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Appendix Table S1. Strains and plasmids used in this study

Strain	Lab strain name	Relevant characteristics and/or purpose	Reference/Source
<i>A. tumefaciens</i>			
C58	EML530	Wild type virulent nopaline type strain, isolated from a cherry gall	Eugene Nester
$\Delta tssL$	EML1073	<i>tssL</i> deletion mutant	[3]
$\Delta tde1\text{-}tdi1$	EML3392	deletion mutant of <i>tde1\text{-}tdi1</i> effector-immunity gene pair	[4]
$\Delta tde2\text{-}tdi2$	EML3551	deletion mutant of <i>tde2\text{-}tdi2</i> effector-immunity gene pair	[4]
$\Delta tae\text{-}tai$	EML3553	deletion mutant of <i>tae\text{-}tai</i> effector-immunity gene pair	[5]
$\Delta tdei$	EML3559	Double deletion mutant of <i>tde1\text{-}tdi1</i> and <i>tde2\text{-}tdi2</i> effector-immunity gene pairs	[4]
$\Delta tde1\text{-}tdi1\Delta tae\text{-}tai$	EML3555	Double deletion mutant of <i>tde1\text{-}tdi1</i> and <i>tae\text{-}tai</i> effector-immunity gene pairs	This study
$\Delta tde2\text{-}tdi2\Delta tae\text{-}tai$	EML3557	Double deletion mutant of <i>tae\text{-}tai</i> and <i>tde2\text{-}tdi2</i> effector-immunity gene pairs	This study
$\Delta 3TIs$	EML3561	Triple deletion mutant of <i>tae\text{-}tai</i> , <i>tde1\text{-}tdi1</i> and <i>tde2\text{-}tdi2</i> effector-immunity gene pairs	[4]
$\Delta tap\text{-}1$	EML4290	Deletion mutant of Tde1 adaptor/chaperone gene <i>tap\text{-}1</i>	[4]
$\Delta atu3641$	EML3406	Deletion mutant of Tde2 adaptor chaperone gene <i>atu3641</i>	[6]

$\Delta G1\Delta G2op$	EML5132	mutant with deletion of <i>vgrG1</i> gene and <i>vgrG2</i> operon	This study
$\Delta G2op$	EML3677	mutant with deletion of <i>vgrG2</i> operon	This study
$\Delta tdei\Delta G1\Delta G2op$	EML5130	mutant with deletion of <i>tdei-tdi1</i> effector-immunity gene pair, <i>vgrG1</i> gene, and <i>vgrG2</i> operon	This study
$\Delta tssB$	EML1109	<i>tssB</i> deletion mutant	[5]
$\Delta tssL\Delta tssB$	EML5156	Double deletion mutant of <i>tssL</i> and <i>tssB</i>	This study
$\Delta tdei\Delta tssB$	EML5159	mutant with deletion of <i>tdei-tdi1</i> and <i>tdei-tdi2</i> effector-immunity gene pairs and <i>tssB</i> gene	This study
12D1	EML490	Wild type virulent strain	Clarence I Kado
12D1 $\Delta tssL$	EML4635	12D1 <i>tssL</i> deletion mutant	[9]
12D1 ΔvI	EML4688	12D1 adaptor deletion mutant	This study
12D1 $\Delta v3-4$	EML4679	12D1 putative effector-immunity pair deletion mutant	This study
1D1108	EML302	Wild type virulent nopaline type strain isolated from a <i>Euonymus</i> gall	Clarence I Kado
1D1108 $\Delta tssL$	EML4631	1D1108 <i>tssL</i> deletion mutant	[9]
1D1108 $\Delta vI-9$	EML4645	1D1108 with deletion of all predicted <i>vgrG</i> -associated genes	This study
1D1108 $\Delta v4$	EML4690	1D1108 adaptor/chaperone gene deletion mutant	This study
1D1108 $\Delta v6$	EML4692	1D1108 PAAR-like domain-encoding gene deletion mutant	This study

