Identification of Tse8 as a Type VI secretion system toxin from *Pseudomonas aeruginosa* that targets the bacterial transamidosome to inhibit protein synthesis in prey cells

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Supplementary Tables

Legend for Supplementary Table 1

Supplementary Table 1. Contains the full TraDIS data set (Tab 1), data above our threshold cut-off (-logFC >2 q<0.05) (Tab 2) and data from Tab 2 limited to genes <600 bp (Tab 3). See 'Generation of TraDIS sequencing libraries, sequencing and downstream analysis' in Methods for details on analyses. P values were corrected for multiple testing using the Benjamini-Hochberg method, and genes with a corrected P value (Q value) of <0.05 (5% false discovery rate) and an absolute log2 fold change (log2FC) of >2 were considered significant.

Supplementary Table 2. Strains and plasmids used in the current study.

Stain or plasmid	Relevant characteristics	Reference/source
Strain		
Escherichia coli		
Dh5α	F - *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80dlacZ* ΔM15 Δ(*lacZYA-argF*)U169, hsdR17 (r K–mK+), λ-	Invitrogen
CC118λ*pir*	Host strain for pKNG101 replication; Δ(*ara leu) araD ΔlacX 74 galE galK -phoA 20 thi-1 rpsE rpoB argE (Am) recA 1 Rfr* λpir	Laboratory collection
SM10	Host strain for pBT20 and replication; *thi-1 thr leu tonA lacY supE recA* ::RP4-2-Tc::Mu λpir , KmR	[5]
BL21 (DE3)	B F^- *ompT gal dcm lon hsdS*$_B$($r_B^-m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K-12}$(λ^S)	Invitrogen
B834	F- *ompT hsdS*B(rB- mB-) *gal dcm met* (DE3)	Invitrogen
K12	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	Laboratory collection
DHM1	*cya-854 recA1 gyrA96 (NaI) thi1 hsdR17 spoT1 rfbD1 glnV44(AS)*	[6]
Agrobacterium tumefaciens		
C58	Wild type virulent strain containing nopaline-type Ti plasmid pTiC58	Eugene Nester
Pseudomonas aeruginosa		
PAK	Wild type	Filloux laboratory strain
PAKΔ*retS*	*retS* deletion mutant	[7]
PAKΔ*retS*Δ*vgrG1a*	*retS* and *vgrG1a* deletion mutant	[7]
PAKΔ*retS*Δ*vgrG1c*	*retS* and *vgrG1c* deletion mutant	[7]
PAKΔ*retS*Δ*vgrG1a*Δ*vgrG1c*	*retS, vgrG1a* and *vgrG1c* deletion mutant	[7]

PAKΔ*retS*ΔH1	*retS* and H1-T6SS cluster (encompassing the PAK genes corresponding to PA0070-PA0095) are deleted	[7]
PAKΔ*retS*ΔH1-H3	*retS*, H1-T6SS cluster (encompassing the PAK genes corresponding to PA0070-PA0095), H2-T6SS cluster (encompassing the PAK genes corresponding to mid PA1657 to PA1662) and H3-T6SS cluster (encompassing the PAK genes corresponding to PA2357 to PA2377) are deleted	Filloux laboratory strain
PAKΔ*retS*ΔH2ΔH3	*retS*, H2-T6SS cluster (encompassing the PAK genes corresponding to mid PA1657 to PA1662) and H3-T6SS cluster (encompassing the PAK genes corresponding to PA2357 to PA2377) are deleted	This study
PAKΔ*retS*Δ*tsei8*	*retS* as well as PA4163 to PA4164 are deleted	This study
PAKΔ*retS*Δ*tse8*	*retS* as well as PA4163 (*tse8*) are deleted	This study
PAKΔ*retS*Δ*tsei8*::*lacZ*	*retS* as well PA4163 to PA4164 are deleted with the *lacZ* gene from miniCTX-lac inserted at the vacant *att* site on the chromosome	This study
PAKΔ*retS*::*tse8S186A*	*retS* deletion mutant with *tse8* (native locus) having S186A substitution	This study
PAKΔ*retS*ΔH1::*tse8S186A*	*retS* and H1-T6SS deletion mutant with *tse8* (native locus) having S186A substitution	This study
Plasmid		
pCR-BluntII-TOPO Blunt	Cloning vector, Zeo^R/Km^R	Invitrogen
pKNG101	Suicide vector, *sacB*, Str^R	[8]
pKNG101:*tsei8* mutator	Mutator construct for deletion of *tsei8* by allelic exchange	This study
pKNG101:*tseS186A* mutator	Mutator construct for generating *tse8S186A* at native site on chromosome by allelic exchange	This study
pKNG101:H2-T6SS	Mutator construct for deletion of H2-T6SS by allelic exchange	Filloux laboratory collection

