Cyclic-di-GMP regulates production of sortase substrates of *Clostridium difficile* and their surface exposure through ZmpI protease-mediated cleavage*

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Supplemental material

Table S1. Strains, plasmids and oligonucleotides used for the present study.

Strain, plasmid or primer	Characteristic/description	Source/construction
Strains		
E. coli NEB5α	General cloning	New England Biolabs
E. coli Rosetta	Protein expression	Novagen
E. coli CA434	Conjugation donor	(1)
C. difficile strains		
630	Wild type Erm^R. Virulent and multidrug-resistant PCR ribotype 01	(2)
630Δ*erm*	Erythromycin-sensitive derivative of wild-type *C. difficile* 630	(3)
Δ*srtB*	630 isogenic *srtB* deletion mutant	This study
Δ*zmpI*	630 isogenic *zmpI* deletion mutant	This study
Δ*srtB*- Δ*zmpI*	630 isogenic double mutant with deletions in the aforementioned genes	This study
P_{tet}-*srtB*	Insertion of the Ptet promoter upstream *srtB* of the 630 strain by allele exchange	This study
Δ$srtB_{comp}$	Δ*srtB* complemented on the chromosome by allele exchange	This study
Plasmids		
pET28a	*E. coli* expression vector. Kan^R	Novagen
pUC19	*E. coli* cloning vector. Amp^R	New England Biolabs
pMTL-SC7215	*E. coli-C. difficile* shuttle vector for *codA*-mediated allele exchange mutagenesis. Tm^R	(4)
pRPF144	*E. coli-C. difficile* shuttle vector for protein expression. P_{cwp2}-*gusA*,Tm^R	(5)
pRPF185	*E. coli-C. difficile* shuttle vector for protein expression. P_{tet}-*gusA*, Tm^R	(5)
pHAS027	CD2831 (32-290)-His_6 in pET28a	This study
pHAS042	His_6-CD27L (1-179) in pET28a	This study
pJKP001	SrtB (27-225)-His_6 in pET28a	This study
pJKP019	pUC19 with construct for *srtB* deletion	This study
pJKP022	pMTL-SC7215 with construct for *srtB* deletion	This study

pJKP029	pMTL-SC7215 with construct for P_{tet}-*srtB* strain creation	This study
pJKP041	pRPF185 derivative carrying P_{tet}-*CD2831* for inducible CD2831 expression	This study
pJKP070	pRFP185 derivative carrying P_{tet}-*CD3392* with addition of a HA-tag encoding sequence into *CD3392* and a functional RBS upstream *CD3392* for inducible $CD3392_{HA}$ expression	This study
pJKP088	pMTL-SC7215 with construct for *zmpI* deletion	This study
pJKP095	pJKP041 with addition of R-HA-tag encoding sequence into *CD2831*	This study
pJKP096	pMTL-SC7215 with construct for *srtB* complementation	This study
pECC12	pRFP144 derivative carrying P_{cwp2}-*dccA* with addition of His-tag encoding sequence into 3'-extremity of dccA for constitutive expression of DccA.	This study
pECC17	pRFP185 derivative carrying P_{tet}-dccA with addition of His-tag encoding sequence into 3'-extremity of *dccA* for inducible expression of DccA.	This study

Primer	**Sequence (5' to 3')***	**Characteristics or use**
NF1614	GGGCCATGGATTCAGAATTAG GAGAGAATAGTCAGATTC	Amplify 5' extremity of CD2831 for pET28a cloning
NF1615	GGGCTCGAGTTCTATTTTTCC AGTAGTTTCAATATGTATTTT TG	Amplify 5' extremity of CD2831 for pET28a cloning
NF1968	ATATCCATGGAACTTACCAAA TACAATCATGATACTA	Amplify *srtB* deleted in the N-terminal hydrophobic domain for pET28a cloning
NF1969	TGAGCTCGAGAATCAATCTAC CATGAATCACCAT	Amplify *srtB* deleted in the N-terminal hydrophobic domain for pET28a cloning
NF2126	GATCGAGCTCGTATTTTATTT TGGAGAAATTAATATGTTTAA AG	Amplify *dccA* with the addition of a C-terminal His-tag encoding sequence for pRFP144 or pRFP185 cloning.
NF2155	GATAGAATTCCCATTTTTAAT ATCTGTACTAACTTATATTAC	Construction of pJKP019 for *srtB* deletion
NF2156	GATAGGATCCGAGAAGACCT GTCCTTAGTAC	Construction of pJKP019 for *srtB* deletion
NF2157	GCCAGAATGGTGATTCATGG	Construction of pJKP019 for *srtB*

