

Supplementary Data

Supplementary Table S1. *C. perfringens* strains used in this study.

Strains	Relevant characteristic(s)	Reference
SM101	Wild-type strain; a transformable derivative of NCTC8798, a food-poisoning type F <i>C. perfringens</i> human strain; carries a chromosomal <i>cpe</i>	[1]
IH101	$\Delta spo0A$ (<i>spo0A::catP</i>) mutant of SM101	[2,3]
NM101	$\Delta sigE$ (<i>sigE::catP</i>) mutant of SM101	[4]
PKT136	$\Delta ylmC$ (<i>ylmC::ermB</i>) mutant of SM101	This study
PKT137	$\Delta bkdR$ (<i>bkdR::ermB</i>) mutant of SM101	This study
PKT139	$\Delta yyaC1$ (<i>yyaC1::ermB</i>) mutant of SM101	This study
PKT140	$\Delta yyaC2$ (<i>yyaC2::ermB</i>) mutant of SM101	This study
PKT141	$\Delta ytaF$ (<i>ytaF::ermB</i>) mutant of SM101	This study
PKT142	$\Delta ytxC$ (<i>ytxC::ermB</i>) mutant of SM101	This study
SM101(pPKT2)	Wild-type strain carrying <i>P_{ylmC}-gusA</i> fusion	This study
SM101(pPKT6)	Wild-type strain carrying <i>P_{bkdR}-gusA</i> fusion	This study
SM101(pPKT10)	Wild-type strain carrying <i>P_{ytaF}-gusA</i> fusion	This study
SM101(pPKT12)	Wild-type strain carrying <i>P_{ytxA}-gusA</i> fusion	This study
SM101(pPKT14)	Wild-type strain carrying <i>P_{ytxA}-gusA</i> fusion	This study
SM101(pPKT16)	Wild-type strain carrying <i>P_{ytxC}-gusA</i> fusion	This study
SM101(pPKT18)	Wild-type strain carrying <i>P_{yyaC1}-gusA</i> fusion	This study
SM101(pPKT20)	Wild-type strain carrying <i>P_{yyaC2}-gusA</i> fusion	This study
PKT140 (pPKT130)	$\Delta yyaC2$ mutant complemented with wild-type <i>yyaC2</i>	This study
PKT142 (pPKT125)	$\Delta ytxC$ mutant complemented with wild-type <i>ytxC</i>	This study

Supplementary Table S2. List of primers used in this study.