pKNG101:H3-T6SS	Mutator construct for deletion of H3-T6SS by allelic exchange	Filloux laboratory collection
pRK2013	Tra+, Mob+, Km^R	[9]
pUX-BF13	OriR6K helper plasmid, mob/oriT, provides Tn7 transposition function in trans, Amp^R	[10]
pBT20	For mariner transposon mutagenesis, Gm^R/Amp^R	[11]
pET-41a-3CD	Vector derived from pET-41a by Novagen produces fusion protein with N-terminal GST-His-S tag, Km^R	[12]
pE221	Coding region of leaderless *E. coli* CcmE (S32-S163) with a C-terminal polyhistidine tag, pET22b, ApR	[13]
pET28a	Expression vector, Km^R	Novagen
pET28a:*tsi8*	Coding region of *tsi8* in frame with N-terminal 6xHis)	This study
pET28a:*gatA*-V5	Coding region of *gatA* with C-terminal V5 tag (out of frame with 6xHis)	This study
pET41a:*gatA*-V5	Coding region of *gatA* with C-terminal V5 tag (out of frame with 6xHis)	This study
pET41a:*tse8*-HA	Coding region of *tse8* with C-terminal HA tag (out of frame with 6xHis)	This study
pET41a:*tse8*-HA-Strep	Coding region of *tse8* with C-terminal HA-Strep tag (out of frame with 6xHis)	This study
pET28a:*gatC*-HA	Coding region of *gatC* with C-terminal HA tag (out of frame with 6xHis)	This study
pET41a-3CD-TEV	pET41a as above but with a TEV site introduced to allow clevage of N-terminal tags	This study
pET41a-3CD-TEV:*tse8*	pET41a with coding region of *tse8* in frame for (cleavable) N-terminal GST-His-S tag	This study
pACYCduet1	Duel expression vector, CmR	Novagen

pACYCduet1-His-*gatB*	Coding region of *gatB* with N-terminal His tag into MCS1	This study
pMMB67HE	Expression vector, Ap^R	Filloux laboratory collection
pMMB67:*tse8*	Coding region of *tse8* with no tag	This study
pMMB67:*tse8*-HA	Coding region of *tse8* with C-terminal HA tag	This study
pMMB67:*vgrG1a*-V5	Coding region of *vgrG1a* (PA0091) with C-terminal V5 tag	This study
pMMB67:*vgrG1b*-V5	Coding region of *vgrG1b* (PA0095) with C-terminal V5 tag	This study
pMMB67:*vgrG1c*-V5	Coding region of *vgrG1c* (PA2685) with C-terminal V5 tag	This study
pBBR1MCS5	Expression vector, Gm^R	[14]
pBBR1:*tsei8*	Coding region of *tsei8* with 500 bp upstream region to include native promoter	This study
pBBR1MCS4	Expression vector, Amp^R	[14]
pBBR1:*tse8*	Coding region of *tse8* (untagged) in constitutively expressed plasmid	This study
miniCTX:*sfgfp*	sfGfp under control of constitutive *pX2* promoter with integration at vacant Tn*7* site on the chromosome	Knut Drescher lab collection
pJN105	Expression vector, Gm^R	[15]
pJN:*tsi8*-V5	Coding region of *tsi8* with C-terminal V5 tag	This study
pJN:*asnS*-His	Coding region of *asnS* from *E. coli* with C-terminal His tag	This study
pTrc200	Sm^R, Sp^R, pVS1 origin $lacI^q$, *trc* promoter expression vector	[16]
pTrC:*tse8*-HA	Coding region of *tse8* with C-terminal HA tag	This study
pUT18c	Vector for Bacterial Two-Hybrid assay	[6]

