Supplementary informations

HpaB-Dependent Secretion of Type III Effectors in the Plant Pathogens *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria*

Fabien Lonjon[1], David Lohou[1], Anne-Claire Cazalé[1], Daniela Büttner[2], Barbara Gomes Ribeiro[1], Claire Péanne[1], Stéphane Genin[1], Fabienne Vailleau[1,]*****

[1]LIPM, Université de Toulouse, INRA, CNRS, INPT, Castanet-Tolosan, France

[2]Institute of Biology, Genetics Department, Martin Luther University Halle-Wittenberg, D-23 06099 Halle (Saale), Germany

*****fabienne.vailleau@inra.fr

Supplementary Table S1: Results of the interactions between *Ralstonia solanacearum* type III effectors and HpaB and HpaD proteins after yeast two-hybrid assays.

***R. solanacearum* type III effector**	***R. solanacearum* corresponding *gene***	**Interaction with HpaB in yeast**	**Interaction with HpaD in yeast**
RipA1	*RSc2139*	-	-
RipA2	*RSp0099*	-	-
RipA3	*RSp0846*	-	-
RipA4	*RSp0847*	-	-
RipA5	*RSp1024*	-	-
RipB	*RSc0245*	-	-
RipG1	*RSp0914*	+	-
RipG3	*RSp0028*	-	-
RipG4	*RSc1800*	+	+
RipG5	*RSc1801*	+	-
RipG6	*RSc1356*	-	-
RipG7	*RSc1357*	-	-
RipH1	*RSc1386*	-	-
RipH2	*RSp0215*	-	-
RipH3	*RSp0160*	-	-
RipI	*RSc0041*	-	-
RipO1	*RSp0323*	+	+
RipP1	*RSc0826*	+	+
RipP2	*RSc0868*	Autoactivation	Autoactivation
RipQ	*RSp1277*	-	-
RipR	*RSp1281*	-	-
RipU	*RSp1212*	-	-
RipV1	*RSc1349*	-	-
RipW	*RSc2775*	-	-
RipZ	*RSp1031*	-	-
RipAD	*RSp1601*	-	-
RipAF1	*RSp0822*	+	+
RipAG	*RSc0824*	-	-
RipAJ	*RSc2101*	-	-
RipAK	*RSc2359*	+	-
RipAM	*RSc3272*	-	-
RipAN	*RSp0845*	-	-
RipAV	*RSp0732*	-	+
RipAW	*RSp1475*	+	+
RipAY	*RSp1022*	-	-
RipAZ1	*RSp1582*	-	-
RipTAL	*RSc1815*	-	+
RipTPS	*RSp0731*	+	+

Supplementary Table S2: Strains and plasmids used in this study.

	Characteristics	Source
***R. solanacearum* strains**		
GMI1000	Wild-type strain	1
GMI1694	GMI1000 *hrcV*::Ω mutant, Sp^r	2
GRS266	GMI1000 *hpaD*::Ω mutant, Sp^r	2
GRS474	GMI1000 *hpaB*::Ω mutant, Sp^r	3
***X. campestris* pv. *vesicatoria* strains**		
85-10	wild type strain; Rif^r	4
85*	85-10 derivative containing the *hrpG** mutation, Rif^r	5
85*Δ*hpaB*	*hpaB* deletion mutant (deleted in codons 13 to 149) of strain 85*, Rif^r	6
Yeast strain		
AH109	Matchmaker™ yeast strain	BD Biosciences, Palo Alto, CA, USA
E.coli strain		
BL21(DE3)	F^- *omp*T *gal dcm lon hsdSB*(r_B^- m_B^-)	Invitrogen, Carlsbad, CA, USA
Rosetta	*F- ompT hsdSB(rB- mB-) gal dcm (DE3) pRARE (CamR)*	Invitrogen, Carlsbad, CA, USA
Plasmids used for cloning		
pACC61	pDONR207 derivative carrying *ripAZ1*, Gm^r	This study
pACC73	pDONR207 derivative carrying *ripAF1*, Gm^r	This study
pACC75	pDONR207 derivative carrying *ripO1*, Gm^r	This study
pACC82	pDONR207 derivative carrying *ripZ*, Gm^r	This study
pACC93	pDONR207 derivative carrying *ripAM*, Gm^r	This study
pACC98	pDONR207 derivative carrying *ripH2*, Gm^r	This study
pACC113	pDONR207 derivative carrying *ripAK*, Gm^r	This study
pACC221	pDONR207 derivative carrying *ripAN*, Gm^r	This study

