SUPPLEMENTARY MATERIAL

Photorhabdus antibacterial Rhs polymorphic toxin inhibits translation through ADP-ribosylation of 23S ribosomal RNA

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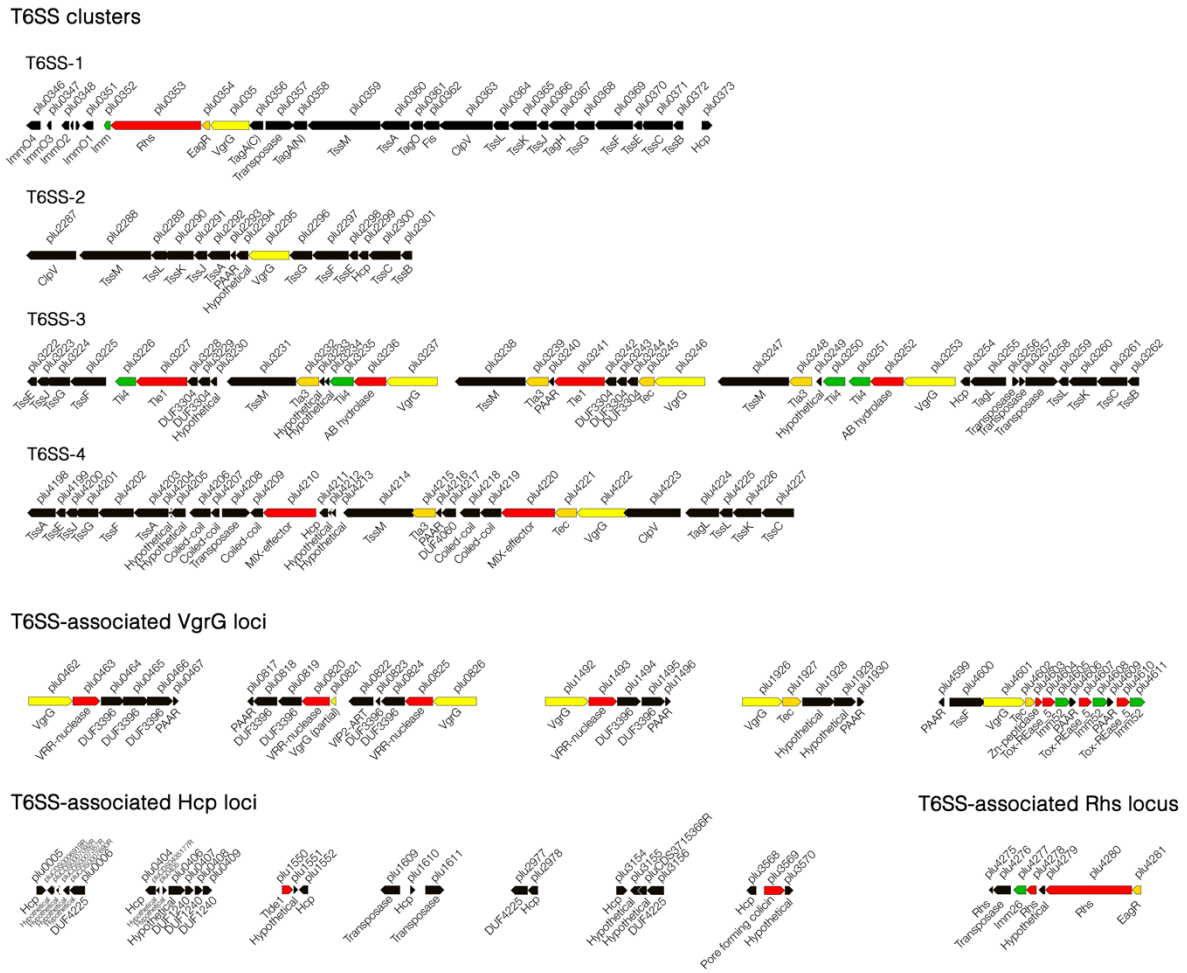
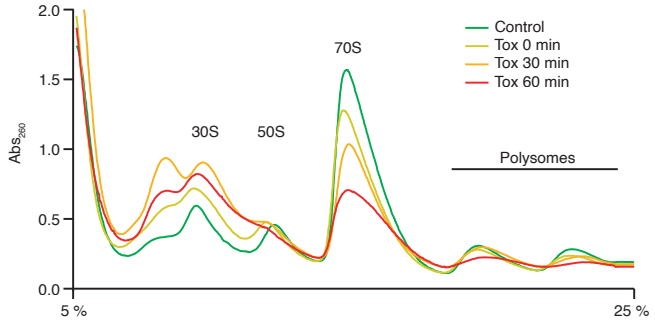
Supplementary Figures and Captions



