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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
<b>Strains</b>		
<i>E. coli</i> NEB5 $\alpha$	General cloning	New England Biolabs
<i>E. coli</i> Rosetta	Protein expression	Novagen
<i>E. coli</i> CA434	Conjugation donor	(1)
<u><i>C. difficile</i> strains</u>		
630	Wild type Erm <sup>R</sup> . Virulent and multidrug-resistant PCR ribotype 01	(2)
630 $\Delta$ <i>erm</i>	Erythromycin-sensitive derivative of wild-type <i>C. difficile</i> 630	(3)
$\Delta$ <i>srtB</i>	630 isogenic <i>srtB</i> deletion mutant	This study
$\Delta$ <i>zmpI</i>	630 isogenic <i>zmpI</i> deletion mutant	This study
$\Delta$ <i>srtB</i> - $\Delta$ <i>zmpI</i>	630 isogenic double mutant with deletions in the aforementioned genes	This study
<i>P</i> <sub>ter</sub> - <i>srtB</i>	Insertion of the P <sub>ter</sub> promoter upstream <i>srtB</i> of the 630 strain by allele exchange	This study
$\Delta$ <i>srtB</i> <sub>comp</sub>	$\Delta$ <i>srtB</i> complemented on the chromosome by allele exchange	This study
<b>Plasmids</b>		
pET28a	<i>E. coli</i> expression vector. Kan <sup>R</sup>	Novagen
pUC19	<i>E. coli</i> cloning vector. Amp <sup>R</sup>	New England Biolabs
pMTL-SC7215	<i>E. coli</i> - <i>C. difficile</i> shuttle vector for <i>codA</i> -mediated allele exchange mutagenesis. Tm <sup>R</sup>	(4)
pRPF144	<i>E. coli</i> - <i>C. difficile</i> shuttle vector for protein expression. <i>P</i> <sub>cwp2</sub> - <i>gusA</i> , Tm <sup>R</sup>	(5)
pRPF185	<i>E. coli</i> - <i>C. difficile</i> shuttle vector for protein expression. <i>P</i> <sub>ter</sub> - <i>gusA</i> , Tm <sup>R</sup>	(5)
pHAS027	CD2831 (32-290)-His <sub>6</sub> in pET28a	This study
pHAS042	His <sub>6</sub> -CD27L (1-179) in pET28a	This study
pJKP001	SrtB (27-225)-His <sub>6</sub> in pET28a	This study
pJKP019	pUC19 with construct for <i>srtB</i> deletion	This study
pJKP022	pMTL-SC7215 with construct for <i>srtB</i> deletion	This study

pJKP029	pMTL-SC7215 with construct for <i>P<sub>ter</sub>-srtB</i> strain creation	This study
pJKP041	pRFP185 derivative carrying <i>P<sub>ter</sub>-CD2831</i> for inducible CD2831 expression	This study
pJKP070	pRFP185 derivative carrying <i>P<sub>ter</sub>-CD3392</i> with addition of a HA-tag encoding sequence into <i>CD3392</i> and a functional RBS upstream <i>CD3392</i> for inducible CD3392 <sub>HA</sub> expression	This study
pJKP088	pMTL-SC7215 with construct for <i>zmpI</i> deletion	This study
pJKP095	pJKP041 with addition of R-HA-tag encoding sequence into <i>CD2831</i>	This study
pJKP096	pMTL-SC7215 with construct for <i>srtB</i> complementation	This study
pECC12	pRFP144 derivative carrying <i>P<sub>cwp2-</sub>dccA</i> with addition of His-tag encoding sequence into 3'-extremity of <i>dccA</i> for constitutive expression of DccA.	This study
pECC17	pRFP185 derivative carrying <i>P<sub>ter</sub>-dccA</i> with addition of His-tag encoding sequence into 3'-extremity of <i>dccA</i> for inducible expression of DccA.	This study

