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Appendix Table S1. List of strains and plasmids

Strain/plasmid Name	Relevant Characteristics	Source/Reference	EML No
A. tumefaciens			
C58	Wild type, virulent strain containing nopaline-type Ti plasmid pTiC58	Eugene Nester	EML530
$\Delta tssL$	<i>tssL</i> deletion mutant	(Ma <i>et al</i> , 2009)	EML1073
$\Delta tde1$	Deletion of <i>atu4350</i>		
$\Delta tdei$	Deletion of <i>tde1-tdi1</i> (<i>atu4350-atu4351</i>) and <i>tde2-tdi2</i> (<i>atu3640-atu3639</i>) toxin immunity pairs	(Ma <i>et al</i> , 2014)	EML3559
$\Delta tdei\Delta tssK$	Deletion of <i>tssK</i> in $\Delta tdei$	This Study	EML5136
E. coli			
DH10B	F- <i>araDJ39</i> Δ <i>ara</i> , <i>leu</i>)7697 Δ <i>lacX74 galU galK rpsL deoR</i> ϕ 80 <i>dlacZ</i> Δ M15 <i>endAI nupG recAI mcrA</i> Δ (<i>mrr hsdRMS mcrBC</i>)	Invitrogen	EML5396
BW25113	<i>rrnB</i> Δ <i>lacZ</i> 4787 <i>hsdR</i> 514 Δ (<i>araBAD</i>)567 <i>DE</i> (<i>rhaBAD</i>)568 <i>rph-1</i> (ME9062)	NBRP, Japan	EML5398
BW25113 (Δ <i>lacY</i>)	<i>lacY</i> deletion mutant of the BW25113	NBRP, Japan	EML5470
Plasmids			
pTrc200	Sm ^R , Sp ^R , pVS1 origin <i>lacIq</i> , <i>trc</i> promoter expression vector	(Schmidt-Eisenlohr <i>et al</i> , 1999)	EML5481
pRL662	Gm ^R , non-conjugal transferable broad host range vector derived from pBBR1MCS-2,	(Vergunst <i>et al</i> , 2000)	EML5452
pRL662- <i>tdi1</i> -Strep	Gm ^R , constitutive expression of Tdi1-Strep by <i>lac</i> promoter on the pRL662 plasmid	(Ma et al., 2014)	EML3398
pTrc- <i>tde1</i> (M)-HA	Sp ^R , C-terminal HA-tagged Tde1(M) driven by <i>trc</i> promoter on pTrc200	This Study	EML5445
pTrc-N- <i>tde1</i> -HA	Sp ^R , N-terminus of Tde1 (1-49) with C-terminal HA tag driven by <i>trc</i> promoter on pTrc200	This Study	EML5482
pTrc-N- <i>tde1</i> ^{GLGL} -HA	Sp ^R , N-terminus of Tde1 (1-49) with amino acid substitutions (G39L, G43L) and HA tag on C-terminus driven by <i>trc</i> promoter on pTrc200	This Study	EML5464
pTrc- <i>tde1</i> (M) ^{GLGL} -HA	Sp ^R , C-terminal HA-tagged Tde1(M) with amino acid substitutions (G39L, G43L) driven by <i>trc</i> promoter on pTrc200	This study	EML5465
pTrc-C1- <i>tde1</i> (M)-HA	Sp ^R , C-terminus of Tde1(M) (49-278) with C-terminal HA tag driven by <i>trc</i> promoter on pTrc200.	This study	EML5479
pTrc-C2- <i>tde1</i> (WT)-HA	Sp ^R , C-terminus of Tde1 (98-278) with C-terminal HA tag driven by <i>trc</i> promoter on pTrc200.	This Study	EML5478
pYTA- <i>lacZ</i>	Amp ^R , full length <i>lacZ</i> gene was cloned on to the YTA vector by TA cloning	This Study	EML1661

