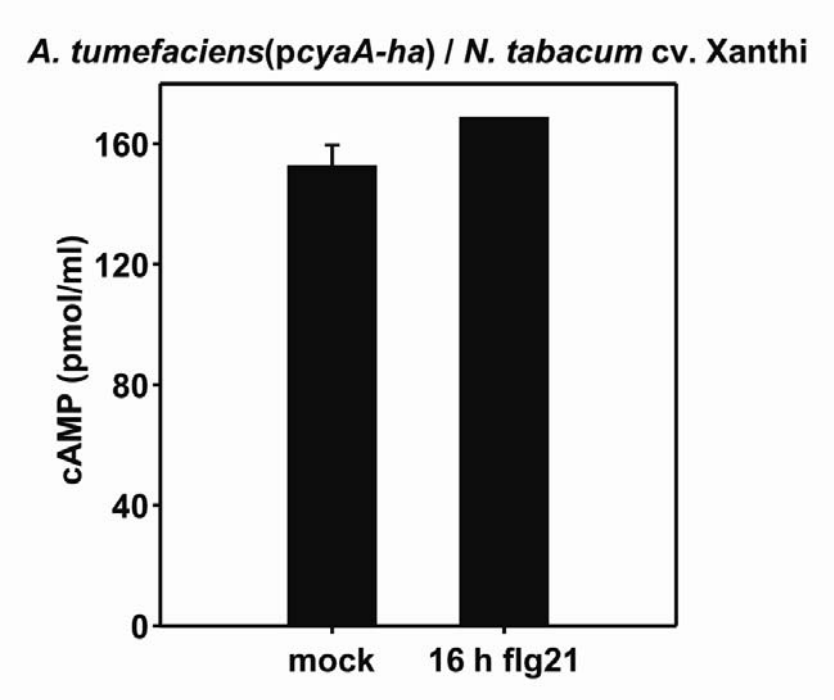
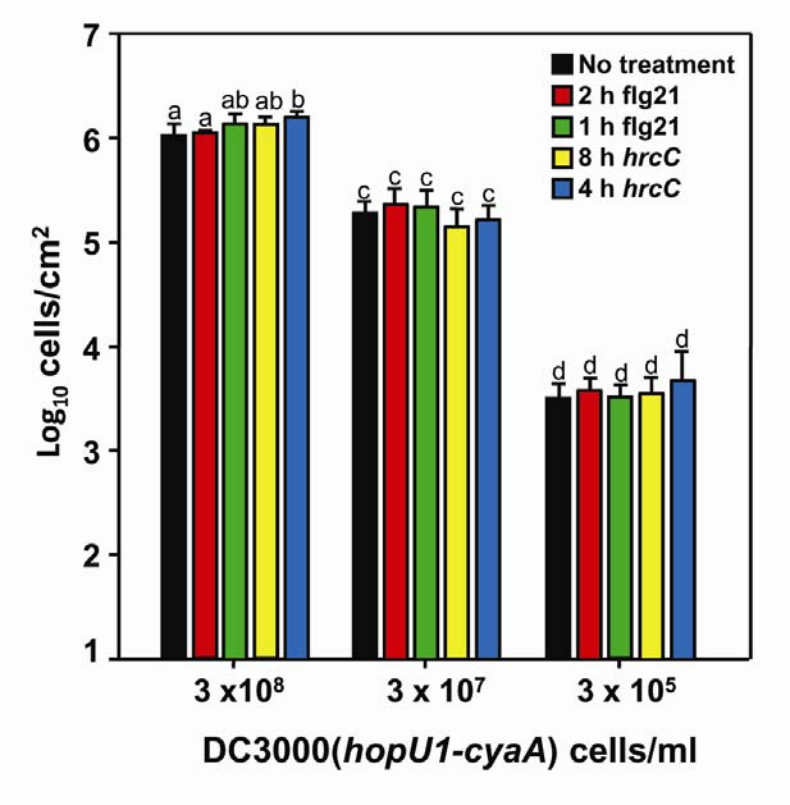
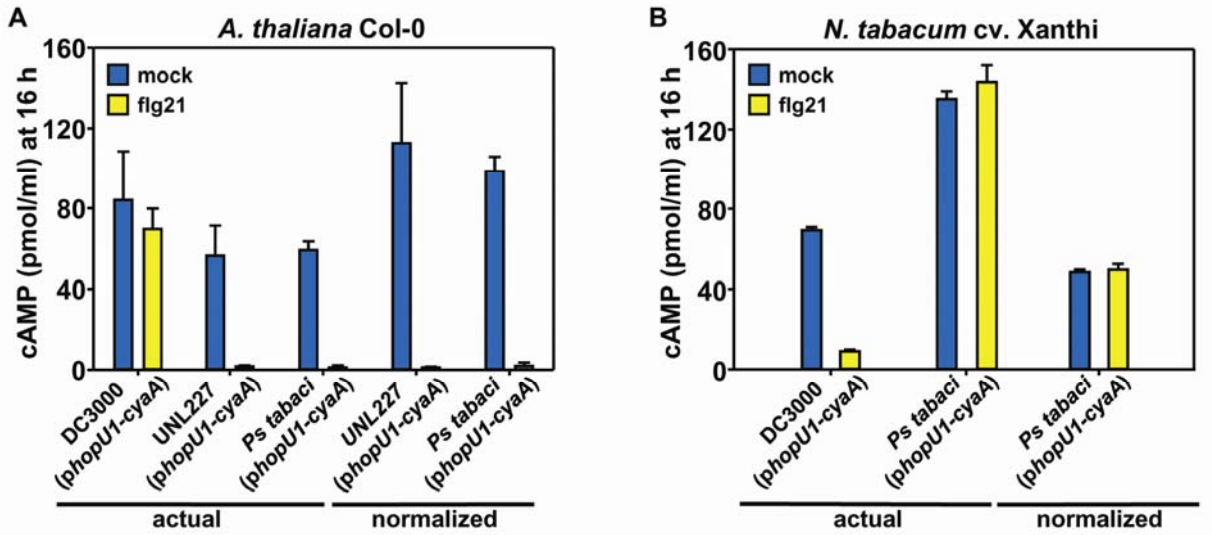