pACC271	pDONR207 derivative carrying *ripAW*, Gm^r	This study
pACC276	pDONR207 derivative carrying *ripQ*, Gm^r	This study
pACC385	pDONR207 derivative carrying *ripAY*, Gm^r	This study
pACC386	pDONR207 derivative carrying *ripH1*, Gm^r	This study
pACC426	pDONR207 derivative carrying *ripR*, Gm^r	This study
pACC429	pDONR207 derivative carrying *ripW*, Gm^r	This study
pACC532	pENTR/D-TOPO derivative carrying *ripTAL*, Km^r	Thomas Lahaye, Universität Tübingen
pACC542	pDONR207 derivative carrying *ripAG*, Gm^r	This study
pACC546	pDONR207 derivative carrying *ripV1*, Gm^r	This study
pACC588	pDONR207 derivative carrying *ripH3*, Gm^r	This study
pACC648	pENTR/SD/D-TOPO derivative carrying *ripAD*, Km^r	This study
pACC652	pDONR207 derivative carrying *ripAJ*, Gm^r	This study
pBR356	Vector carrying *avrBs3Δ2* downstream of the lac promoter and the lacZ fragment flanked by *BsaI* sites	7
pDONR207	Gateway™ entry vector, Gm^r Cm^r	Invitrogen, Carlsbad, CA, USA
pENTR/SD/D-TOPO	Gateway™ entry vector, Shine–Dalgarno sequence, Km^r	Invitrogen, Carlsbad, CA, USA
pFL47	pENTR/SD/D-TOPO derivative carrying $hpaB_{Xcv85\text{-}10}$, Km^r	This study
pFL92	pGEM-T derivative carrying *ripP1* flanked by *BsaI* site	This study
pGAD	Matchmaker™ pGADT7 yeast expression vector made Gateway™ compatible, N-terminal AD fusion, Ap^r, Cm^r	8
pGBG	Matchmaker™ pGADT7 yeast expression vector made Gateway™ compatible, N-terminal BD fusion, Ap^r, Cm^r	8
pGEX	Gateway™ expression vector, N-terminal GST fusion, Ap^r	Invitrogen, Carlsbad, CA, USA
pGEM-T	TA cloning vector, lacZα,f1 ori, Amp^r	Promega, Madison, WI, USA
pLBy1	pDONR207 derivative carrying *hpaD*, Gm^r	This study
pLBy3	pDONR207 derivative carrying *ripI*, Gm^r	This study

pLBy5	pDONR207 derivative carrying *hpaB*, Gm^r	3
pLby7	pDONR207 derivative carrying *ripU*, Gm^r	This study
pLBy13	pDONR207 derivative carrying *ripAV*, Gm^r	This study
pMP12	pDONR207 derivative carrying *ripTPS*, Gm^r	9
pMS130	pENTR/SD/D-TOPO derivative carrying *ripA2*, Km^r	10
pMS132	pDONR207 derivative carrying *ripA5*, Gm^r	10
pMS456	pDONR207 derivative carrying *ripA3*, Gm^r	10
pMS457	pDONR207 derivative carrying *ripA1*, Gm^r	10
pMS459	pENTR/SD/D-TOPO derivative carrying *ripA4*, Km^r	10
pNP200	pDONR207 derivative carrying *ripG7*, Gm^r	11
pNP329	pRCG derivative with the *ripG7* promoter followed by a Gateway™ destination cassette and a triple HA epitope tag, Gm^r Km^r	8
pPR122	pDONR207 derivative carrying *ripG6*, Gm^r	11
pPR126	pDONR207 derivative carrying *ripG4*, Gm^r	11
pPR134	pDONR207 derivative carrying *ripG5*, Gm^r	11
pPR156	pDONR207 derivative carrying *ripG3*, Gm^r	11
pPR181	pDONR207 derivative carrying *ripG1*, Gm^r	11
pSC192	pENTR/SD/D-TOPO derivative carrying ripB, Km^r	This study
pTH19	Gateway™ expression vector, N-terminal 6His tag, Ap^r	Invitrogen, Carlsbad, CA, USA
Plasmids for yeast two hybrid		
pDL6	pGBG derivative carrying *hpaB*, Km^r	This study
pDL7	pGBG derivative carrying *hpaD*, Km^r	This study
pDL9	pGAD derivative carrying *ripI*, Amp^r	This study
pDL10	pGAD derivative carrying *ripU*, Amp^r	This study
pDL12	pGAD derivative carrying *ripAV*, Amp^r	This study