		deletion
NF2158	CTGTAAAGTATTACTAATACTAGTATAATGT	Construction of pJKP019 for *srtB* deletion
NF2169	GGATTTCACATTTGCCGTTTTGTAAAC	Screen for insert into pMTL-SC7215
NF2170	GATCTTTTCTACGGGGTCTGAC	Screen for insert into pMTL-SC7215
NF2171	GCTACATCTTATATCATATCATATTATTTACATC	Screen for single and double crossover for *srtB* deletion
NF2172	CGGTCCCTATCCGTCGC	Screen for single and double crossover for *srtB* deletion
NF2199	GATCGGATCCTTAATGGTGATGGTGATGGTGCTCGAGATAATCATTTTTATCAAATTTTTTCTTGTTTTTC	Amplify dccA with the addition of a C-terminal His-tag encoding sequence for pRFP144 or pRFP185 cloning.
NF2214	AAACTCCTTTTTGATAATCTCATGACC	Linearize pMTL-SC7215 by inverse PCR at the PmeI site
NF2215	AAACTTAGGGTAACAAAAAACACCG	Linearize pMTL-SC7215 by inverse PCR at the PmeI site
NF2254	gttttttgttaccctaagtttGTAATATTTACATTGTGCAAAGTTG	Construction of pJKP029 for creation of P_{tet}-*srtB* strain
NF2257	atgtagtttaGGAGCTCAGATCTGTTAAC	Construction of pJKP029 for creation of P_{tet}-*srtB* strain
NF2258	tgagctccTAAACTACATTTAACTCGAGGTG	Construction of pJKP029 for creation of P_{tet}-*srtB* strain
NF2259	gattatcaaaaaggagtttCAATCTACCATGAATCACCATTC	Construction of pJKP029 for creation of P_{tet}-*srtB* strain
NF2260	CACTCAAAACCTTCACTCCTTAAATC	Screen for single and double crossover forcreation of P_{tet}-*srtB* strain
NF2261	CAGTTTAAAATTATTGTTTTAAACTGTGTCTG	Screen for single and double crossover for creation of P_{tet}-*srtB* strain
NF2266	cagaattcgTATATCTATATTACACTATTTTTGAATAAATTTAAATTTTTAG	Construction of pJKP029 for creation of P_{tet}-*srtB* strain
NF2267	atatagatataCGAATTCTGCATCAAGCTAG	Construction of pJKP029 for creation of P_{tet}-*srtB* strain
NF2378	GATAGAGCTCAAAATGAAAGGAGCATTAGATTTATG	Amplify *CD3392* for pRFP185 cloning
NF2379	GATAGGATCCTTATGATTTCTTCATTTTACGGC	Amplify *CD3392* for pRFP185 cloning
NF2451	GGATCCTATAAGTTTTAATAAAACTTTAAATAG	Remove CD3392 coding sequence by inverse PCR
NF2501	GATAGAGCTCGGAAGATTAGATTATAAATTATAGGTAAC	Amplify *CD2831* for pRFP185 cloning
NF2502	GATAGGATCCTAATTTGTATTTTTATTTCTTCTTAATACGATAAG	Amplify *CD2831* for pRFP185 cloning
NF2726	TTCCAGATTATGCTGGTCAGA	Add a sequence encoding for HA

	CACCAGATGTAGA	tag into the CD3246 coding sequence by inverse PCR
NF2727	CATCATATGGATAATTTGATT GTTCTTCTCCATTAAGTATTG	Add a sequence encoding for HA tag into the CD3246 coding sequence by inverse PCR
NF2858	AAATCTAATGCTCCTTTCATT TTGAGC	Remove CD3392 coding sequence by inverse PCR
NF2860	gaaaggagcattagatttTTGAAAACAA AAATTAAAAAATCAAGTATA ATC	Amplify *CD3246* for pRFP185 cloning
NF2861	attaaaacttataggatccTTAATTTCTT TTTATTTTACATTGATACGC	Amplify *CD3246* for pRFP185 cloning
NF2922	gttttttgttaccctaagtttCATACCATAT TATTCCAATATGCT	Construction of pJKP088 for *zmpI* deletion
NF2923	atcaatattcCAAACTTATAGCTTTT TGCAAAATTTAG	Construction of pJKP088 for *zmpI* deletion
NF2924	tataagtttgGAATATTGATATTAT AGCTATTAATAATTTTTTACTT G	Construction of pJKP088 for *zmpI* deletion
NF2925	gattatcaaaaaggagtttGGGGAGACT ATACGAAATG	Construction of pJKP088 for *zmpI* deletion
NF2926	CCTGTGCAAAGTTGGGTTAC	Screen for single and double crossover for *zmpI* deletion
NF2956	CTTTACCAGCATTTGAAAAAT TAGCC	Screen for single and double crossover for *zmpI* deletion
NF2958	CAGATTATGCTGTAAATCCAC CAGTACCACC	Add a sequence encoding for the R-HA tag into the CD2831 encoding gene of pJKP041 by inverse PCR
NF2959	GAACATCATATGGATACCGCA AAGTATCATCTTTAACTTTAT TATTTTTTTC	Add a sequence encoding for the R-HA tag into the CD2831 encoding gene of pJKP041 by inverse PCR
NF2966	gttttttgttaccctaagtttCGTCTTCTTC TCTCTGATAG	Construction of pJKP096 for complementation of *srtB* mutant
NF2967	tcaatctacCATGAATCACCATTCT GGC	Construction of pJKP096 for complementation of *srtB* mutant
NF2968	tgattcatgGTAGATTGATTTAGAA ACTCACAGACAC	Construction of pJKP096 for complementation of *srtB* mutant
NF2969	gattatcaaaaaggagtttCGGAAAGAC CCCATGGAG	Construction of pJKP096 for complementation of *srtB* mutant
NF2970	GAAGGTCTTTATATAGGTTTA AAACC	Screen for single and double crossover for complementation of *srtB* mutant
NF2971	CGGATACCTGCGACAGG	Screen for single and double crossover for complementation of *srtB* mutant

*Underlined bases indicate engineered restriction sites; lowercase bases indicate overlapping sequences

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