pKT25	Vector for Bacterial Two-Hybrid assay	[6]
pUT18c-Zip	N-terminal T18 fusion on leucine zipper of GCN4	[6]
pKT25-Zip	N-terminal T25 fusion on leucine zipper of GCN4	[6]
pUT18c-*tse8*	N-terminal T18 fusion to coding region of *tse8*	This study
pKT25-*tse8*	N-terminal T25 fusion to coding region of *tse8*	This study
pUT18c-*tsi8*-V5	N-terminal T18 fusion to coding region of *tsi8* with C-terminal V5 tag	This study
pKT25-*tsi8*-V5	N-terminal T25 fusion to coding region of *tsi8* with C-terminal V5 tag	This study
pUT18c-*vgrG1a*	N-terminal T18 fusion to coding region of *vgrG1a*	This study
pKT25c-*vgrG1a*	N-terminal T25 fusion to coding region of *vgrG1a*	This study
pUT18c-*vgrG1b*	N-terminal T18 fusion to coding region of *vgrG1b*	This study
pKT25c-*vgrG1b*	N-terminal T25 fusion to coding region of *vgrG1b*	This study
pUT18c-*vgrG1c*	N-terminal T18 fusion to coding region of *vgrG1c*	This study
pKT25c-*vgrG1c*	N-terminal T25 fusion to coding region of *vgrG1c*	This study

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Supplementary Table 3. Primers used in the current study.

Primer list	**Name**	**Oligonucleotide sequence (5'-3')**	**Description**
OAL2939	Primer 1 *tsei8* left F	GGCATCCACGGCGCTTTCCG	Generate deletion mutant of *tsei8* primer 1
OAL2940	Primer 2 *tsei8* left R	TCAGTCGCGCTCGATCATGCTGTCACC	Generate deletion mutant of *tsei8* primer 2
OAL2941	Primer 3 *tsei8* right F	ATGATCGAGCGCGACTGAGCGCTT	Generate deletion mutant of *tsei8* primer 3
OAL2942	Primer 4 *tsei8* right R	GCTCTACATCGGCACGTTCACC	Generate deletion mutant of *tsei8* primer 4
OAL2943	Primer 5 upstream *tsei8*	GCGTTGACCGTGATGCCCAG	Generate deletion mutant of *tsei8* primer 5
OAL2944	Primer 6 downstream *tsei8*	GAGGACCCGGCCTACTACGG	Generate deletion mutant of *tsei8* primer 6
OAL2939	Primer 1 *tse8* left F	GGCATCCACGGCGCTTTCCG	Generate deletion mutant of *tse8* primer 1
OAL4770	Primer 2 *tse8* left R	TCACTTGCTCTCGATCATGCTGTCACC	Generate deletion mutant of *tse8* primer 2
OAL4771	Primer 3 *tse8* right F	ATGATCGAGAGCAAGTGAAAGGCGCGG	Generate deletion mutant of *tse8* primer 3
OAL4772	Primer 4 *tse8* right R	GCTTCGCCGCCTACACCACGG	Generate deletion mutant of *tse8* primer 4
OAL2943	Primer 5 upstream *tse8*	GCGTTGACCGTGATGCCCAG	Generate deletion mutant of *tse8* primer 5