15955	EML459	Wild type virulent agropine type strain isolated from a tomato gall	Clarence I Kado
15955 Δ <i>tssL</i>	EML4629	15955 <i>tssL</i> deletion mutant	[9]
15955 Δ <i>v1-7</i>	EML4650	15955 with deletion of all predicted <i>vgrG</i> -associated genes	This study
Δ <i>tap-1</i> Δ <i>atu3641</i>		Double deletion mutant of <i>tap-1</i> and <i>atu3641</i>	This study
12D1 Δ <i>tae-tai</i>		12D1 deletion mutant of <i>tae-tai</i> effector-immunity gene pair	This study
12D1 Δ <i>v3-4</i> Δ <i>tae-tai</i>		12D1 double deletion mutant of <i>tae-tai</i> and <i>v3-v4</i> effector-immunity gene pairs	This study
Δ <i>tae-tai</i> *		New deletion mutant of <i>tae-tai</i> effector-immunity gene pair	This study
Δ <i>tap-1</i> Δ <i>atu3641</i> Δ <i>tae-tai</i> *		Triple deletion mutant of <i>tap-1</i> , <i>atu3641</i> and <i>tae-tai</i> effector-immunity gene pair	This study
Δ 3TIs*		New triple deletion mutant of <i>tae-tai</i> , <i>tde1-tdi1</i> and <i>tde2-tdi2</i> effector-immunity gene pairs	This study
<i>E. coli</i>			
DH10B	EML455	Host for molecular cloning and target cell for bacterial competition assay	Invitrogen
Plasmids			
pRL662	EML315	Broad host range vector derived from pBBR1MCS-2, Gm ^R	[7]
pTrc200	EML904	pVS1 origin, <i>lacI^q</i> , <i>trc</i> promoter, Sp ^R	[8]

pTdei1	EML4277	<i>tde1</i> and <i>tdi1</i> in pTrc200, Sp ^R	This study
pTde2*	EML4797	<i>tde2</i> catalytic site mutant in pRL662, Gm ^R	This study
pTap-1	EML4255	<i>tap-1</i> in pTrc200, Sp ^R	[4]
p3641	EML4785	<i>atu3641</i> in pTrc200, Sp ^R	[6]
pTrc-TssB-GFP	EML5154	<i>tssB</i> fused at its C-terminal coding portion to <i>gfp</i> in pTrc200, Sp ^R	This study
pTrc-TssB	EML4043	<i>tssB</i> in pTrc200, Sp ^R	This study
pBBR1-GFP	EML3	Broad-host range vector	[1]
pTae	EML1616	<i>tae</i> in pRL662, Gm ^R	[4]
pTai	EML4228	<i>tai</i> in pTrc200, Sp ^R	[5]
pG1 ⁸¹²	EML4567	<i>vgrG1</i> encoding VgrG1 variants with amino acid residue 813 to 816 deleted in pRL662, Gm ^R	[6]
pG1 ⁸⁰⁴	EML4568	<i>vgrG1</i> encoding VgrG1 variants with amino acid residue 805 to 816 deleted was cloned into pRL662, Gm ^R	[6]
pG1 ⁷⁸⁵	EML4599	<i>vgrG1</i> encoding VgrG1 variants with amino acid residue 786 to 816 deleted was cloned into pRL662, Gm ^R	[6]
pG1 ⁷⁸¹	EML4598	<i>vgrG1</i> encoding VgrG1 variants with amino acid residue 782 to 816 deleted was cloned into pRL662, Gm ^R	[6]
pJQ200KS- <i>tssB</i>	EML949	<i>tssB</i> -flanking sequences in pJQ200KS for generating in-frame deletion mutant, Gm ^R	[5]