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

NF2158	CTGTAAAGTATTACTAATACT AGTATAATGT	deletion Construction of pJKP019 for <i>srtB</i> deletion
NF2169	GGATTTACATTTGCCGTTTT GTAAAC	Screen for insert into pMTL- SC7215
NF2170	GATCTTTTCTACGGGGTCTGA C	Screen for insert into pMTL- SC7215
NF2171	GCTACATCTTATATCATATCA TATTATTTACATC	Screen for single and double crossover for <i>srtB</i> deletion
NF2172	CGGTCCCTATCCGTCGC	Screen for single and double crossover for <i>srtB</i> deletion
NF2199	GATCGGATCCTTAATGGTGAT GGTGATGGTGCTCGAGATAAT CATTTTTATCAAATTTTTTCTT GTTTTTC	Amplify <i>dccA</i> with the addition of a C-terminal His-tag encoding sequence for pRFP144 or pRFP185 cloning.
NF2214	AAACTCCTTTTTGATAATCTC ATGACC	Linearize pMTL-SC7215 by inverse PCR at the PmeI site
NF2215	AAACTTAGGGTAACAAAAAA CACCG	Linearize pMTL-SC7215 by inverse PCR at the PmeI site
NF2254	gTTTTgttaccctaagtttGTAATATTTA CATTGTGCAAAGTTG	Construction of pJKP029 for creation of $P_{tet}$ - <i>srtB</i> strain
NF2257	atgtagttaaGGAGCTCAGATCTGT TAAC	Construction of pJKP029 for creation of $P_{tet}$ - <i>srtB</i> strain
NF2258	tgagctccTAAACTACATTTAACT CGAGGTG	Construction of pJKP029 for creation of $P_{tet}$ - <i>srtB</i> strain
NF2259	gattatcaaaaaggagtttCAATCTACCA TGAATCACCATTC	Construction of pJKP029 for creation of $P_{tet}$ - <i>srtB</i> strain
NF2260	CACTCAAAACCTTCACTCCTT AAATC	Screen for single and double crossover for creation of $P_{tet}$ - <i>srtB</i> strain
NF2261	CAGTTTAAAATTATTGTTTTA AACTGTGTCTG	Screen for single and double crossover for creation of $P_{tet}$ - <i>srtB</i> strain
NF2266	cagaattcgTATATCTATATTACAC TATTTTTGAATAAATTTAAAT TTTTAG	Construction of pJKP029 for creation of $P_{tet}$ - <i>srtB</i> strain
NF2267	atatagatataCGAATTCTGCATCAA GCTAG	Construction of pJKP029 for creation of $P_{tet}$ - <i>srtB</i> strain
NF2378	GATAGAGCTCAAAATGAAAG GAGCATTAGATTTATG	Amplify <i>CD3392</i> for pRFP185 cloning
NF2379	GATAGGATCCTTATGATTTCT TCATTTTACGGC	Amplify <i>CD3392</i> for pRFP185 cloning
NF2451	GGATCCTATAAGTTTTAATAA AACTTTAAATAG	Remove <i>CD3392</i> coding sequence by inverse PCR
NF2501	GATAGAGCTCGGAAGATTAG ATTATAAATTATAGGTAAC	Amplify <i>CD2831</i> for pRFP185 cloning
NF2502	GATAGGATCCTAATTTGTATT TTTATTTCTTCTTAATACGATA AG	Amplify <i>CD2831</i> 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 AAATTA AAAAATCAAGTATA ATC	Amplify <i>CD3246</i> for pRFP185 cloning
NF2861	attaaaacttataggatccTTAATTTCTT TTTATTTTACATTGATACGC	Amplify <i>CD3246</i> for pRFP185 cloning
NF2922	gtttttgtaccctaagtttCATACCATAT TATCCAATATGCT	Construction of pJKP088 for <i>zmpI</i> deletion
NF2923	atcaatattcCAAACCTTATAGCTTTT TGCAA AATTTAG	Construction of pJKP088 for <i>zmpI</i> deletion
NF2924	tataagtttgGAATATTGATATTAT AGCTATTAATAATTTTTTACTT G	Construction of pJKP088 for <i>zmpI</i> deletion
NF2925	gattatcaaaaaggagtttGGGGAGACT ATACGAAATG	Construction of pJKP088 for <i>zmpI</i> deletion
NF2926	CCTGTGCAAAGTTGGGTTAC	Screen for single and double crossover for <i>zmpI</i> deletion
NF2956	CTTTACCAGCATTGAAAAAT TAGCC	Screen for single and double crossover for <i>zmpI</i> 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	gtttttgtaccctaagtttCGTCTTCTTC TCTCTGATAG	Construction of pJKP096 for complementation of <i>srtB</i> mutant
NF2967	tcaatctacCATGAATCACCATTCT GGC	Construction of pJKP096 for complementation of <i>srtB</i> mutant
NF2968	tgattcatgGTAGATTGATTTAGAA ACTCACAGACAC	Construction of pJKP096 for complementation of <i>srtB</i> mutant
NF2969	gattatcaaaaaggagtttCGGAAAGAC CCCATGGAG	Construction of pJKP096 for complementation of <i>srtB</i> mutant
NF2970	GAAGGTCTTTATATAGGTTTA AAACC	Screen for single and double crossover for complementation of <i>srtB</i> mutant
NF2971	CGGATACCTGCGACAGG	Screen for single and double crossover for complementation of <i>srtB</i> mutant

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

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