pDL13	pGAD derivative carrying *ripAG*, Amp^r	This study
pDL14	pGAD derivative carrying *ripAD*, Amp^r	This study
pDL15	pGAD derivative carrying *ripAZ1*, Amp^r	This study
pDL16	pGAD derivative carrying *ripAJ*, Amp^r	This study
pDL17	pGAD derivative carrying *ripAF1*, Amp^r	This study
pDL18	pGAD derivative carrying *ripO1*, Amp^r	This study
pDL19	pGAD derivative carrying *ripZ*, Amp^r	This study
pDL20	pGAD derivative carrying *ripAM*, Amp^r	This study
pDL21	pGAD derivative carrying *ripH2*, Amp^r	This study
pDL22	pGAD derivative carrying *ripAK*, Amp^r	This study
pDL23	pGAD derivative carrying *ripAN*, Amp^r	This study
pDL24	pGAD derivative carrying *ripAW*, Amp^r	This study
pDL25	pGAD derivative carrying *ripQ*, Amp^r	This study
pDL26	pGAD derivative carrying *ripV1*, Amp^r	This study
pDL27	pGAD derivative carrying *ripAY*, Amp^r	This study
pDL28	pGAD derivative carrying *ripH1*, Amp^r	This study
pDL29	pGAD derivative carrying *ripR*, Amp^r	This study
pDL30	pGAD derivative carrying *ripW*, Amp^r	This study
pDL33	pGAD derivative carrying *ripA2*, Amp^r	This study
pDL34	pGAD derivative carrying *ripA4*, Amp^r	This study
pDL35	pGAD derivative carrying *ripA3*, Amp^r	This study
pDL36	pGAD derivative carrying *ripA5*, Amp^r	This study
pDL37	pGAD derivative carrying *ripA1*, Amp^r	This study
pDL38	pGAD derivative carrying *ripTPS*, Amp^r	This study
pDL40	pGAD derivative carrying *ripG4*, Amp^r	This study

pDL41	pGAD derivative carrying *ripG5*, Amp^r	This study
pDL42	pGAD derivative carrying *ripG6*, Amp^r	This study
pDL43	pGAD derivative carrying *ripG7*, Amp^r	This study
pDL44	pGAD derivative carrying *ripB*, Amp^r	This study
pDL45	pGAD derivative carrying *ripG1*, Amp^r	This study
pDL46	pGAD derivative carrying *ripG3*, Amp^r	This study
pDL47	pGAD derivative carrying *ripH3*, Amp^r	This study
pDL48	pGAD derivative carrying *ripTAL*, Amp^r	This study
pDL50	pGAD derivative carrying *hpaB*, Amp^r	This study
pDL51	pGAD derivative carrying *hpaD*, Amp^r	This study
pDL53	pGAD derivative carrying *ripP1*, Amp^r	This study
pDL54	pGAD derivative carrying *ripP2*, Amp^r	This study
pAD-T-Ag	AD-t-antigen fusion	BD Biosciences, Palo Alto, CA, USA
pBD-p53	BD-p53 fusion, Km^r	BD Biosciences, Palo Alto, CA, USA
Plasmids for GST-Pulldown		
pDL175	pGEX derivative carrying *hpaD*, Amp^r	This study
pDL188	pGEX derivative carrying KQGS*, Apr	8
pDL193	pTH19 derivative carrying *hpaD*, Amp^r	This study
pFL37	pTH19 derivative carrying *hpaB*, Amp^r	This study
pFL67	pGEX derivative carrying *ripAF1*, Amp^r	This study
pFL68	pGEX derivative carrying *ripAK*, Amp^r	This study
pFL69	pGEX derivative carrying *ripG5*, Amp^r	This study
pFL70	pGEX derivative carrying *ripG1*, Amp^r	This study
pFL71	pGEX derivative carrying *ripG7*, Amp^r	This study
pFL72	pGEX derivative carrying *ripTPS*, Amp^r	This study