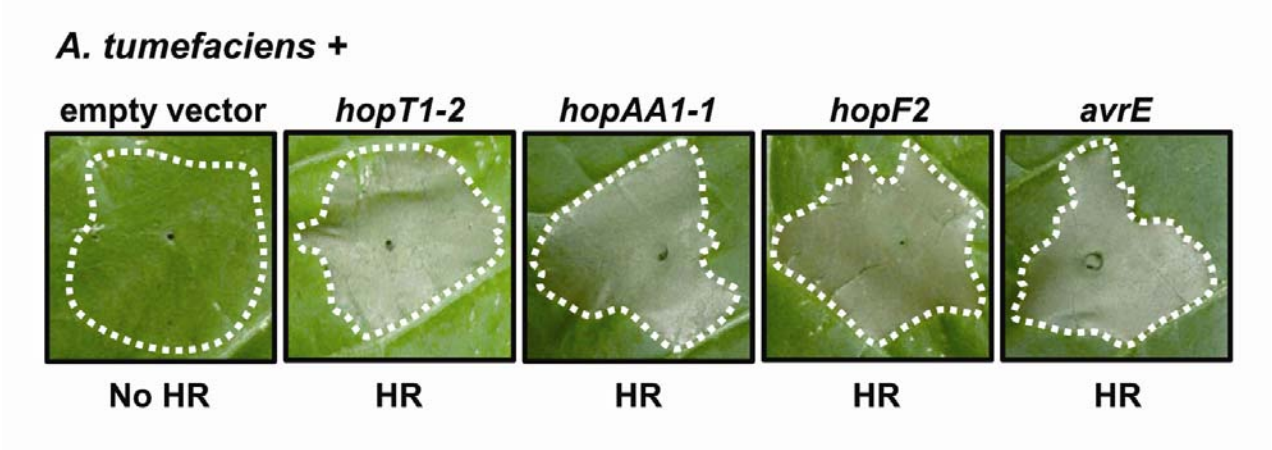
Supplemental Figure 1. CyaA remains functional in PTI-induced tissue. *N. tabacum* cv. Xanthi leaves that were infiltrated with *A. tumefaciens* transiently expressing *cyaA-ha*. 24 h post infiltration the leaves were infiltrated again with either water (mock) or a 1 µM flg21 solution. Samples were taken 16 h after the second infiltration and a CyaA assay was performed to measure cAMP levels. This experiments were repeated three times with similar results.



