265=2	0	0	41.666667	1.38E-11	-6.759961

TRG`4`

Gene levels	Cla/Mod	Mod/Cla	Global	p.value	v.test
RipG3.RSp0028.=3	88.888889	100	18.75	2.39E-08	5.581461
ripU.PTO3560.=2	88.888889	100	18.75	2.39E-08	5.581461
RipW.RSc2775.=4	100	87.5	14.58333	1.09E-07	5.311624
PTO3558=2	80	100	20.83333	1.19E-07	5.294637
phylotype=III	80	100	20.83333	1.19E-07	5.294637
RipAU.RSp1460.=1	87.5	87.5	16.66667	8.53E-07	4.92276
BA7003=2	57.142857	100	29.16667	7.96E-06	4.466307

RipN.RSp1130.=1	47.058824	100	35.41667	6.44E-05	3.995995
RipG5.RSc1801.=1	66.666667	75	18.75	1.72E-04	3.756306
RSp0854=1	42.105263	100	39.58333	2.00E-04	3.718641
ripV2.PTO1326.=2	42.105263	100	39.58333	2.00E-04	3.718641
PTO1265=2	40	100	41.66667	3.34E-04	3.587527
RipA5_2.PTO1391.=2	38.095238	100	43.75	5.39E-04	3.460456
RipS5.RSp0296.=1	46.666667	87.5	31.25	5.97E-04	3.433037
RSc1723=1	41.176471	87.5	35.41667	1.73E-03	3.133638
PTO7001=2	33.333333	100	50	1.95E-03	3.097889
RipP2.RSc0868.=1	55.555556	62.5	18.75	3.39E-03	2.930059
RipAZ1.RSp1582.=1	42.857143	75	29.16667	5.10E-03	2.800718
RipAS.RSp1384.=1	40	75	31.25	8.16E-03	2.645261
RipAX2.RSp0572.=1	35.294118	75	35.41667	1.86E-02	2.353953
RipG4.RSc1800.=1	38.461538	62.5	27.08333	2.81E-02	2.196456
ripE2.PTO4834.=2	35.714286	62.5	29.16667	4.13E-02	2.040394
RipAX2.RSp0572.=2	6.451613	25	64.58333	1.86E-02	-2.353953
ripE2.PTO4834.=3	3.846154	12.5	54.16667	1.34E-02	-2.471743
RipAS.RSp1384.=2	6.25	25	66.66667	1.25E-02	-2.496729
RipAZ1.RSp1582.=2	5.882353	25	70.83333	5.10E-03	-2.800718
RipG4.RSc1800.=2	5.882353	25	70.83333	5.10E-03	-2.800718
RipS5.RSp0296.=3	0	0	45.83333	4.14E-03	-2.867282
PTO7001=1	0	0	50	1.95E-03	-3.097889
RSc1723=2	3.225806	12.5	64.58333	1.73E-03	-3.133638
RipG3.RSp0028.=4	0	0	56.25	5.39E-04	-3.460456
RipA5_2.PTO1391.=1	0	0	56.25	5.39E-04	-3.460456
PTO1265=1	0	0	56.25	5.39E-04	-3.460456
phylotype=IIB	0	0	58.33333	3.34E-04	-3.587527
RSp0854=3	0	0	60.41667	2.00E-04	-3.718641
ripV2.PTO1326.=1	0	0	60.41667	2.00E-04	-3.718641
RipG5.RSc1801.=2	5.128205	25	81.25	1.72E-04	-3.756306
RipN.RSp1130.=2	0	0	64.58333	6.44E-05	-3.995995
BA7003=1	0	0	66.66667	3.41E-05	-4.144159
RipW.RSc2775.=1	2.702703	12.5	77.08333	3.32E-05	-4.150108
RipAU.RSp1460.=2	0	0	75	1.31E-06	-4.83796
PTO3558=1	0	0	79.16667	1.19E-07	-5.294637
ripU.PTO3560.=1	0	0	81.25	2.39E-08	-5.581461