Supplementary Figure 1. Type VI secretion genes in *Photorhabdus laumondii.* Schematic representation of the *Photorhabdus laumondii* subsp. *laumondii* TT01 T6SS clusters and associated islands. Gene identifiers are indicated above the arrows, predicted proteins are indicated below. Spike proteins (VgrG), effector-associated chaperones, putative effectors and putative immunity proteins are indicated in yellow, orange, red and green, respectively.



Supplementary Figure 2. Tre23 reduces the fraction of actively translating ribosomes. Polysome analysis of *E. coli* cells harboring the empty vector (green line) or producing Tre23 at time 0 (yellow line), 30 (orange line) or 60 min (red line) post-induction. Cell lysates were resolved by ultracentrifugation on 5-25% sucrose gradients and detected at A_{260}. The positions of the 30S, 50S, 70S and polysomes peaks are indicated on top.

Supplemental Table S1. Strains and Plasmids used in this study.

Strains	Description and genotype	Source/References
Escherichia coli K12		
DH5α	F-, Δ(*argF*-lac)U169 *phoA supE44 lacZ*ΔM15 *recA relA endA thi hsdR gyr*	Laboratory collection
BW25113	F-, Δ(*araBAD*) *lacZ*(del)::*rrnB*-3 *rph1* Δ(*rhaD-rhaB*) *hsdR*	Barry Wanner
BL21(DE3)	*dcm ompT hsdS gal λDE3*	Laboratory collection
WM3064	*thrB pro thi rpsL hsdS lacZ*ΔM15 RP4- Δ(*araBAD*) Δ*dapA*::[*erm* pir (wt)]	(1)
MRE600	*rna*, *hsdM* pColE1	(2)
Photorabdus laumondii		
TT01	WT *Photorhabdus laumondii*	(3)
TT01-contr.	Cam_Plac_GFP cassette (from pJQ200-*glm-rpm*) inserted at g*lmS/rpmE* locus in TT01	(4)
TT01-ΔP_{T6SS}	Replacement of the T6SS-1 promoter region by the Cam_Plac_GFP cassette (from pJQ200-ΔP_{T6SS}) locus in TT01	This study
TT01-ΔP_{T6SS}	Replacement of the T6SS-1 *tre23-tri23* by the Cam_Plac_GFP cassette (from pJQ200-ΔMutEff) locus in TT01	This study
MRE600	*rna*, *hsdM* pColE1	(2)

Plasmid	Description and main characteristics	Source/References
pJQ200	Mobilizable vector, suicide in *P. laumondii*, Gm^R	(5)
pJQ200-*glm-rpm*	pJQ200 vector to insert Cam_Plac_GFP cassette into the g*lmS/rpmE* locus by homologous recombination	(4)
pJQ200-ΔP_{T6SS}	pJQ200 vector to exchange the T6SS-1 promoter by the Cam_Plac_GFP cassette by homologous recombination	This study
pJQ200-ΔMutEff	pJQ200 vector to exchange *tre23-tri23* by the Cam_Plac_GFP cassette by homologous recombination	This study
pBAD33	Expression vector, AraC, arabinose-inducible, Cm^R	(6)
pBAD-Tre23	*P. laumondii tre23* (corresponding to Rhs amino-acids 1343-1471) cloned into pBAD33	This study
pBAD-Tre23-H8A	His8-to-Ala (His-1350 in Rhs) substitution in pBAD-Tre23	This study
pBAD-Tre23-Y33A	Tyr33-to-Ala (Tyr-1375 in Rhs) substitution in pBAD-Tre23	This study
pBAD-Tre23-HY	Tyr33-to-Ala substitution in pBAD-Tre23-H8A	This study
pBAD-Tri23	*P. laumondii tri23* (*plu0352*) cloned into pBAD33	This study
pNDM220	Mini-R1, single-copy vector, $LaqI^q$, $P_{A1/04/03}$, Amp^R	(7)
pNDM-Tre23	*P. laumondii tre23* (corresponding to Rhs amino-acids 1343-1471) cloned into pNDM220	This study
pET-Duet1	pBR322 ColE1 *ori*, T7 promoter, Amp^R	Novagen