OAL4773	Primer 6 downstream *tse8*	GCGAGCGCCTGGGATTCC	Generate deletion mutant of *tse8* primer 6
OAL996	Primer 1 H2-T6SS left F	GACTGGTTGAAAATCCTGGAAAAC	Generate deletion mutant in H2-T6SS primer 1
OAL997	Primer 2 H2-T6SS left F	TCAGGCGAACGGCCTCCTGCTGGGCGC	Generate deletion mutant in H2-T6SS primer 2
OAL998	Primer 3 H2-T6SS left F	AGGAGGCCGTTCGCCTGAGGTGGGTGC	Generate deletion mutant in H2-T6SS primer 3
OAL999	Primer 4 H2-T6SS left F	CAACACGGTATAGGGGTTGTG	Generate deletion mutant in H2-T6SS primer 4
OAL1000	Primer 5 H2-T6SS left F	GAATTGTTAAGATATTCATTGGCGCAC	Generate deletion mutant in H2-T6SS primer 5
OAL1001	Primer 6 H2-T6SS left F	TCGAGCAGCAGGGTTCCGCCATCCGCG	Generate deletion mutant in H2-T6SS primer 6
OAL1002	Primer 1 H3-T6SS left F	ATTTCCGACATATGGTGAAACATC	Generate deletion mutant in H3-T6SS primer 1
OAL1003	Primer 2 H3-T6SS left F	TGCTGATCAGAAGCGCAGCTCGACGTT	Generate deletion mutant in H3-T6SS primer 2
OAL1004	Primer 3 H3-T6SS left F	CTGCGCTTCTGATCAGCATCAACCTCT	Generate deletion mutant in H3-T6SS primer 3

OAL1005	Primer 4 H3-T6SS left F	GTGAATGGCACGAATAAATAGTTCATA	Generate deletion mutant in H3-T6SS primer 4
OAL1006	Primer 5 H3-T6SS left F	AACTGCTGCCGGTAGTCGCGGCGGTAC	Generate deletion mutant in H3-T6SS primer 5
OAL1007	Primer 6 H3-T6SS left F	CACCCTTTCCAGTAGTCGCACATCAGC	Generate deletion mutant in H3-T6SS primer 6
OAL3095	*tsei8*comp_F	GGATCCCAGCGTCTCGGCGGTTTG	Generate complementation construct of *tsei8* with native promoter (500 bp upstream of *tse8*) (BamHI site)
OAL3096	*tsei8*comp_R	AAGCTTTCAGTCGCGCAGGGCGTA	Generate complementation construct of *tsei8* (HindIII site)
OAL3099	*tse8*comp_F	CCGGCGGATCCTAACAGGAGGAATTAACCATGATCGAGGTCACCGAGGTT	Generate complementation of *tse8* with C-terminal V5 tag (BamHI site)
OAL3100	*tse8*comp_R	CCGGGCTCGAGTCACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCCTTGCTTGCCAGCGGTGG	Generate complementation of *tse8* with C-terminal V5 tag (XhoI site)
OAL3792	*tse8*_BTH_F	GAGCTCTCACTTGCTTGCCAGCGG	Generate *tse8* with N-terminal T18/T25 fusion in BTH vector (BamHI site)

OAL3792	*tse8*_BTH_R	GAGCTCTCACTTGCTTGCCAGCGG	Generate *tse8* with N-terminal T18/T25 fusion in BTH vector (SacI site)
OAL4774	*tse8* no tag_F	TCTAGAATGATCGAGGTCACCGAGGTT	Generate *tse8* with no tag (XbaI site)
OAL4775	*tse8* no tag_R	GAGCTCTCACTTGCTTGCCAGCGGT	Generate *tse8* with no tag (SacI site)
OAL3560	*tsi8* BTH_F	GCGCTCTAGAATGCAGCGACTCTTCGTCTACGGCAGC	Generate *tse8* with N-terminal T18/T25 fusion and C-terminal V5 tag in BTH vector (XbaI site)
OAL3561	*tsi8* BTH_R	GGATCCTCACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCGTCGCGCAGGGCGTAGAC	Generate *tse8* with N-terminal T18/T25 fusion and C-terminal V5 tag in BTH vector (BamHI site)
OAL4061	His-*tsi8*_F	GGATCCATGCAGCGACTCTTCGTCTA	Generate *tsi8* with N-terminal His tag in pET28a (BamHI site)
OAL4062	His-*tsi8*_R	GAGCTCTCAGTCGCGCAGGGCGT	Generate *tsi8* with N-terminal His tag in pET28a (SacI site)
OAL1511	Tn7-*glmS*	AATCTGGCCAAGTCGGTGAC	Combined with Tn7-RR109 produces a fragment of approx. 150bp that can be used to check any Tn7 insertion