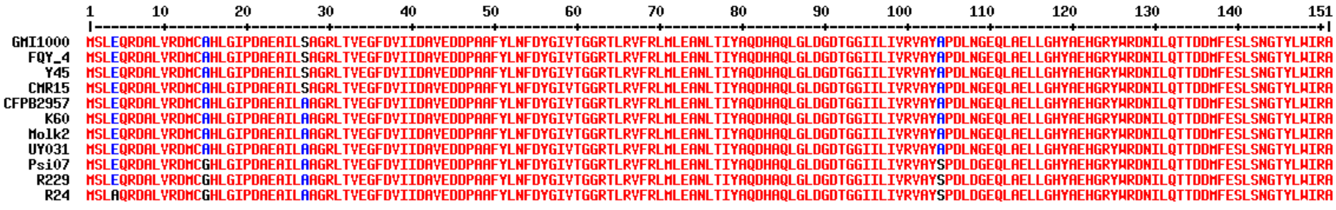
pFL75	pGEX derivative carrying *ripAW*, Amp^r	This study
pFL76	pGEX derivative carrying *ripO1*, Amp^r	This study
pFL77	pGEX derivative carrying *ripW*, Amp^r	This study
pFL83	pGEX derivative carrying *ripP1*, Amp^r	This study
pFL84	pGEX derivative carrying *hpaB*, Amp^r	This study
pFL116	pTH19 derivative carrying $hpaB_{Xcv}$, Amp^r	This study
pGEX-RipP1	pGEX derivative carrying *ripP1*, Amp^r	Laurent Deslandes, LIPM
pGEX-RipP2	pGEX derivative carrying *ripP2*, Amp^r	12
Plasmids transformed in *R. solanacearum*		
pAM5	pLAFR3 carrying 2-kb fragment containing *hrpB*,Tc^r	13
pEG11	pNP329 derivative carrying $hpaB_{\mathrm{GMI1000}}$, Km^r, Gm^r	3
pFL49	pNP329 derivative carrying $hpaB_{Xcv\ \mathrm{85\text{-}10}}$, Km^r, Gm^r	This study
Plasmids transformed in *X. campestris* pv. *vesicatoria*		
pFL98	pBR356 derivative carrying *ripP1*, Gm^r	This study

Supplementary Table S3: List of primers used in this study.

	Gene	Recipient Plasmid	Plasmid ID	Primers	Primer Sequences
RipAZ1	*RSp1582*	pDONR207	pACC61	Oacc31 Oacc32	GGAGATAGAACCATGCCCCCATCGATCCG CAAGAAAGCTGGGTCTCMCACCGGGAATGCTTTCC
RipAF1	*RSp0822*	pDONR207	pACC73	Oacc19 Oacc20	GGAGATAGAACCATGGGTTTGCCACGGATC CAAGAAAGCTGGGTCTCMTCGCGTTGACGTGGACG
RipO1	*RSp0323*	pDONR207	pACC75	Oacc17 Oacc18	GGAGATAGAACCATGCCAAAAATCCCAAAAAACC CAAGAAAGCTGGGTCTCMGGCGGCGGGGCTGGCC
RipZ	*RSp1031*	pDONR207	pACC82	Oacc23 Oacc24	GGAGATAGAACCATGCCGCGTTTTTCAAACTG CAAGAAAGCTGGGTCTCMTCGGCGCATCGACGGCG
RipAM	*RSc3272*	pDONR207	pACC93	Oacc11 Oacc12	GGAGATAGAACCATGAAACGAATCGACGTCCAC CAAGAAAGCTGGGTCTCMGGCCGGCCGCGTCGTTG
RipH2	*RSp0215*	pDONR207	pACC98	Oacc13 Oacc14	GGAGATAGAACCATGCTTGGCGGAAACATCACGGA CAAGAAAGCTGGGTCTCMATGCGCCCCCGGCGCGC
RipAK	*RSc2359*	pDONR207	pACC113	Oacc9 Oacc10	GGAGATAGAACCATGCGCCCTACCGCCCC CAAGAAAGCTGGGTCTCMCAGGTGCGCGATGGCTC
RipAN	*RSp0845*	pDONR207	pACC221	Oacc21 Oacc22	GGAGATAGAACCATGCGCCCCCTCTCTTTTC CAAGAAAGCTGGGTCTCMGGCTTCCGGGGCGCCG
RipAW	*RSp1475*	pDONR207	pACC271	Oacc29 Oacc30	GGAGATAGAACCATGGTTTTCCTTGTCCGGAGC CAAGAAAGCTGGGTCTCMTCCGCCGCGCGCGGCGAC
RipQ	*RSp1277*	pDONR207	pACC276	Olb69 Olb70	GGAGATAGAACCATGCTGCGAACCTCCCTTGAC CAAGAAAGCTGGGTCTCMCACCATGTCGATGGCCG
RipAY	*RSp1022*	pDONR207	pACC385	Olb79 Olb80	GGAGATAGAACCATGGAAAGAATCTCGACAA CAAGAAAGCTGGGTCTCMGTCGGGTTTGGGC
RipH1	*RSc1386*	pDONR207	pACC386	Oacc3 Oacc4	GGAGATAGAACCATGGCAGGAGGACGAGTCG CAAGAAAGCTGGGTCTCMTCCGGCCGGGCGCACGC
RipR	*RSp1281*	pDONR207	pACC426	Oacc57 Oacc58	GGAGATAGAACCATGAACATAAGAAAATACTTAC CAAGAAAGCTGGGTCTCMTTCCAGCGACTTGCTAC