Supplemental Figure 2. Growth of DC3000(p*hopU1-cyaA*) was not affected by pre-treatment with either flg21 or *hrcC*. DC3000(p*hopU1-cyaA*) was infiltrated into tobacco at 3 x 10^8, 3 x 10^7, or 3 x 10^5 cells/ml either alone, 1 or 2 h after 1 μM flg21 infiltration, or 4 or 8 h after infiltration of 3 x 10^8 cells/ml of *hrcC*. Bacteria were enumerated in the overlapping area 7 h after DC3000(p*hopU1-cyaA*) infiltration. PTI-induction does not alter the growth of DC3000(p*hopU1-cyaA*) in tobacco within 7 h. These experiments were repeated three times with similar results and standard error bars are shown.



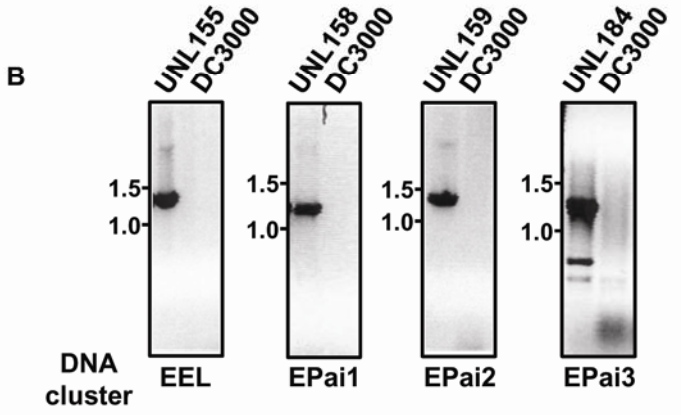
Supplemental Figure 3. The growth of DC3000(p*hopU1-cyaA*), UNL227(p*hopU1-cyaA*), and *P. syringae pv. tabaci*(p*hopU1-cyaA*) was determined at 0 and 16 h after infiltration in *A. thaliana* Col-0 or *N. tabacum* cv. Xanthi leaves pretreated for 2 h with 1 µM flg21 or water (mock). The levels of cAMP were determined at 16 h in *A. thaliana* Col-0 (A) and *N. tabacum* cv. Xanthi (B) plant tissue. The cAMP levels were normalized with respect to the differences in growth between DC3000 and the other strains. The cAMP amounts are shown before (actual) and after normalization (normalized). While there were significant changes in cAMP levels in UNL227(p*hopU1-cyaA*) and *P. syringae* pv. *tabaci*(p*hopU1-CyaA*) after normalization, there was little change in the differences in cAMP amounts between flg21-induced and mock samples. Therefore, the growth differences between the *P. syringae* strains are not responsible for the dramatic differences in cAMP levels that occur after PTI induction. These experiments were repeated twice with similar results and standard error bars are shown.

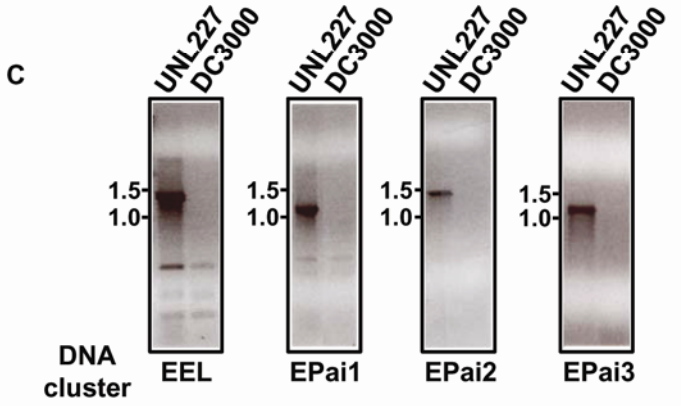


Supplemental Figure 4. Tobacco leaves were infiltrated with *Agrobacterium* carrying binary constructs containing each of the indicated T3E genes. After 24-48 h HR-like responses developed within the infiltrated zone for these T3E genes suggesting that they encode T3Es that induce ETI. Pictures were taken 48 h after infiltration.