OAL1512	Tn7-RR109	CAGCATAACTGGACTGATTTCAG	Combined with Tn7-glmS produces a fragment of approx. 150bp that can be used to check any Tn7 insertion
OAL3231	*vgrG1a*_F	ATGCAACTGACCCGCCTGGTCCAGGTGGA	Generation of *vgrG1a* (PA0091) with C-terminal V5 tag
OAL3232	*vgrG1a*_R	TCAGCACGCGTAGTCCGGCACGTCGTACGGGTAGCCCTTCGCCGGCGGCGGAA	Generation of *vgrG1a* (PA0091) with C-terminal V5 tag
OAL3233	*vgrG1b*_F	ATGGCACTTGCGCAACAGACCCGCCTGGT	Generation of *vgrG1b* (PA0095) with C-terminal V5 tag
OAL3234	*vgrG1b*_R	TCAGCACGCGTAGTCCGGCACGTCGTACGGGTAGTTCTGGAGGATCTTGCGT	Generation of *vgrG1b* (PA0095) with C-terminal V5 tag
OAL3235	*vgrG1c*_F	GTGGCTATTGGCCAGCCTTTCGCGACGGC	Generation of *vgrG1c* (PA2685) with C-terminal V5 tag
OAL3236	*vgrG1c*_R	TCAGCACGCGTAGTCCGGCACGTCGTACGGGTAACAGTTGATATCGACATTGG	Generation of *vgrG1c* (PA2685) with C-terminal V5 tag

OAL1740	*vgrG1a* BTH_F	GCGCGGGATCCCATGCAACTGACCCGCCTG	Generate *vgrG1a* with N-terminal T18/T25 fusion in BTH vector (BamHI site)
OAL1741	*vgrG1a* BTH_R	GCGCGGAATTCTCAGCCCTTCGCCGGCGG	Generate *vgrG1a* with N-terminal T18/T25 fusion in BTH vector (EcoRI site)
OAL1860	*vgrG1b* BTH_F	GCGCGTCTAGAGATGGCACTTGCGCAACAGACC	Generate *vgrG1b* with N-terminal T18/T25 fusion in BTH vector (XbaI site)
OAL1861	*vgrG1b* BTH_R	GCGCGGAATTCTCAGTTCTGGAGGATCTTGCG	Generate *vgrG1b* with N-terminal T18/T25 fusion in BTH vector (EcoRI site)
OAL2390	*vgrG1c* BTH_F	GCGCGTCTAGAATGCAACACACCCGCCTGGTACACG	Generate *vgrG1c* with N-terminal T18/T25 fusion in BTH vector (XbaI site)
OAL2391	*vgrG1c* BTH_R	GCGCGGAATTCTCAACAGTTGATATCGACATTGGGC	Generate *vgrG1c* with N-terminal T18/T25 fusion in BTH vector (EcoRI site)
OAL2458	*vgrG6* BTH_F	GCGCGTCTAGAATGTTCGCCCCCGCCAACCAGACGC	Generate *vgrG6* with N-terminal T18/T25 fusion in BTH vector (XbaI site)
OAL2459	*vgrG6* BTH_R	GCGCGGAATTCTCATGGCGTGGGCTCATCCTTGTCG	Generate *vgrG6* with N-terminal T18/T25 fusion in BTH vector (EcoRI site)
OAL3099	*tse8*-HA_F	CCGGCGGATCCTAACAGGAGGAATTAACCATGATC	Generate *tse8*-HA (BamHI site and Shine-Dalgarno)