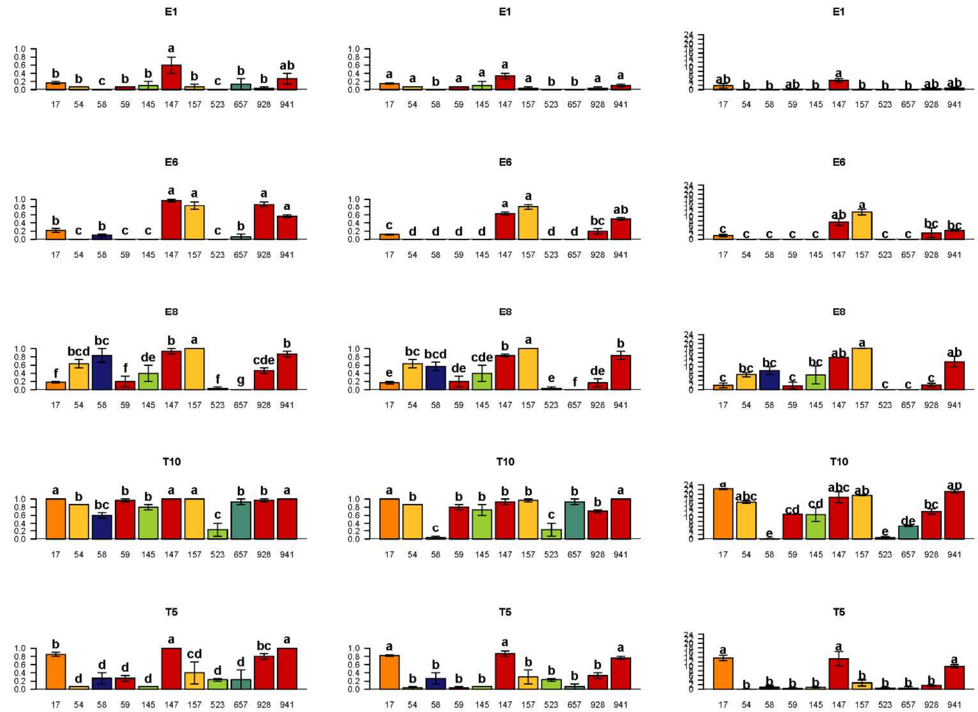
TRG`5`

Gene levels	Cla/Mod	Mod/Cla	Global	p.value	v.test
phylotype=I	85.714286	100	14.583333	5.70E-07	5.00097
RipG3.RSp0028.=1	100	83.33333	10.416667	3.50E-06	4.638819
RipW.RSc2775.=3	100	66.66667	8.333333	7.71E-05	3.953274
RipG4.RSc1800.=1	46.153846	100	27.083333	1.40E-04	3.808458
RipAZ1.RSp1582.=1	42.857143	100	29.166667	2.45E-04	3.66773
BA7003=2	42.857143	100	29.166667	2.45E-04	3.66773

RipS5.RSp0296.=1	40	100	31.25	4.08E-04	3.534948
RipN.RSp1130.=1	35.294118	100	35.416667	1.01E-03	3.288141
RSc1723=1	35.294118	100	35.416667	1.01E-03	3.288141
RSp0854=1	31.578947	100	39.583333	2.21E-03	3.060324
ripV2.PTO1326.=2	31.578947	100	39.583333	2.21E-03	3.060324
RipP1.RSc0826.=1	57.142857	66.66667	14.583333	2.48E-03	3.025745
PTO1265=2	30	100	41.666667	3.16E-03	2.951872
RipA5_2.PTO1391.=2	28.571429	100	43.75	4.42E-03	2.846379
RipAX2.RSp0572.=1	29.411765	83.33333	35.416667	1.76E-02	2.372901
RipW.RSc2775.=1	5.405405	33.33333	77.083333	2.08E-02	-2.312121
RipS5.RSp0296.=3	0	0	45.833333	1.88E-02	-2.350239
RipAX2.RSp0572.=2	3.225806	16.66667	64.583333	1.76E-02	-2.372901
RipAS.RSp1384.=2	3.125	16.66667	66.666667	1.27E-02	-2.492201
RipG3.RSp0028.=4	0	0	56.25	4.42E-03	-2.846379
RipA5_2.PTO1391.=1	0	0	56.25	4.42E-03	-2.846379
PTO1265=1	0	0	56.25	4.42E-03	-2.846379
phylotype=IIB	0	0	58.333333	3.16E-03	-2.951872
RipP1.RSc0826.=2	4.878049	33.33333	85.416667	2.48E-03	-3.025745
RSp0854=3	0	0	60.416667	2.21E-03	-3.060324
ripV2.PTO1326.=1	0	0	60.416667	2.21E-03	-3.060324
RipN.RSp1130.=2	0	0	64.583333	1.01E-03	-3.288141
RSc1723=2	0	0	64.583333	1.01E-03	-3.288141
BA7003=1	0	0	66.666667	6.53E-04	-3.408768
RipAZ1.RSp1582.=2	0	0	70.833333	2.45E-04	-3.66773
RipG4.RSc1800.=2	0	0	70.833333	2.45E-04	-3.66773

TRG`6`

Gene levels	Cla/Mod	Mod/Cla	Global	p.value	v.test
RipG3.RSp0028.=5	100	50	2.083333	0.0416667	2.036834
PTO1265=3	100	50	2.083333	0.0416667	2.036834
souche=930	100	50	2.083333	0.0416667	2.036834
souche=657	100	50	2.083333	0.0416667	2.036834



Virulence of the 11 R.solanacearum strains on the eggplants E1, E6 (resistant) and E8 (susceptible) and tomatoes T10 (susceptible) and T5 (resistant), as determined by the colonization index (left), final wilting rate (middle), and AUDPC (right). Strains, named after their RUN number (abscissa), are representative of the TRG 1 (orange), TGR2 (dark blue), TRG3 (red), TRG4 (green), TRG5 (yellow), TRG6 (dark turquoise). Values marked with similar letters within each barplot are not significantly different from each other (Tukey test, threshold = 0.05).