SUPPORTING INFORMATION LEGENDS

Supplemental Table SI. **Identification of proteins from secretome of *R. solanacearum* by LC-MS/MS (Q Exactive) and the X!TandemPipeline.** GMI1000 wild-type strain.

Supplemental Table SII. **Statistical significance of the quantitative differences of the secreted proteins between the GMI1000 wild-type strain and the *hrcV* and *hpa* mutants.** P-values obtained for each protein after glht test are shown. Grey cells highlight p-values < 0.05.

Supplemental Table SIII. **Identification of proteins from secretome of *R. solanacearum* by LC-MS/MS (Q Exactive) and the X!TandemPipeline.** GMI1000 wild-type strain, mutants : *hrcV, hpaB, hpaD, hpaG.*

Supplemental Table SIV**. MW, pI and gene expression of Rips detected in this study.** Gene expression data obtained by RNA-Seq and expressed in rpkm correspond to the mean of three biological replicates in the GMI1000 wild-type strain.

Supplemental FIG. S1. S**ecreted and bacterial cell proteins from *R. solanacearum* wild-type and mutant strains**. Proteins from an equal amount of cultures were loaded in each lane and separated by SDS-PAGE followed by silver staining. Experiments were repeated twice using protein extracts from the untagged (pictures) and HA-tagged strains.

Supplemental FIG. S2. **Experimental procedure to study *R. solanacearum* secretome and involvement of some mutants in bacterial pathogenicity.** A, procedure scheme to generate, analyze and validate secretome data. B, schematic representation of the extracellular part of the T3SS that comprises pilin subunits (HrpY) and translocon proteins (RipF1_1 and RipF1_2). The T3SS spans bacterium membranes (IM: inner membrane; P: periplasm; OM: outer membrane) and host plasma membrane (PM) to deliver T3Es also called Rips for Ralstonia injected proteins, with the help of T3C (type 3 chaperone). C, schematic representation of the *hrp* cluster and localization of the *hpa* genes and of *hrcV* gene, for which the corresponding mutants were analyzed. Transcriptional regulators are in purple, and the *hrp/hrc* genes in grey. At the left border we find Rips in orange and genes of unknown function in white.

Supplemental FIG. S3. **Summary of the proteins detected secreted by the wild-type strain, the *hrcV* mutant and the three *hpa* mutants.** Venn diagram showing the sets of proteins detected and their intersections in the secretomes of the *R. solanacearum* strains studied. Venn diagram was generated thanks to http://bioinformatics.psb.ugent.be/webtools/Venn/ website.

Supplemental FIG. S4. **PCA with *R. solanacearum* LC-MS/MS secretomes of the GMI1000 wild-type strain and of the *hrcV* mutant, excluding genome-annotated T3-associated proteins out of the analysis.** A, the most discriminating component is the strain. B, bar plots show that the first component explains 30% of the variance, much less than the

explained variance when T3-proteins are kept in the analysis (see Fig. 3*A*). C, almost all detected proteins appear clustered. Four biological replicates were used for each strain.

Supplemental FIG. S5. **RipBJ is not required for pathogenicity on *M. truncatula and A. thaliana,* and for HR on *N. tabacum*.** Kaplan–Meier survival analysis on 16 *A. thaliana* plants (A) and on 16 *M. truncatula* plants (B) inoculated with *R. solanacearum* wild-type strain (red) and *ripBJ* mutant (purple). Gehan–Breslow–Wilcoxon tests indicate that the two curves are not significantly differents (p-value = 0.6699 and 0.3021, for (A) and (B), respectively). C, tobacco leaf infiltration of the GMI1000 wild-type strain and of the *ripBJ* mutant, 24 hpi. Both strains are able to trigger HR. Three independent biological replicates were done for each experiment. D, RipBJ N-terminal sequence is highly homologous to RipAG (and to a lesser extent to RipAH). This N-terminal sequence is also shared with the effectors AvrRpm1 and AvrPto of *Pseudomonas syringae*, acylated in order to be targeted to the host membrane (56). Essential residues which are targets for myristoylation are the Glycine in position 2 or the Cysteine in position 3 or 5. Both are conserved in the three Rip effectors.

Supplemental FIG. S6. **PCA with *R. solanacearum* LC-MS/MS secretomes of the wild-type as well as T3-mutant strains, excluding T3-associated proteins out of the analysis.** A, PCA shows that all strains occupy the same space, except for the *hrcV* mutant, which seems to remain distinct from the rest. B, in comparison with PCA presented in Fig. 5, variance barplot shows no highly discriminating components, the first component showing a standard deviation of 0.21 and accounting for 21% of the total variance. C, almost all detected proteins appear clustered. Only one protein (RSc3359) appears to be responsible for *hrcV* mutant discrimination along PC1. Four biological replicates were used for each strain.

Supplemental FIG. S7. **Distribution of Rips detected by MS** (black histogram bar) **and of Rips not detected** (grey histogram bar) according to the isoelectric point (pI) (A, B), molecular weight (MW) (C, D) and gene expression obtained by RNA-Seq (rpkm) (E, F). Each distribution is represented with absolute amount of Rips in each class (A, C, E) or according to their percentage in each category, detected or not (B, D, F). G, exponential relation between the transcriptomics data and the proteomics data: the plot shows the relationship between the rpkm obtained on the RNA-Seq experiment and the log-normalized PAI data, for genes/proteins detected and quantified with both approaches. The red line shows the fitted exponential model between both datasets (*i.e.* $\log(\text{normalized PAI}) = 0.1509 + 0.0093 * (\log \text{rpkm})^2$).

Supplemental FIG. S8. **HpaD and HpaG are not required for HR, and HpaD is not required for *R. solanacearum* pathogenicity on *A. thaliana* and *M. truncatula*.** A, *N. tabacum* leaves infiltrated with *R. solanacearum* wild-type strain, *hpaD* and *hpaG* mutants show a similar HR 24 hpi. B, Kaplan–Meier survival analysis of 16 *A. thaliana* plants inoculated with the wild-type strain (red) and the *hpaD* mutant (black). Gehan-Breslow-Wilcoxon test indicates that the two curves are not significantly different (p-value = 0.7208). C, Kaplan–Meier survival analysis of 20 *M. truncatula* plants inoculated with the wild-type strain (red) and the *hpaD* mutant (black). Gehan-Breslow-Wilcoxon test indicates that the two curves are not significantly different (p-value = 0.5354). Each experiment was repeated at least three times.