pET-His-VgrG	*P. laumondiis vgrG* gene (*plu0355*) under pT7 control in pET-Duet1, N-terminal 6×His tag, TEV cleavage	This study
pRSF-Duet1	RSF1030 (NTP1) *ori*, T7 promoter, Kan^R	Novagen
pRSF-EagR-ST	*P. laumondii eagR* gene (*plu0354*) under pT7 control in pRSF-Duet1, C-terminal Strep-tag	This study
pCDF-Duet1	CloDF13 *ori*, T7 promoter, $Strep^R$	Novagen
pCDF-Rhs*-FL	*P. laumondii rhs* gene (*plu0353)* under pT7 control in pCDF-Duet1, C-terminal FLAG tag, Tyr1351-to-Ala, Tyr1375-to-Ala, and Asp1338-to-Asn substitutions	This study
pPJ23104-gfp-lva	*GFP-lva* cloned under synthetic constitutive promoter in pBAD33, Cm^R	This study

Oligonucleotide	**Sequence (5' to 3')**	**Purpose**
Construction of mutations in *Photorhabdus* [a]		
MutClus_Left_F_SalI	GTGC**GTCGAC**CTGACGCTCTTTCCAGGAAC	amplify flanking regions for T6SS promoter mutation in *Photorhabdus*
MutClus_Left_R_AatII	GTGC**GACGTC**GCTGGAATCATCATGGCGTT	amplify flanking regions for T6SS promoter mutation in *Photorhabdus*
MutClus_Right_F_SacI	GCGT**GAGCTC**ACTGTCTTCAACTTCTTTCAGCT	amplify flanking regions for T6SS promoter mutation in *Photorhabdus*
MutClus_Right_R_SpeI	GCGT**ACTAGT**TCTTCACGCAAAACTCTGGC	amplify flanking regions for T6SS promoter mutation in *Photorhabdus*
MutEff_Left_F_SalI	GTGC**GTCGAC**TTCAACCGGTTCCATATGAA	amplify flanking regions for Tre23-Tri23 mutation in *Photorhabdus*
MutEff_Left_R_AatII	GTGC**GACGTC**TTAATAATTAACAAAGCCGG	amplify flanking regions for Tre23-Tri23 mutation in *Photorhabdus*
MutEff_Right_F_SacI	GCGT**GAGCTC**GGTCCGGTTCTCTTCATCCA	amplify flanking regions for Tre23-Tri23 mutation in *Photorhabdus*
MutEff_Right_R_SpeI	GCGT**ACTAGT**CGTCATCCGCCTCAAAAGAG	amplify flanking regions for Tre23-Tri23 mutation in *Photorhabdus*
VerMutT6SS_PlumEff_LFwd	GAGAGCATGAGATGACAATAGCC	check mutations in *Photorhabdus* genome
VerMutT6SS_PlumEff_RRev	CAGGGAATTCAGGGGGTATT	check mutations in *Photorhabdus* genome
VerMutT6SS_PlumClus_LFwd	TGTTTCAGCCAGGTGTTCAG	check mutations in *Photorhabdus* genome
VerMutT6SS_PlumClus_RRev	TCAGTGGTGGCAATTGTGAT	check mutations in *Photorhabdus* genome
VerMutT6SS_LRevCam	TGCTCATGGAAAACGGTGTA	check mutations in *Photorhabdus* genome
VerMutT6SS_RFwdGFP	CCACACAATCTGCCCTTTCG	check mutations in *Photorhabdus* genome
Plasmid construction [a,b]		
R-pBAD-SD-Sal	GATCA**GTCGAC**CCTCCTTCTAGAGGATCCCCGGGTACCG	introduce synthetic ribosome binding site into pBAD33