A

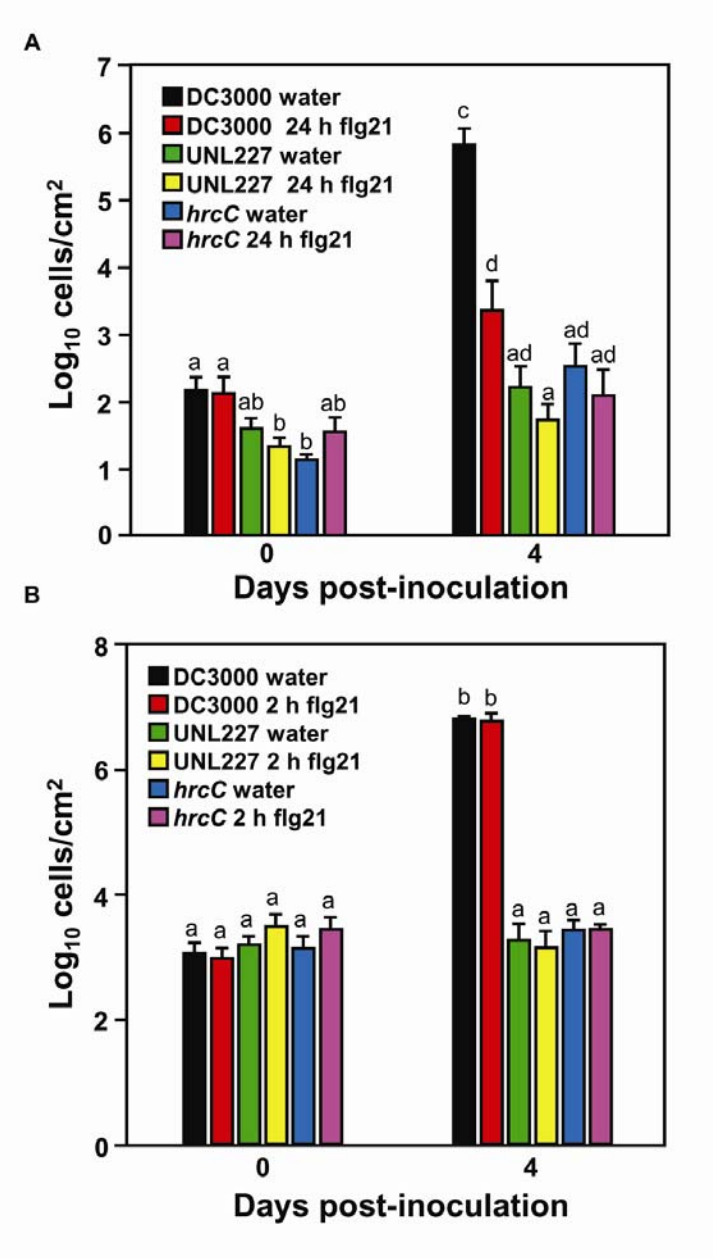
DNA cluster	Primer set	Predicted band length in DC3000	Predicted band length in mutant	Observed band length in mutant
EEL	P2087/P2088	7 kb	1.2 kb	1.2 kb
EPai1	P2095/P2096	26 kb	1.3 kb	1.3 kb
EPai2	P2101/P2102	20 kb	1.2 kb	1.2 kb
EPai3	P2447/P2448	4 kb	1.3 kb	1.3 kb