pJQ200KS- <i>vgrG1</i>	EML954	<i>vgrG1</i> -flanking sequences in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	[5]
pJQ200KS- <i>vgrG2</i> operon	EML2689	<i>vgrG2</i> operon-flanking sequences in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	[5]
pJQ200KS- <i>G1_785</i>		<i>vgrG1</i> -flanking sequences in pJQ200KS plasmid to generate truncated VgrG1_785 mutant, Gm ^R	This study
pJQ200KS- <i>G1_781</i>		<i>vgrG1</i> -flanking sequences in pJQ200KS plasmid to generate truncated VgrG1_781 mutant, Gm ^R	This study
pJQ200KS- <i>tae-tai*</i>		<i>tae-tai</i> -flanking sequences in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study
pJQ200KS- <i>v1</i> (12D1)	EML4670	<i>v1</i> -flanking sequences from 12D1 in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study
pJQ200KS- <i>v3-4</i> (12D1)	EML4671	<i>v3-4</i> -flanking sequences from 12D1 in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study
pJQ200KS- <i>tae-tai</i> (12D1)		<i>tae-tai</i> -flanking sequences from 12D1 in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study
pJQ200KS- <i>v4</i> (1D1108)	EML4686	<i>v4</i> -flanking sequences from 1D1108 in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study

pJQ200KS- <i>v6</i> (1D1108)	EML4687	<i>v6</i> -flanking sequences from 1D1108 in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study
pJQ200KS- <i>vI-9</i> (1D1108)	EML4645	<i>vI-9</i> -flanking sequences from 1D1108 in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study
pJQ200KS- <i>vI-7</i> (15955)	EML4644	<i>vI-7</i> -flanking sequences from 15955 in pJQ200KS plasmid to generate in-frame deletion mutant, Gm ^R	This study
pDONR222		Gateway cloning Donor vector, Km ^R	Invitrogen
pRL662_RfC.1		Gateway reading frame cassette C.1 (RfC.1) cloned in pRL662, Gm ^R	[9]
pTrc200_RfC.1		Gateway reading frame cassette C.1 (RfC.1) cloned in pTrc200, Sp ^R	[9]
pTdei2		<i>tde2</i> and <i>tdi2</i> were cloned into pTrc200, Sp ^R	This study
pV1 (12D1)		<i>vI</i> from 12D1 was cloned into pRL662, Gm ^R	This study
pV3-4 (12D1)		<i>v3-4</i> from 12D1 was cloned into pRL662, Gm ^R	This study
pV4 (1D1108)		<i>v4</i> from 1D1108 was cloned into pRL662, Gm ^R	This study
pV6 (1D1108)		<i>v6</i> from 1D1108 was cloned into pRL662, Gm ^R	This study

Appendix Table S2. Primers used in this study

Name	Sequence (5' to 3') ^a	Related construct(s)
<i>tssB</i> _XmaI_F	<u>CCCGGG</u> AAACCCGGCGGA GACAATAA	pTrc-TssB-GFP
<i>GFP</i> _HindIII_R	<u>AAGCTT</u> CTATTTGTATAGTTC ATCCATGCCA	pTrc-TssB-GFP
<i>tssB-GFP</i> _F	AAAAGAACGGCGCGAGCGAA ATGAGTAAAGGAGAAGAACT TTT	pTrc-TssB-GFP
<i>tssB-GFP</i> _R	AAAAGTTCTTCTCCTTTACTC ATTTTCGCTCGCGCCGTTCTTTT	pTrc-TssB-GFP
<i>tssB</i> _F	TTGGATACACAGCATGTAA	$\Delta tssL\Delta tssB$, $\Delta tdei\Delta tssB$
<i>tssB</i> _R	GTTTTCTCCTCCGCACAGGCGA	$\Delta tssL\Delta tssB$, $\Delta tdei\Delta tssB$
<i>G1</i> _F	ATGCGCGTTAACTTTGACAC	$\Delta tdei\Delta vgrG1$
<i>G1</i> _R	ATTATGGGTGTGTCGTTTCAT	$\Delta tdei\Delta vgrG1$
<i>G2op</i> _F	GAACAGCCTGACAATCCTGT	$\Delta tdei\Delta vgrG1\Delta vgr$ G2 operon
<i>G2op</i> _R	CAGTGCCTGATAGACGTTGT	$\Delta tdei\Delta vgrG1\Delta vgr$ G2 operon
<i>XbaI</i> _G1_F	ATATATTCTAGAGACACCCTC TATAGCAATTA	<i>vgrG1</i> _785 $\Delta vgrG2$ operon
<i>BamHI</i> _G1_R	ATATATGGATCCAGGTCCTAA AAACCGG	<i>vgrG1</i> _785 $\Delta vgrG2$ operon