F-pBAD-MCS	CTGCAGGCATGCAAGCTTGGC	introduce synthetic ribosome binding site into pBAD33
F-plART-sal	GACT**GTCGAC**ATGGCTGGTGAAAATGTATTTATTCAC	clone *tre23* into pBAD33 through *Sal*I and *Hind*III sites
R-plART-hind	GACT**AAGCTT**TTATTTTCCTCCGCACTTTCTG	clone *tre23* into pBAD33 through *Sal*I and *Hind*III sites
F-imART-Sal	GACT**GTCGAC**ATGAAGCACGAATCTATTCAA	clone *tri23* into pBAD33 through *Sal*I and *Hind*III sites
R-A-plART-hind	GACT**AAGCTT**CTACCACTTATATGGATATGCATG	clone *tri23* into pBAD33 through *Sal*I and *Hind*III sites
F-pBAD-bam	ACCCGG**GGATCC**TCTAGAAG	amplify *tre23* from pBAD33 with RBS and clone into pNDM220 through *BamH*I and *EcoR*I sites
R-pBAD-eco	CAGT**GAATTC**CCGCCAAAACAGCCAAGCTT	amplify *tre23* from pBAD33 with RBS and clone into pNDM220 through *BamH*I and *EcoR*I sites
F-gfp-eco	ATGC**GAATTC**ATGAGTAAAGGAGAAGAACTTTTCAC	clone *gfp-lva* through *EcoR*I and *Hind*III in pBAD33
R-GFP-LVA-Hind	GGCC**AAGCTT**TTAAGCTACTAAAGCGTAGTTTT	clone *gfp-lva* through *EcoR*I and *Hind*III in pBAD33
R-pBAD-sac	GGAG**GAGCTC**TTGGTAACGAATCAGACAAT	remove *araC* and *ara* promoter and introduce synthetic promoter J23104 in front of *gfp-lva* into pBAD33-GFP-LVA
F-p33-J32104	TTGACAGCTAGCTCAGTCCTAGGTATTGTGCTAGCACCCGTTTTTTTGGGCTA	remove *araC* and *ara* promoter and introduce synthetic promoter J23104 in front of *gfp-lva* into pBAD33-GFP-LVA
F-pETd-synth	GCCAGGATCCGAATTCGAGCTC	introduce TEV protease site after 6×His-tag and *Bmt*I site for in frame cloning
R-pETd-tev-bmt	ACAA**GCTAGC**GCCCTGGAAGTACAGGTTTTC*GTGGTGATGATGGTGATG*GCTGCTG	introduce TEV protease site after 6×His-tag and *Bmt*I site for in frame cloning
F-pRSFd-strep-sal	GACT**GTCGAC***TGGAGCCACCCGCAGTTCGAAAAATAA*AAGCTTGCGGCCGCATAATGC	introduce Streptag for in frame cloning at the C terminus
F-pACYCd-flag-sal	GATC**GTCGAC***GATTACAAGGACGACGATGACAAGTAA*AAGCTTGCGGCCGCATAATG	introduce FLAG tag for in frame cloning at the C terminus
R-pRSF-sac	GATC**GAGCTC**CTCCTTATTAAAGTTAAACAAAATTATTTCTACAGGGG	amplify Duet1 vectors for cloning in fusion with Strep of FLAG tags at the C terminus
R-duet-strepTEV	ACTG**GAGCTC**AGACTGGAAGTACAGGTTTTC*TTTTTCGAACTGCGGGTGGCTCCAC*ATGGTATATCTCCTTATTA AAGT	amplify Duet1 vectors for cloning with Streptag and TEV protease cleavage site at the N terminus
F-duet-sal	CATG**GTCGAC**TAATGCTTAAGTCGAACAGAAA	amplify Duet1 vectors for cloning with Streptag and TEV protease cleavage site at the N terminus