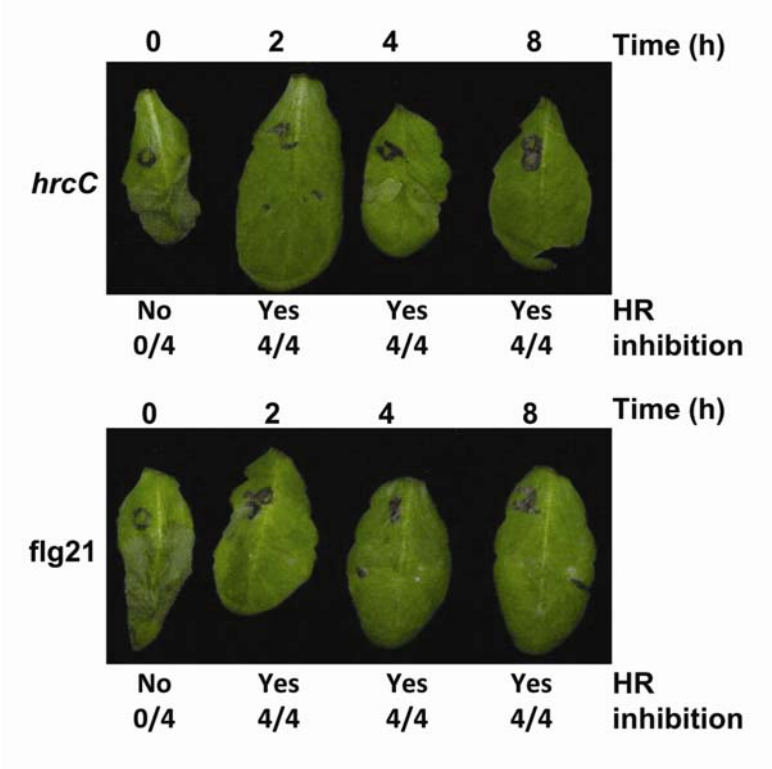


Supplemental Figure 5. Confirmation of DC3000 DNA cluster mutants. Colony PCR was done for each of the mutants and compared to wild type bacterial samples to confirm that a T3E-related DNA cluster was deleted from the different bacterial mutants. (A) The DNA cluster names, primer sets used, and expected and observed PCR band lengths are indicated. Primers were made approximately 600 base pairs upstream and downstream of the DNA cluster, which would result in a PCR product of about 1.2 kb if the putative mutant carried the correct mutation. (B) DNA agarose gels of PCR reactions from the single T3E-related DNA clusters. (C) DNA agarose gels of PCR reactions from the poly DNA cluster mutant UNL227. In DC3000 the DNA

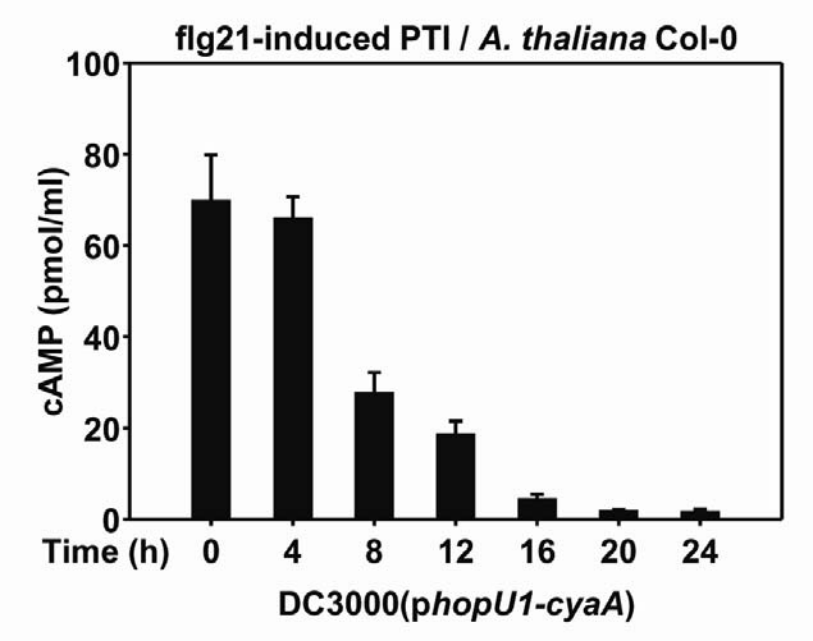
clusters were too big to be amplified in the PCR conditions used and resulted in the absence of bands of the predicted length.