RipW	*RSc2775*	pDONR207	pACC429	Oacc61 Oacc62	GGAGATAGAACCATGTCCATCCAGATTGATCGC CAAGAAAGCTGGGTCTCMGCCCGAGTAGGCCTTGTAG
RipTAL	*Rsc1815*	pENTR/D-TOPO	pACC532	Oacc177 Oacc178	CACCATGAGAATAGGCAAATCAAGCGGTTGGTTGAAC TCACGTTTCCAATATTTGCAGAAGCCAGTCG
RipAG	*RSc0824*	pDONR207	pACC542	Oacc1 Oacc2	GGAGATAGAACCATGGGATGCGTTAATGTC CAAGAAAGCTGGGTCTCMGTCAGAACGGCCGATC
RipV1	*RSc1349*	pDONR207	pACC546	Opr53 Opr52	GGAGATAGAACCATGCCAACCCGCGTTCCGTCCCC CAAGAAAGCTGGGTCTCMGCGGCTGCCTTGCGAA
RipH3	*Rsp0160*	pDONR207	pACC588	Olb53 Olb54	GGAGATAGAACCATGTTGAAAGGACGTATCGACG CAAGAAAGCTGGGTCTCMCCCGGAAGCCGGCAGCTTGA
RipAJ	*RSc2101*	pDONR207	pACC652	Oacc5 Oacc6	GGAGATAGAACCATGCGCCGGACGGCGACTTC CAAGAAAGCTGGGTCTCMCGCTGCGCGGGGCTCGC
RipAD	*Rsp1601*	pENTR/SD/D-TOPO	pACC648	Oacc217 Oacc34	GGAGATAGAACCATGTTGAAAATAGGCCGTATC CAAGAAAGCTGGGTCTCMCCAGTCGAGCTGCGGATC
HpaB$_{\mathit{Xcv}\ 85\text{-}10}$	*XCV0416*	pENTR/SD/D-TOPO	pFL47	Ofl82 Ofl83	CACCATGAGCAGCGCGCGATTC TCAGGCGCGTAACCACAG
HpaD	*Rsp0848*	pDONR207	pLBy1	Olb17 Olb18	GGAGATAGAACCATGCCCTCCGCCGAAACCG CAAGAAAGCTGGGTCTCMATGCGCCTCCCACT
RipI	*RSc0041*	pDONR207	pLBy3	Olb9 Olb10	GGAGATAGAACCATGCCTCTTACCAAGATCAATCCTTCGG CAAGAAAGCTGGGTCTCMCTCGGTCGTCCG
RipU	*RSp1212*	pDONR207	pLby7	Olb83 Olb84	GGAGATAGAACCATGTCACGCATCTTCAGGTC CAAGAAAGCTGGGTCTCMGCGCGCCTCGTGCG
RipAV	*RSp0732*	pDONR207	pLBy13	Olb77 Olb78	GGAGATAGAACCATGATCCCGAAAACACTC CAAGAAAGCTGGGTCTCMCCCCACCGCCTC
RipB	*Rsc0245*	pENTR/SD/D-TOPO	pSC192	RipB-Fwd RipB-Rev	CACCATGAAGGCCGTCACCCGA TCAGCGCGTACCCGGCGT
RipP1 flanked by BsaI sites	*Rsc0826*	pGEM-T	pFL92	Ofl162 Ofl163	TTTGGTCTCTTATGAAAAGACTATTCAGAGCATTGG TTTGGTCTCTGATCCGACTCCAGGGCATGTCGA