785_F	CCTGAAGATCGATATCGAA TGATTATGAACGACACACC	<i>vgrG1_785</i> Δ <i>vgrG2</i> operon
785_R	GGTGTGTCGTTTCATAATCATT CGATATCGATCTTCAGG	<i>vgrG1_785</i> Δ <i>vgrG2</i> operon
781_F	GCTCTTTGCCCTGAAG TGATTATGAACGACACACC	<i>vgrG1_781</i> Δ <i>vgrG2</i> operon
781_R	GGTGTGTCGTTTCATAATCACT TCAGGGCAAAGAGC	<i>vgrG1_781</i> Δ <i>vgrG2</i> operon
<i>tai</i> _Up_F	<u>ATCGAATTCCTGCAGACATCG</u> CAAGTGGATGGATATT	pJQ200KS- <i>tae-tai</i> (new)
<i>tai</i> _Up_R	ATATAGGGAGCGCATGAAA	pJQ200KS- <i>tae-tai</i> (new)
<i>tae</i> _Down_F	ATGCGCTCCCTATATGCCTGC TACTACAATTCG	pJQ200KS- <i>tae-tai</i> (new)
<i>tae</i> _Down_F	<u>AGAACTAGTGGATCCCGAAA</u> CGGCGAGTGAGATAA	pJQ200KS- <i>tae-tai</i> (new)
Tde2_F	CAAAAAAGCAGGCTCCTGCA AGGGAGACAGCATGAGT	pTdei2

Tdi2_R	GAAAGCTGGGTGTCACCTCGC CGAACCGATTTTCCT	pTdei2
V1_12D1_F	CAAAAAAGCAGGCTCCGAAG TAGGGGCCGACGTGGAA	pV1 (12D1)
V1_12D1_R	GAAAGCTGGGTGTCATGACG ATATCCTCTCAACTGC	pV1 (12D1)
V3_12D1_EcoRI_F	ATCGAATTCCTGCAGATAGAG GAGGAGTTCGAGC	pV3-4 (12D1)
V4_12D1_BamHI_R	AGAACTAGTGGATCCGCATTT ATATCTCATCGACC	pV3-4 (12D1)
V4_1D1108_F	CAAAAAAGCAGGCTCCTCTG AATTGACCAGTATGTGG	pV4 (1D1108)
V4_1D1108_R	GAAAGCTGGGTGTCATGAGA CCACCCCAGCCATTTG	pV4 (1D1108)
V6_1D1108_F	CAAAAAAGCAGGCTCCCGGT GGGATAGACGCATGAAG	pV6 (1D1108)
V6_1D1108_R	GAAAGCTGGGTGCTACCAGCT TGGTATCGGAG	pV6 (1D1108)
V1_12D1_Up_F	GCGTCTAGATTGGTGCTGGCG GTATTTC	pJQ200KS- <i>vl</i> (12D1)
V1_12D1_Up_R	CTAGGATCCGCTGTTGAGTTC CACGTCG	pJQ200KS- <i>vl</i> (12D1)
V1_12D1_Down_F	TCAGGATCCGAGAGGATATC GTCATGATC	pJQ200KS- <i>vl</i> (12D1)
V1_12D1_Down_R	AAGCCCGGGTCGAACTCCTCC TCTATGTCG	pJQ200KS- <i>vl</i> (12D1)