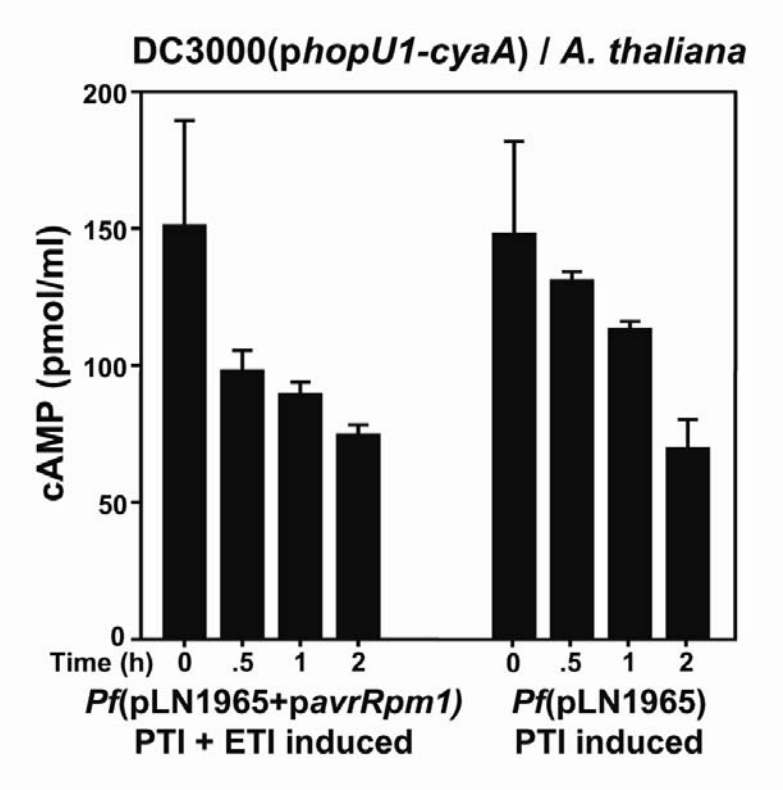
Supplemental Figure 6. The induction of PTI by flg21 at 24 h but not 2 h before inoculation with DC3000 inhibits bacterial growth in *A. thaliana*. *A. thaliana* Col-0 plants were infiltrated with 1μM flg21 or a water control at 24 h (A) or 2 h (B) prior to spray-inoculation with 2 x 10^8 cells/ml of DC3000, UNL227 or *hrcC*. Bacteria were enumerated at 0 and 4 d post-inoculation. Letters a-d are statistically different ($p<0.05$) and standard error bars are shown. These experiments were repeated at least three times with similar results.



Supplemental Figure 7. PTI-induction inhibits AvrRpm1 induced HR in *A. thaliana* by *hrcC* and flg21 PTI-inducers. Infiltration of the leaves of *A. thaliana* with 1 x 10^7 cells/ml of *P. fluorescens*(pLN1965 + p*avrRpm1*) was unable to cause an HR in PTI-induced leaves pretreated with the *hrcC* mutant or flg21. With the exception of the zero time point, the HRs were inhibited in leaves at the remaining time points. The fraction below each leaf indicates the number of times that the HR was inhibited over the total number of times the assay was performed. Photos were taken after 48 hours after *P. fluorescens*(pLN1965 + p*avrRpm1*) infiltration.



Supplemental Figure 8. Time course of the restriction of HopU1-CyaA injection into PTI-induced *A. thaliana* plant cells. PTI was induced with 1 µM flg21 and at the indicated times DC3000(p*hopU1-cyaA*) at a cell density of 3 x 10^8 cells/ml was infiltrated into the pretreated leaf regions. After 16 h cAMP levels were determined. This experiment was repeated three times with similar results.