pBBRMCS2- <i>mCherry</i>	Km ^R , full length <i>mCherry</i> gene was cloned on to pBBRMCS2 plasmid	This Study	EML3022
pTrc200- <i>lacY</i>	Sp ^R , <i>lacY</i> from BW25113 with C-terminal HA tag driven by <i>trc</i> promoter on pTrc200 plasmid.	This Study	EML6350
pTrc- <i>N-tde1-sfGFP</i>	Sp ^R , N-terminus of Tde1 (1-49) fused to the sfGFP on the C-terminal with a GGS linker driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5489
pTrc- <i>N-tde1^{GLGL}-sfGFP</i>	Sp ^R , N-terminus of Tde1 (1-49) with point mutation (G39L, G43L) fused to the sfGFP on the C-terminal with a GGS linker driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5490
pTrc- <i>tde1(M)-sfGFP</i>	Sp ^R , full length of Tde1(M) fused to the sfGFP on the C-terminal with a GGS linker driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5493
pTrc- <i>tde1(M)^{GLGL}-sfGFP</i>	Sp ^R , full length of Tde1(M) with point mutation on (G39L, G43L) fused to the sfGFP on the C-terminal with a GGS linker driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5494
pTrc- <i>C1-tde1(M)-HA</i>	Sp ^R , C-terminus of Tde1 (49-278) fused to the sfGFP on the C-terminal with a GGS linker driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5492
pTrc- <i>C2-tde1(WT)-HA</i>	Sp ^R , C-terminus of Tde1 (98-278) fused to the sfGFP on the C-terminal with a GGS linker driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5491
pTrc200- <i>sfGFP</i>	Sp ^R , sfGFP driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5495
pTrc- <i>tde1-tdi1</i>	Sp ^R , expressopm of Tde1-Tdi1 driven by <i>trc</i> promoter on pTrc200 plasmid	(Wu et al., 2020)	EML4277
pTrc- <i>tde1^{GLGL}-tdi1</i>	Sp ^R , expression of Tde1-Tdi1 driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML5513
pTrc- <i>tde1^{G39L}-tdi1</i>	Sp ^R , expression Tde1-Tdi1 with point mutation on (G39L) driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML6358
pTrc- <i>tde1^{G43L}-tdi1</i>	Sp ^R , expression Tde1-Tdi1 with point mutation on (G43L) driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML6359
pTrc- <i>tde1(M)^{GLGL}-tdi1</i>	Sp ^R , expression Tde1-Tdi1 with point mutation on (G43L) driven by <i>trc</i> promoter on pTrc200 plasmid	This Study	EML6360
pJN105	Gm ^R , arabinose-inducible gene expression vector derived from pBBRMCS-1, araC-P _{BAD}	(Newman & Fuqua, 1999)	EML4220
pJN105- <i>tde1</i>	Gm ^R , pJN105 expressing C-terminal 6xHis tagged Tde1	This study	EML6331
pJN105- <i>tde1^{G39L}</i>	Gm ^R , pJN105 expressing C-terminal 6xHis tagged Tde1 with the substitution of G39L	This study	EML3211

pJN105- <i>tde1</i> ^{G43L}	Gm ^R , pJN105 expressing C-terminal 6xHis tagged Tde1 with the substitution of G43L	This study	EML3212
pJN105- <i>tde1</i> ^{GLGL}	Gm ^R , pJN105 expressing C-terminal 6xHis tagged Tde1 with the substitutions of G39L and G43L	This study	EML6337
pJN105- <i>tde1</i> (M)	Gm ^R , pJN105 expressing C-terminal 6xHis tagged Tde1 with the substitutions of H190A and D193A	This study	EML4289