V3-4_12D1_Up_F	GAGTCTAGAGATGAATGCGA TGTTGCACG	pJQ200KS-v3-4 (12D1)
V3-4_12D1_Up_R	GAGGGATCCGAGGTGCTGGC TCATTGAAC	pJQ200KS-v3-4 (12D1)
V3-4_12D1_Down_F	GAAGGATCCAAGGTCGATGA GATATAAATGC	pJQ200KS-v3-4 (12D1)
V3-4_12D1_Down_R	CTACCCGGGCTCTATCTCAGG AAAGAAGG	pJQ200KS-v3-4 (12D1)
<i>tai</i> _12D1_Up_F	ATCGAATTCCTGCAGGAGCTT ACAGCATGCCAATTTAT	pJQ200KS- <i>tae-tai</i> (12D1)
<i>tai</i> _12D1_Up_R	CGCATATAGGGAGCGCAT	pJQ200KS- <i>tae-tai</i> (12D1)
<i>tae</i> _12D1_Down_F	<u>CGCTCCCTATATGCGGCCTGC</u> <u>TACTACAATTCG</u>	pJQ200KS- <i>tae-tai</i> (12D1)
<i>tae</i> _12D1_Down_R	AGAACTAGTGGATCCCGTGGC TGAACCAGTAGAA	pJQ200KS- <i>tae-tai</i> (12D1)
V4_1D1108_Up_F	GAATCTAGAAGTCGGTGACGT GAATAAGG	pJQ200KS-v4 (1D1108)
V4_1D1108_Up_R	TAAGGATCCGCTGACAGCCCA CATACTGG	pJQ200KS-v4 (1D1108)
V4_1D1108_Down_F	TAAGGATCCGCTGGGGTGGTC TCATGA	pJQ200KS-v4 (1D1108)
V4_1D1108_Down_R	GATCCCGGGTGTCAACGACCG CAACAAGG	pJQ200KS-v4 (1D1108)
V6_1D1108_Up_F	AGTTCTAGATGCCTATCACAG TGAAGTGC	pJQ200KS-v6 (1D1108)

V6_1D1108_Up_R	TGT <u>GGATCC</u> GATTGTTTCCTT CATGCGTC	pJQ200KS-v6 (1D1108)
V6_1D1108_Down_F	TAAGGATCCCCGATACCAAGC TGGTAGG	pJQ200KS-v6 (1D1108)
V6_1D1108_Down_R	GAT <u>CCCGGG</u> TCGTCTGGTACA TCAGGTGC	pJQ200KS-v6 (1D1108)
V1-9_1D1108_Up_F	ACCATCTAGAGATAAATCGAC GGAAGTTGG	pJQ200KS-v1-9 (1D1108)
V1-9_1D1108_Up_R	GAGTGGATCCTGCTTCTCAAT TCAAATCTATC	pJQ200KS-v1-9 (1D1108)
V1-9_1D1108_Down_F	CTGAGGATCCGACCCCACTGA GGACTGATC	pJQ200KS-v1-9 (1D1108)
V1-9_1D1108_Down_R	AGCCCGGGAGAAAGCCAACC TCTGCACCC	pJQ200KS-v1-9 (1D1108)
V1-7_15955_Up_F	GACGATCTAGACGAAACAAT CACAGCGAACG	pJQ200KS-v1-7 (15955)
V1-7_15955_Up_R	CGGGATCCTGCTTCTCAATTC AAATCTATC	pJQ200KS-v1-7 (15955)
V1-7_15955_Down_F	CGGGATCCGACCCCACTGAG GACTGATC	pJQ200KS-v1-7 (15955)
V1-7_15955_Down_R	TCCCCCGGGCAACATGGATC ACGTCAAAC	pJQ200KS-v1-7 (15955)

^aRestriction enzyme sites are underlined.

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