		GAGGTCACCGAGGTT	
OAL3310	*tse8*-HA_R	GCCGGCTGCAGTCAGCACGCGTAGTCCGGCACGTCGTACGGGTACTTGCTTGCCAGCGGTGGAG	Generate *tse8*-HA (PstI site)
OAL3669	*tse8* exp_F	GCGCGGATCCATCGAGGTCACCGAGGTTTC	Generate *tse8* with N-terminal GST-His-S tag for purification (BamHI site)
OAL3670	*tse8* exp_R	GCGGCCGCTCACTTGCTTGCCAGCGG	Generate *tse8* with N-terminal GST-His-S tag for purification (NotI site)
OAL5140	*tse8*-HA-Strep_F	CATATGATCGAGGTCACCGAGGTTTCCATCGCCGAGCTGCGTG	Generate *tse8* with C-terminal HA and Strep tags (NdeI site) with pET41a:*tse8*-HA as template; use with primer *gatC*-HA-Strep^{R}
OAL3301	*tsi8*-V5_F	CCGCCTCTAGATAACAGGAGGAATTAACCATGCAGCGACTCTTCGTCTAC	Generate *tsi8* with C-terminal V5 tag (XbaI site and Shine-Dalgarno)
OAL3302	*tsi8*-V5_F	CTGCAGTCACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCCGTAGAAT	Generate *tsi8* with C-terminal V5 tag (PstI site)

		CGAGACCGAGG AGAGGGTTAGG GATAGGCTTACC GTCGCGCAGGGC GTAGA	
OAL3273	*gatA*-V5_F	CCGGCCTCGAGT AACAGGAGGAA TTAACCATGCTG CATCAATTGACC CT	Generate *gatA* with C-terminal V5 tag (XhoI site and Shine-Dalgarno)
OAL3274	*gatA*-V5_R	CCGGGATGCATT TACGTAGAATCG AGACCGAGGAG AGGGTTAGGGAT AGGCTTACCGAA GCCGGCCGGGGT GCG	Generate *gatA* with C-terminal V5 tag (NsiI site)
OAL3275	*gatB*_F	GCGCGGGATCCG CAATGGGAAACC GTGATC	Generate *gatB* with N-terminal His tag (BamHI site)
OAL3276	*gatB*_R	AAGCTTTCACGC TTCGAGCTTTTT	Generate *gatB* with N-terminal His tag (HindIII site)
OAL3277	*gatC*-HA_F	CATATGGCGCTT GAACGCTCCGAC	Generate *gatA* with C-terminal HA tag (NdeI site)
OAL3278	*gatC*-HA_R	AATTACTCGAGT CAGCACGCGTAG TCCGGCACGTCG	Generate *gatA* with C-terminal HA tag (XhoI site)

		TACGGGTATGACTCGATGACTTTCGG	
OAL3913	*asnS*-His_F	GCGCGCTGCAGTAACAGGAGGAATTAACCATGAGCGTTGTGCCTGTAGCCGA	Generate *asnS* (asparagine tRNA snythase from *E. coli* MG1655) with C-terminal His tag (SpeI site and Shine-Dalgarno)
OAL3914	*asnS*-His_R	GGCGCACTAGTTTAGTGATGGTGATGGTGATGGAAGCTGGCGTTACGCGG	Generate *asnS* (asparagine tRNA snythase from *E. coli* MG1655) with C-terminal His tag (NotI site)

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Supplementary Table 4. Output of bioinformatic analyses of relevant bacteria possessing GatCAB, AsnS and GlnS.

Transamidosome (GatCAB Components)		
GatA (pBLAST)	**GatB (pBLAST)**	**GatC (pBLAST)**
Agrobacterium tumefaciens	*Agrobacterium tumefaciens*	*Agrobacterium tumefaciens*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
Operons containing gatCAB (nBLAST)		
Agrobacterium tumefaciens		
Pseudomonas aeruginosa		
Cognate tRNA synthases (AsnS and/or GlnS)		
AsnS (pBLAST)	**GlnS (pBLAST)**	
Escherichia coli	*Escherichia coli*	
	Pseudomonas aeruginosa	

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