F-plVgrG-bmt	GGGC**GCTAGC**TCATTTCAGAAAAAAACCGCCA	clone *vgrG* into pET-Duet1 through *Bmt*I and *Hind*III sites fusing to 6×His-TEV site at the N terminus
R-plVgrG-hind	GACT**AAGCTT**TCAGTTCACATTGACCTGTTTG	clone *vgrG* into pET-Duet1 through *Bmt*I and *Hind*III sites fusing to 6×His-TEV site at the N terminus
F-pl1795-Sac	GGAGGAGCTCATGGAAAACACAACCTATCAGA	clone *eagR* into pRSF-Duet1 through *Sac*I and *Sal*I sites fusing to Streptag at the C terminus
R-pl1795-Sal	GATC**GTCGAC**GGCTGCCTGAAAACTGTGGA	clone *eagR* into pRSF-Duet1 through *Sac*I and *Sal*I sites fusing to Streptag at the C terminus
F-plRhs-Sac	GGAG**GAGCTC**ATGTCACTTGGTGATGAAATCG	clone *rhs* into pCDF-Duet1 through *Sac*I and *Sal*I sites fusing to FLAG tag at the C terminus
R-plRhs-Sal	GATC**GTCGAC**TTTTCCTCCGCACTTTCTGATT	clone *rhs* into pCDF-Duet1 through *Sac*I and *Sal*I sites fusing to FLAG tag at the C terminus
F-pl-Imm-Sac	GGAG**GAGCTC**ATGAAGCACGAATCTATTCAATT	clone *tri23* immunity protein into pCDF-Duet1 through *Sac*I and *Sal*I sites in fusion with Streptag and TEV cleavage site at the N terminus
R-pl-Imm-Sal	GATC**GTCGAC**CCACTTATATGGATATGCAT	clone *tri23* immunity protein into pCDF-Duet1 through *Sac*I and *Sal*I sites in fusion with Streptag and TEV cleavage site at the N terminus or with Streptag at the C terminus
R-pl-ART-ATG-Sac	CATG**GAGCTC**ATGGCTGGTGAAAATGTATTTATT	in pair with above indicated R-plRhs-Sal, to clone Tre23 toxic protein domain into pRSF-Duet1 through *Sac*I and *Sal*I sites in fusion with Streptag at the C terminus
Site directed mutagenesis[c]		
F-plART-H8A	GCGTATACAAATAAAGCGGGTTTTG	introduce H8A substitution in *tre23*
R-plART-H8	AATAAATACATTTTCACCAGCC	introduce H8A substitution in *tre23*
F-plART-Y9A	GCGACAAATAAAGCGGGTTTTGAC	introduce Y1351A substitution in *rhs* (Y9A at the Tre23)
R-plART-Y9	GTGAATAAATACATTTTCACCAGC	introduce Y1351A substitution in *rhs* (Y9A at the Tre23)
F-plART-Y33A	GCGATAACGGATGTACTAATGTCTC	introduce Y1375A substitution in *rhs* (Y33A at the Tre23)
R-plART-Y33	AACTTTTCCACTGACGTTTG	introduce Y1375A substitution in *rhs* (Y33A at the Tre23)
F-plRhs-D1338N	AACCCTCTAGGGCTTGCTGGT	introduce D1338N substitution in *rhs*
R-plRhs-D1338	AATAAATTTCGTGGGATTTTGCACATAGC	introduce D1338N substitution in *rhs*