a



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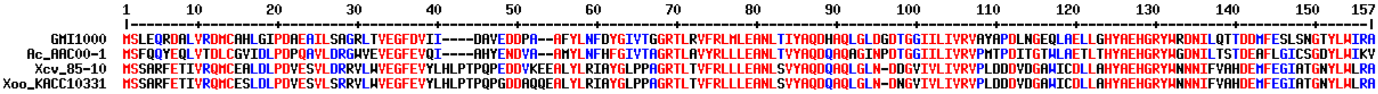
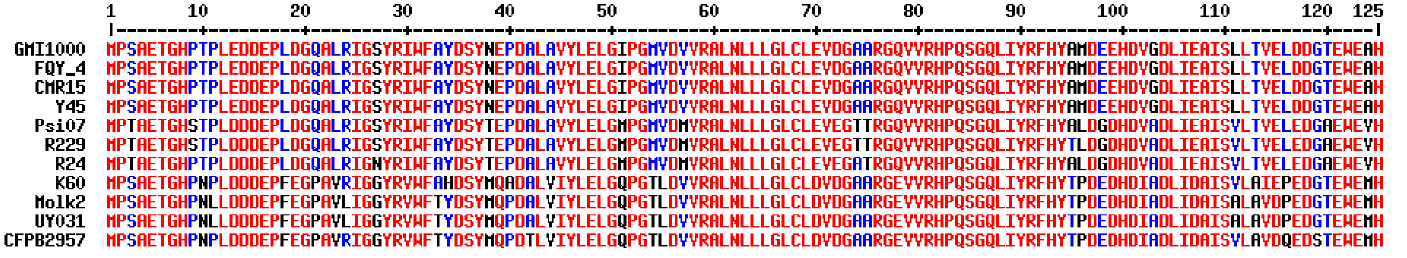


Supplementary Figure S1. (a) Amino acid sequence alignment of HpaB from *R. solanacearum* GMI1000 and its homologs in 10 other wild-type sequenced strains belonging to all four phylotypes. Sequences were aligned using multalin 5.4.1 (http://multalin.toulouse.inra.fr/multalin/). Conserved residues are colored in red. (b) Amino acid sequence alignment of HpaB from *R. solanacearum* GMI1000 and its homologs in *Acidovorax citruli* AAC00-1, *Xanthomonas campestris* pv. *vesicatoria* 85-10 and *Xanthomonas oryzae* pv. *oryzae* KACC10331. Sequences were aligned using multalin 5.4.1 (http://multalin.toulouse.inra.fr/multalin/). Conserved residues are colored in red.