Supplemental Figure 9. PTI- and ETI-induced *A. thaliana* plants appear to restrict T3E injection more quickly than when PTI only is induced in *A. thaliana* plants. *A. thaliana* plants were pretreated with 3 x 10^8 cells/ml *P. fluorescens*(pLN1965), which induces PTI, or *P. fluorescens*(pLN1965 + p*avrRpm1*), which induces PTI and ETI. At the indicated times DC3000(p*hopU1-cyaA*) at a cell density of 3 x 10^8 cells/ml was infiltrated into the pretreated leaves and the level of T3E injection was determined by quantifying cAMP in the overlapping infiltration area 16 h later. These experiments were repeated at least three times with similar results.

Supplemental Table 1. Strains and plasmids used in this study

Strain or plasmid	Characteristics	Reference or source
E. coli DH5α	*supE44 ΔlacU169(Φ80lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1,* Nal^r	(Hanahan, 1983); Life Technologies, Gaithersburg, MD, USA
E. coli DB3.1	*F⁻ gyrA462 endA1 Δ(sr1-recA) mcrB mrr hsdS20 (r_B^-, r_B^-) supE44 ara-14 galK2 lacY1 proA2 rpsL20* (Sm^r) *xyl-5 λ⁻ leu mtl-1*	Invitrogen, Carlsbad, CA, USA
E. coli HB101	*supE44 hsdS20* (r_B^-, m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 Δleu mtl-1*	New England Biolabs, Beverly, MA, USA
Pseudomonas fluorescens 55	Nal^r	M. Sasser
Pseudomonas syringae pv. *tomato* DC3000	Wild type, Rf^r	(Cuppels, 1986)
DC3000 *hrcC*	*hrcC* mutant defective in T3SS, Rf^r Cm^r	(Yuan and He, 1996)
Pseudomonas syringae pv. *tabaci* 11528	Wild type, Nal^r	American Type Culture Collection
UNL155	DC3000 EEL mutant lacking T3E *hopB1*, Rf^r	This work
UNL158	DC3000 EPai1 mutant lacking T3E genes *hopD1*, *hopQ1-1*, and *hopR1*, Rf^r	This work
UNL159	DC3000 EPai2 mutant lacking T3E genes *hopAA1-2*, *hopV1/shcV*, *hopAO1*, *hopG1*, and *hopQ1-2*, Rf^r	This work
UNL184	DC3000 EPai3 mutant lacking T3E genes *hopF2/shcF* and *hopU1*, Rf^r	This work
UNL227	DC3000 EPai1, EPai2, EPai3, EEL mutant, Rf^r	This work
pBH474	Suc^s derivative of pTH474	(House et al., 2004)
pHIR11	Cosmid pLAFR3 derivative carrying T3SS DNA from *P. syringae* pv. *syringae* 61, Tc^r	(Huang et al., 1988)
pENTR/D-TOPO	Gateway system entry vector, Km^r	Invitrogen, Carlsbad, CA, U.S.A.
pLN525	pPZP212 derivative carrying *hopF2*, Km^r	This work
pLN953	pPZP212 derivative carrying *hopT1-2*, Km^r	This work
pLN956	pPZP212 derivative carrying *avrE1*, Km^r	This work
pLN958	pPZP212 derivative carrying *hopAA1-2*, Km^r	This work
pLN1965	pHIR11 derivative containing a deletion of *shcA/hopA1* operon replaced by a sp resistant cassette, Tc^r Sp^r	(Guo et al., 2009)
pLN2193	pML123 derivative gateway destination vector containing a CyaA tag for C-terminal fusions, Gm^r	(Fu et al., 2006)
pLN2194	pMK2017 derivative carrying 2.5 kb sequence US of EPai1, Tc^r, Sp^r	This work
pLN2195	pMK2017 derivative carrying 2.5 kb sequence US of EPai2, Tc^r, Sp^r	This work
pLN2204	pMK2016 derivative carrying 2.0 kb sequence DS of EPai1, Sp^r	This work
pLN2205	pMK2016 derivative carrying 2.0 kb sequence DS of EPai2, Sp^r	This work
pLN2250	pLN2193 derivative carrying *avrPtoB*, Gm^r	This work
pLN2254	pLN2193 derivative carrying *hopU1*, Gm^r	This work
pLN2616	pMK2017 derivative carrying 1.5 kb sequence US of EPai3, Tc^r, Sp^r	This work
pLN2617	pMK2016 derivative carrying 2.0 kb sequence DS of EPai3, Sp^r	This work
pLN2637	pML123 derivative carrying *avrRpt2-ha*, Gm^r	This work
pLN2665	pMK2017 derivative carrying 2.4 kb sequence US of EEL, Tc^r, Sp^r	This work
pLN2666	pMK2016 derivative carrying 2.5 kb sequence DS of EEL, Sp^r	This work
pMK2016	Sp^r St^r *oriV* $oriT_{ColE1}$ with FRT cassette from pMK2014	(House et al., 2004)