DNA fragments for *in vitro* transcription-translation[b]		
3'UTR-plART	GCGAATTAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGGCTGGTGAAAATGTATT	amplify DNA fragment for *in vitro* synthesis of Tre23-Streptag
5'UTR-plART-strep	AAACCCCTCCGTTTAGAGAGGGGTTATGCTAGTTATTA*TTTTTCGAACTGCGGGTGGCTCCA*TTTTCCTCCGCACTTTCTGA	amplify DNA fragment for *in vitro* synthesis of Tre23-Streptag
5'UTR-GFP	GCGAATTAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAAAATATGAGTAAAGGAGAAGAACTTTTCAC	amplify DNA fragment for *in vitro* synthesis of GFP-Streptag
3'UTR-GFP-strep	AAACCCCTCCGTTTAGAGAGGGGTTATGCTAGTTATTA*TTTTTCGAACTGCGGGTGGCTCCA*TTTGTATAGTTCATCCATGCCA	amplify DNA fragment for *in vitro* synthesis of GFP-Streptag
rRNA library construction[a]		
Universal miRNA Linker	rAppCTGTAGGCACCATCAAT-NH2	Universal miRNA Cloning linker (NEB)
RT-uni-NEB-Hind	AGCC**AAGCTT**ATTGATGGTGCCTACAG	Reverse transcription against universal cloning linker
ss-linker	pACTCTCTACTGTTTCTCCAT	second linker ligated to copy DNA
F-sslinker-Eco	GGAG**GAATTC**ATGGAGAAACAGTAGAGAGT	PCR primer to amplify copy DNA ligated to linkers, introduces *EcoR*I site
R-NEB-Hind	AGCC**AAGCTT**ATTGATGGTG	PCR primer to amplify copy DNA ligated to linkers, introduces *Hind*III site
F-pKK-seq	GCACTCCCGTTCTGGATAAT	universal pKK223.3 oligo for sequencing
R-pKK-seq	GTTTCACTTCTGAGTTCGGCATG	universal pKK223.3 oligo for sequencing
Hybridization to 23S fragments		
SRL-control	TCCACTCCGGTCCTCTCGTACTAGGAGCAG	hybridizes to 2647-2676 nucleotides of 23S
23S-lib-L	AAATGATGGCTGCTTCTAAGCCAACATCCTGGCTGTCTGGG	hybridizes to 1043-1083 nucleotides of 23S
23S-lib-R	CGCGCAGGCCGACTCGACCAGTGAGCTATTACGCTTTCTTT	hybridizes to 1084-1124 nucleotides of 23S
23S-from1093	CGCAGGCCGACTCGACCAGTGAGCTATTAC	hybridizes to 1093-1122 nucleotides of 23S
23S-from1095	CGCGCAGGCCGACTCGACCAGTGAGCTATT	hybridizes to 1095-1124 nucleotides of 23S
23S-from1099	CTTCCGCGCAGGCCGACTCGACCAGTGAGC	hybridizes to 1099-1128 nucleotides of 23S

[a] Restriction site in bold.
[b] 6×His-, Strep- or FLAG-coding sequence italicized.
[c] introduced mutation underlined.

References

1. Løbner-Olesen,A. and von Freiesleben,U. (1996) Chromosomal replication incompatibility in Dam methyltransferase deficient Escherichia coli cells. *EMBO J*, **15**, 5999–6008.
2. Kurylo,C.M., Alexander,N., Dass,R.A., Parks,M.M., Altman,R.A., Vincent,C.T., Mason,C.E. and Blanchard,S.C. (2016) Genome Sequence and Analysis of Escherichia coli MRE600, a Colicinogenic, Nonmotile Strain that Lacks RNase I and the Type I Methyltransferase, EcoKI. *Genome Biol Evol*, **8**, 742–752.
3. Duchaud,E., Rusniok,C., Frangeul,L., Buchrieser,C., Givaudan,A., Taourit,S., Bocs,S., Boursaux-Eude,C., Chandler,M., Charles,J.-F., *et al.* (2003) The genome sequence of the entomopathogenic bacterium Photorhabdus luminescens. *Nat Biotechnol*, **21**, 1307–1313.
4. Payelleville,A., Blackburn,D., Lanois,A., Pagès,S., Cambon,M.C., Ginibre,N., Clarke,D.J., Givaudan,A. and Brillard,J. (2019) Role of the Photorhabdus Dam methyltransferase during interactions with its invertebrate hosts. *PLoS One*, **14**, e0212655.
5. Quandt,J. and Hynes,M.F. (1993) Versatile suicide vectors which allow direct selection for gene replacement in gram-negative bacteria. *Gene*, **127**, 15–21.
6. Guzman,L.M., Belin,D., Carson,M.J. and Beckwith,J. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol*, **177**, 4121–4130.
7. Gotfredsen,M. and Gerdes,K. (1998) The Escherichia coli relBE genes belong to a new toxin-antitoxin gene family. *Mol Microbiol*, **29**, 1065–1076.