a



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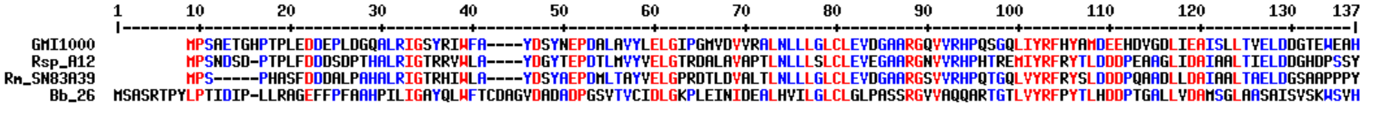
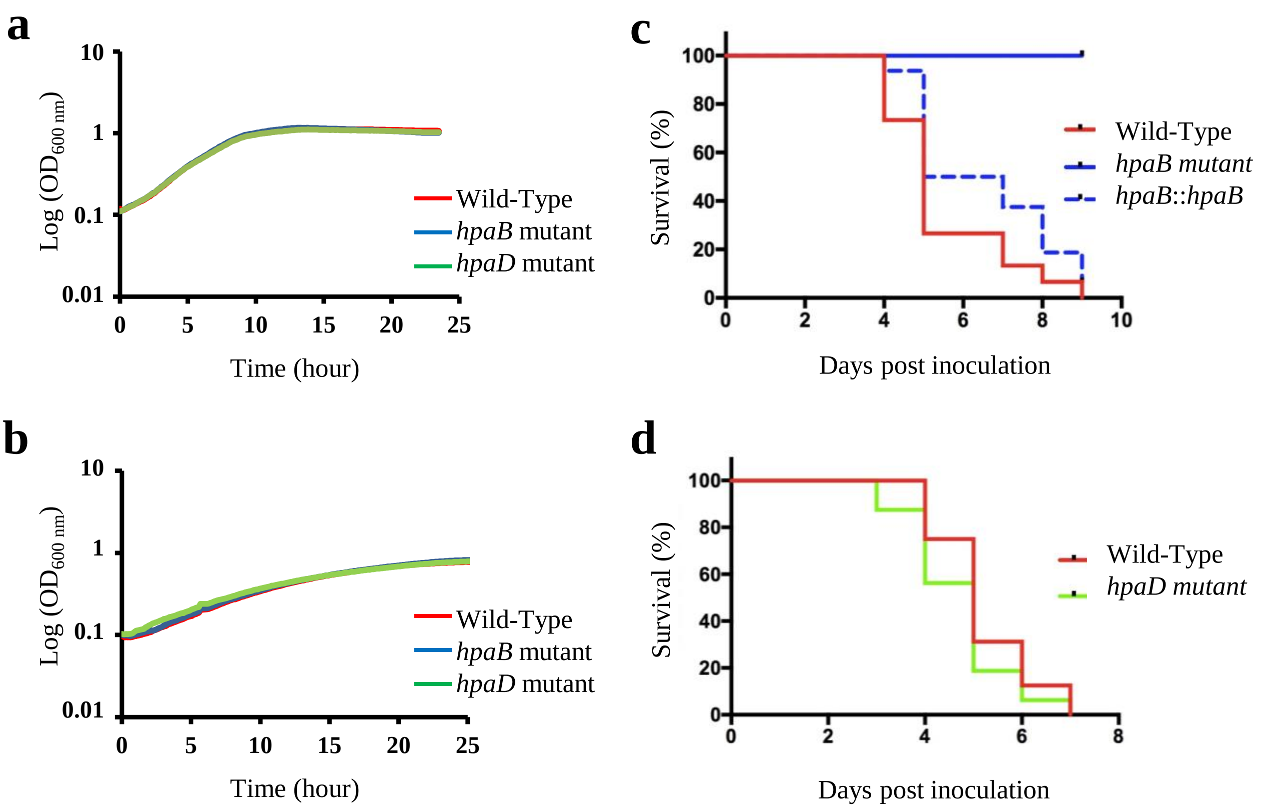


Supplementary Figure S2. (a) Amino acid sequence alignment of HpaD from *R. solanacearum* GMI1000 and its homologs in 10 other wild-type sequenced strains belonging to all four phylotypes. Sequences were aligned using multalin 5.4.1 (http://multalin.toulouse.inra.fr/multalin/). Conserved residues are colored in red. (b) Amino acid sequence alignment of HpaD from *R. solanacearum* GMI1000 and its homologs in *Ralstonia sp* A12, *Ralstonia mannitolilytica* SN83A39 and *Burkholderiaceae bacterium* 26. Sequences were aligned using multalin 5.4.1 (http://multalin.toulouse.inra.fr/multalin/). Conserved residues are colored in red.



Supplementary Figure S3. HpaB is strictly required for *R. solanacearum* pathogenicity on tomato, not HpaD. *In vitro* growth in complete (a) or minimal medium (b), of the wild-type strain (red), and of the *hpaB* (blue) and *hpaD* (green) mutants. *In vitro* growth was investigated by monitoring OD_{600} during 25 hours starting at 0.1 as described in material and methods. These experiments have been repeated twice. (c) Kaplan-Meier survival analysis of 16 tomato inoculated plants. Gehan-Breslow-Wilcoxon test indicates that the wild-type strain curve (red) is significantly different from the *hpaB* mutant curve (blue) (p value < 0.001), contrary to the complemented *hpaB* curve (dotted blue) (p value = 0.0678). (d) Kaplan-Meier survival analysis of 16 tomato inoculated plants. Gehan-Breslow-Wilcoxon test indicates that the wild-type strain curve (red) is not significantly different from the *hpaD* curve (green) (p value = 0.2689). These experiments have been repeated three times.

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