pMK2017	Tc^r *oriV*$_{R6K}$ *oriT*$_{RP4}$ with FRT cassette from pMK2015	(House et al., 2004)
pRK2013	Km^r mobilization helper plasmid	
pRK2073	Sp^r mobilization helper plasmid	
pVSP61::*avrRpm1*	pVSP61 derivative containing *avrRpm1*, Km^r	(Mackey et al., 2002)
pPZP212	*Agrobacterium tumefaciens* binary vector, Km^r	(Hajdukiewicz et al., 1994)

Supplemental Table 2. Primers used in this study

Primer	Sequence	Use[a]
P1789	5'-GAACTTCAAGATCCCCTGATTCCCTT-3'	pMK2016 insert
P1790	5'-GAGCGCTTTTGAAGCTGATGTGC-3'	pMK2017 insert
P2087	5'-GCACGTTGGGTACGCTGCAAG-3'	confirm EEL
P2088	5'-CGCCGCCGCCATCGATC-3'	confirm EEL
P2091	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTGCGCTTCTCCCTGGC-3'	pLN2194
P2092	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTGCCTGCGGGCTGGATG-3'	pLN2194
P2093	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCTCCTGGGCATTCTTCAGACG-3'	pLN2204
P2094	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTGGCGTTGACACCTACGTCATAC-3'	pLN2204
P2095	5'-CCGTCCGCAGTTCAGGCG-3'	confirm EPai1
P2096	5'-CCGGCAAGCGGGTATGC-3'	confirm EPai1
P2097	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTGCTCTATTATCGCAGCCCCCTG-3'	pLN2195
P2098	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTGCTACTCAGCGTATGGGGCGAG-3'	pLN2195
P2099	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTGACAACCCCCAAGACAAACTCC-3'	pLN2205
P2100	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTGCAACTGGGGTTTGCGGAGC-3'	pLN2205
P2101	5'-GAAGAGTTTTCCCAGGGGCTGC-3'	confirm EPai2
P2102	5'-CGGTGAAACTGCTTCCCCTATTCC-3'	confirm EPai2
P2404	5'-CACCGTTTTTCATAATGCATCTCCTCAT-3'	pLN2617
P2405	5'-CCCTCCTACCTGGCATCGAAATG-3'	pLN2617
P2406	5'-CACCGTCATTCGTTCCAGGATTCATCAG-3'	pLN2616
P2407	5'-CAGATTTGAGTCCATGAAGGAGGCC-3'	pLN2616
P2443	5'-CACCCTCAATGGTGGTGCCCCGAG-3'	pLN2665
P2444	5'-GTATAAAAAGCAGGAAAAACTCGTTC-3'	pLN2665
P2445	5'-CACCCGATCTCGATCATTTTTTCTGG-3'	pLN2666
P2446	5'-CGCGGAGATTCAATCATG-3'	pLN2666
P2447	5'-GAACAAGGAATGGGGCGAGC-3'	confirm EPai3
P2448	5'-GGCGATGTTGCTGACGACCAAATAC-3'	confirm EPai3

[a]Primers were used to make a construct, confirm a deletion, or confirm a sequence insertion.

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