

Supplementary Materials: A Highly Specific Holin-Mediated Mechanism Facilitates the Secretion of Lethal Toxin TcsL in *Paeniclostridium sordellii*

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Table S1. Bacterial isolates and plasmids used in this study.

Strain/ plasmid	Characteristics	Reference
<i>P. sordellii</i>		
ATCC9714	Bovine, myonecrosis, contains pCS1-3, <i>tcsH-tcsL</i> ⁺	[1]
DLL5002 [<i>tcsLTT</i>]	ATCC9714Δ <i>tcsL</i> ::TT, Em ^R	[2]
DLL5143 [pCS1-1]	ATCC9714 cured of pCS1-1, <i>tcsL</i> ⁻	This study, derived from [3].
DLL5036 [<i>tcsETT1</i>]	ATCC9714Δ <i>tcsE</i> ::TT, independent mutant 1, Em ^R	This study
DLL5037 [<i>tcsETT2</i>]	ATCC9714Δ <i>tcsE</i> ::TT, independent mutant 2, Em ^R	This study
DLL5159 [<i>tcsETT1(tcsE⁺)</i>]	DLL5036(pDLL101), Em ^R , Tm ^R	This study
DLL5240 [<i>tcsETT1(V)</i>]	DLL5036(pRPF185), Em ^R , Tm ^R	This study
<i>E. coli</i>		
MC1061/ λC1857Sam7	Lysogenic MC1061 carrying λC1857Sam7, does not encode a functional holin	[4]
MC1061/ λCm ^r Δ(SR)	Lysogenic MC1061 carrying λCm ^r Δ(SR), does not encode a functional holin or endolysin	[5]
DH5α	F ⁻ φ80 <i>lacZ</i> ΔM15 Δ(<i>lacZYA-argF</i>)U169 <i>recA1 endA1 hsdR17(rk⁻, mk⁺) phoA supE44 λ⁻ thi-1 gyrA96 relA1</i> , used for subcloning	Thermo Fisher Scientific
HB101(pVS520)	<i>recA123</i> , Res ⁻ , Mod ⁻ , Str ^R containing pVS520, Tc ^R , used as conjugation donor for <i>P. sordellii</i>	[6]
Plasmids		
pBRQ(ΔRBS)	pJN4 derivative; λS gene with a deletion in its ribosome binding site (RBS) under the control of the late transcription regulatory (LTR) elements of phage λ	[7]
pJN5	pBR322 derivative; carries S105 under the control of the LTR elements of phage λ	[8]
pRG32	pBRQ(Δrbs) carrying <i>tcdE</i> with its own RBS	[7]
pDIA6884	pBRQ(Δrbs) carrying <i>tcsE</i> with its own RBS	This study
pDIA6883	pBRQ(Δrbs) carrying <i>tpeE</i> with its own RBS	This study
pDIA6885	pBRQ(Δrbs) carrying <i>tcnE</i> with its own RBS	This study
pDLL46	Clostridial TargetTron vector, contains RP4 and Tn916 <i>oriTs</i> and <i>lacZα</i> within retargeting region for blue white screening	[3]
pVS520	Tra ⁺ , Mob ⁺ , RP1 derivative	[6]
pRPF185	Clostridial tetracycline inducible expression vector	[9]
pDLL101	pRPF185 carrying <i>tcsE</i> with its predicted RBS under a tetracycline inducible promoter	This study

Table S2. Oligonucleotide primers used in PCR.

Primer	Sequence (5'-3')	Use
OBD778	GCTCTAGATGAATATAACAATATCTTTTTATCAAAA	Amplify <i>tcsE</i> and predicted RBS (+) for cloning into pBRQ(Δ RBS)
OBD779	CCCAAGCTTCATTATTTTATCTATCCTCAATTTTAC	Amplify <i>tcsE</i> and predicted RBS (-) for cloning into pBRQ(Δ RBS)
OBD776	GCTCTAGAGTGGATTGAGAACTGTTTAAATTATG	Amplify <i>tpeE</i> and predicted RBS (+) for cloning into pBRQ(Δ RBS)
OBD777	CCCAAGCTTCTAATTATTATCAATTTATTTTTATGCTTC	Amplify <i>tpeE</i> and predicted RBS (-) for cloning into pBRQ(Δ RBS)
OBD780	GCTCTAGAATGGATAAACAGAAATATTTTAAACAC	Amplify <i>tcnE</i> and predicted RBS (+) for cloning into pBRQ(Δ RBS)
OBD781	CCCAAGCTTGCAAGACTATTTGTCCTGCTC	Amplify <i>tcnE</i> and predicted RBS (-) for cloning into pBRQ(Δ RBS)
JRP3867	CGAAATTAGAACTTGCCTTCAGTAAAC	Targetron EBS universal
DLP965	AAAAAAGCTTATAATTATCCTTAAATATCCAAGCTGTGCGCCCAGATAGGGTG	<i>tcsE</i> Targetron IBS, 161/162 antisense
DLP966	CAGATTGTACAAATGTGGTGATAACAGATAAGTCCAAGCTATTAACCTAC-CTTTCCTTGT	<i>tcsE</i> Targetron EBS1d, 161/162 antisense
DLP967	TGAACGCAAGTTTCTAATTTTCGGTTATATTCCGATAGAGGAAAGTGCT	<i>tcsE</i> Targetron EBS2, 161/162 antisense
DLP525	AAAGAGCTCCTAGGAGGCATTATGAATATAAC	Amplify <i>tcsE</i> and predicted RBS (+) for complementation - introduces SacI site, screening of mutants, generation of probe
DLP526	AAAGGATCCATATTTTCATTATTTTATCTATCCTC	Amplify <i>tcsE</i> and predicted RBS (-) for complementation - introduces BamHI site, screening of mutants, generation of probe
DLP968	AAGAACTCAGCGAAACAAATGAC	Internal <i>tcsL</i> (+), RT-ddPCR
DLP969	TTACTAAACTTGGTATCCCTGCTG	Internal <i>tcsL</i> (-), RT-ddPCR
DLP970	GAAGCACAAGGACCATGTACAG	Internal <i>P. sordellii</i> <i>rpoA</i> (+), RT-ddPCR
DLP971	CTTCCTTTATTACAGATATTTCCATG	Internal <i>P. sordellii</i> <i>rpoA</i> (-), RT-ddPCR

(+) forward primer, (-) reverse primer.

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