Appendix Table S2. List of primers and plasmids

Name	Sequence (5' to 3')	Related construct(s)
NcoI_sfGFP_F HindIII_sfGFP_R	TGGACCCATGGACCGTAAAGGCGAAGAGCTGTTCA TCGACAAGCTTTTTGTACAGTTCATCCA	pTrc200_sfGfp
NCoI_tde1_F	AAAAACCATGGACATGAGTGCGACGACAACCTGTCG	pTrc200_tde1(M)-HA pTrc200_N-tde1-HA pTrc200_N-tde1 ^{GLGL} -HA pTrc200_tde1 ^{G39L} -Tdi1 pTrc200_N-tde1-sfgf pTrc200_tde1(M)-sfgf pTrc200_tde1(M) ^{GLGL} -sfgf
XbaI-tde1_R	AAAAATCTAGACACCGGGACGTCAGGATTGCC	pTrc200_tde1(M)-HA pTrc200_C2-tde1(M)-HA pTrc200_C1-tde1(M)-HA pTrc200_tde1(M) ^{GLGL} -HA
XbaI_N-tde1_R	AAAAATCTAGA A GCT CCC TCA GGC GGA CTA AAG CAA T	pTrc200_N-tde1-HA pTrc200_N-tde1 ^{GLGL} -HA
NCoI_C2-tde_F	AAAAACCATGGACACGCGCTAAGCAAGTTGAAGAGT TTAGG	pTrc200_C2-tde1(M)-HA
NCoI_C2-tde_R	AAAAACCATGGACGCGGGTAGAGCGGCTGCCTCC GCAA	pTrc200_C2-tde1(M)-HA
NcoI_C1_tde1_F	AAAAACCATGGACGCGGGTAGAGCGGCTGCCTCC GCAA	pTrc200_C1-tde1(M)-HA
G39L_G43L_F G39L_G43L_R	TGGAGGTACAGCGGTTTTAGCATGGATACTTGGGC AAGTCGGCGCTAT ATAGCGCCGACTTGCCCAAGTATCCATGCTAAAC CGCTGTACCTCCAG	pTrc200_N-tde1 ^{GLGL} -HA pTrc200_tde1(M) ^{GLGL} -HA pTrc200_tde1(M) ^{GLGL} -tdi1
NcoI_lacY_F XbaI_lacY_R	AAAAACCATGGACATGTACTATTTAAAAAACACAAA C AAAAATCTAGAAGCGACTTCATTACCTGACGAC	pTrc200_lacY-HA
N-tde1_GGGS _sfGFP_F sfGFP _GGGS_N- tde1_R	TTAGTCCGCCTGAGGGAGCTTCTAGAGGTGGTGGT TCTCGTAAAGGCGAAGAGCTGTT ACAGCTCTTCGCCTTTACGAGAACCACCACCTCTA GAAGCTCCCTCAGGCGGACTAA	pTrc200_N-tde1-sfGFP
Hind III_sfGFP_R	AAA AAA AGC TTT TAT TTG TAC AGT TCA TCC ATA C	pTrc200_N-tde1-sfgf pTrc200_N-tde1 ^{GLGL} -sfgf pTrc200_tde1(M)-sfgf pTrc200_tde1(M) ^{GLGL} -sfgfp pTrc200_C1-tde1(M)-sfgfp
pET-rbs-F1-XbaI pET-R1-SacI	CCCCTCTAGAAATAATTTTGTTTAAC GGAAGAGCTCCCTTTGCGGGCTTTGTTAG	pJN105_tde1
Tde1_G39L_F Tde1_G39L_R	ACAGCGGTTCTCGCATGGATAGGTGGGCAATC GACTTGCCACCTATCCATGCGAGAACCGCTGT	pTrc-tde1 ^{G39L} -tdi1 pJN105_tde1 ^{G39L}
Tde1_G43L_F Tde1_G43L_R	ACAGCGGTTGGAGCATGGATACTCGGG CAAGTC GACTTGCCCGAGTATCCATGCTCCAACCGCTGT	pTrc-tde1 ^{G43L} -tdi1 pJN105_tde1 ^{G43L}

Appendix References

Ma LS, Hachani A, Lin JS, Filloux A, Lai EM (2014) *Agrobacterium tumefaciens* deploys a superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta. *Cell Host Microbe* 16: 94-104

Ma LS, Lin JS, Lai EM (2009) An IcmF family protein, ImpLM, is an integral inner membrane protein interacting with ImpKL, and its Walker A motif is required for type VI secretion system-mediated Hcp secretion in *Agrobacterium tumefaciens*. *J Bacteriol* 191: 4316-4329

Newman JR, Fuqua C (1999) Broad-host-range expression vectors that carry the L-arabinose-inducible *Escherichia coli* araBAD promoter and the araC regulator. *Gene* 227: 197-203

Schmidt-Eisenlohr H, Domke N, Baron C (1999) TraC of IncN plasmid pKM101 associates with membranes and extracellular high-molecular-weight structures in *Escherichia coli*. *Journal of Bacteriology* 181: 5563-5571

Vergunst AC, Schrammeijer B, den Dulk-Ras A, de Vlaam CM, Regensburg-Tuink TJ, Hooykaas PJ (2000) VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. *Science* 290: 979-982