Primer name	Primer sequence (5'-3') ^a	Gene	Nucleotide position ^b	Use ^c
CPP1016	GCA GCG <u>TCG ACT</u> ATA TAT AAC AGC AAT TTG CGT GCC	<i>bkdR</i>	-383 to -358	GUS
CPP1017	GAC <u>GCT GCA</u> GAG TAT CTA GTG TGT GTA ATC CTA AGG	<i>bkdR</i>	+31 to +55	GUS
CPP1020	GCA GCG <u>TCG ACG</u> GAA CCT ACT GTA TCT CAA ATA GC	<i>ylmC</i>	-501 to -477	GUS
CPP1021	GAC <u>GCT GCA</u> GCC TTC ACT TAC ATC TAT CAC TTC C	<i>ylmC</i>	+41 to +65	GUS
CPP1018	GCA GCG <u>TCG ACA</u> AGA AGA ATA GTA CCT TAT CTC C	<i>ylxY</i>	-348 to -325	GUS
CPP1019	GAC <u>GCT GCA</u> GTG CCC AAT TCA CAT CAA AGG	<i>ylxY</i>	+166 to +186	GUS
CPP1022	GCA GCG <u>TCG ACG</u> AAG GTG AGA AAT TAG CTA GTG	<i>ylzA</i>	-441 to -419	GUS
CPP1023	GAC <u>GCT GCA</u> GTA AGC ATA CCT CTA TCT CTA GC	<i>ylzA</i>	+108 to +130	GUS
CPP1033	GCA GCG <u>TCG ACG</u> ATC ACA TAA GTA ATA GAG AAA GCC	<i>ytaF</i>	-231 to -206	GUS
CPP1034	GAC <u>GCT GCA</u> GGT TCC TAA AGC AGA TAT GGA TGC	<i>ytaF</i>	+117 to +140	GUS
CPP1162	GCA GCG <u>TCG ACT</u> CTG GAT GAC CAT CTA TAG CA	<i>ytxC</i>	-262 to -241	GUS
CPP1163	GAC <u>GCT GCA</u> GCT GAG ATA CTT TCT GAC ACA CCA A	<i>ytxC</i>	+94 to +117	GUS
CPP1182	GCA GCG <u>TCG ACT</u> TTT GAT GAT TCC TCC TCA GC	<i>yyaCl</i>	-468 to -447	GUS
CPP1183	GAC <u>GCT GCA</u> GGG TAC CAA CTA ATG GAC CTA AGC A	<i>yyaCl</i>	+126 to +150	GUS
CPP1184	GCA GCG <u>TCG ACC</u> CCT GTG CTA GGT ATG CCC TT	<i>yyaC2</i>	-332 to -311	GUS
CPP1185	GAC <u>GCT GCA</u> GAT CTC CTG TGG AGC GAT CAG A	<i>yyaC2</i>	+126 to +146	GUS
CPP1210	AAA AAA GCT TAT AAT TAT CCT TAC AGG TCC TTG AAG TGC GCC CAG ATA GGG TG	<i>bkdR</i>	IBS 357/358s	MP
CPP1211	CAG ATT GTA CAA ATG TGG TGA TAA CAG ATA AGT CCT TGA AAG TAA CTT ACC TTT CTT TGT	<i>bkdR</i>	EBS1d 357/358s	MP
CPP1212	TGA ACG CAA GTT TCT AAT TTC GGT TAC CTG TCG ATA GAG GAA AGT GTC T	<i>bkdR</i>	EBS2 357/358s	MP
CPP1207	AAA AAA GCT TAT AAT TAT CCT TAA GAA TCT TAT CTG TGC GCC CAG ATA GGG TG	<i>ylmC</i>	IBS 126/127s	MP
CPP1208	CAG ATT GTA CAA ATG TGG TGA TAA CAG ATA AGT CTT ATC TAT TAA CTT ACC TTT CTT TGT	<i>ylmC</i>	EBS1d 126/127s	MP
CPP1209	TGA ACG CAA GTT TCT AAT TTC GAT TAT TCT TCG ATA GAG GAA AGT GTC T	<i>ylmC</i>	EBS2 126/127s	MP
CPP1186	AAA AAA GCT TAT AAT TAT CCT TAA GGG TCC TTG AAG TGC GCC CAG ATA GGG TG	<i>ytaF</i>	IBS 98/99a	MP
CPP1187	CAG ATT GTA CAA ATG TGG TGA TAA CAG ATA AGT CCT TGA AAA TAA CTT ACC TTT CTT TGT	<i>ytaF</i>	EBS1d 98/99a	MP
CPP1188	TGA ACG CAA GTT TCT AAT TTC GAT TAC CCT TCG ATA GAG GAA AGT GTC T	<i>ytaF</i>	EBS2 98/99a	MP
CPP1189	AAA AAA GCT TAT AAT TAT CCT TAG TAG ACG ATT ATG TGC GCC CAG ATA GGG TG	<i>ytxC</i>	IBS 519/520s	MP
CPP1190	CAG ATT GTA CAA ATG TGG TGA TAA CAG ATA AGT CGA TTA TAT TAA CTT ACC TTT CTT TGT	<i>ytxC</i>	EBS1d 519/520s	MP
CPP1191	TGA ACG CAA GTT TCT AAT TTC GAT TTC TAC TCG ATA GAG GAA AGT GTC T	<i>ytxC</i>	EBS2 519/520s	MP
CPP1216	AAA AAA GCT TAT AAT TAT CCT TAA CCA TCA TAG TAG TGC GCC CAG ATA GGG TG	<i>yyaCl</i>	IBS 96/97s	MP
CPP1217	CAG ATT GTA CAA ATG TGG TGA TAA CAG ATA AGT CAT AGT ATG TAA CTT ACC TTT CTT TGT	<i>yyaCl</i>	EBS1d 96/97s	MP
CPP1218	TGA ACG CAA GTT TCT AAT TTC GAT TAT GGT TCG ATA GAG GAA AGT GTC T	<i>yyaCl</i>	EBS2 96/97s	MP
CPP1219	AAA AAA GCT TAT AAT TAT CCT TAG TAG GCC ACG TAG TGC GCC CAG ATA GGG TG	<i>yyaC2</i>	IBS 342/343s	MP
CPP1220	CAG ATT GTA CAA ATG TGG TGA TAA CAG ATA AGT CCA CGT ACT TAA CTT ACC TTT CTT TGT	<i>yyaC2</i>	EBS1d 342/343s	MP

CPP1221	TGA ACG CAA GTT TCT AAT TTC GAT TCC TAC TCG ATA GAG GAA AGT GTC T CGA AAT TAG AAA CTT GCG TTC AGT AAA C	<i>yyaC2</i>	EBS2 342/343s EBS Universal	MP MP
CPP1031	GCT TGC AAT ACT TTC TCT TAA GGT GT	<i>bkdR</i>		Detect
CPP1204	TGA GGA AGC TGA GAT GAT GGA	<i>bkdR</i>		Detect
CPP1092	GCG GGT ACC CGG TAT ATC TCA AGC GCA AGT TTC	<i>ylmC</i>		Detect
CPP1094	GCA GCG TCG ACA TCC ACG CCT TTC TCT AAG TTG	<i>ylmC</i>		Detect
CPP1194	TGG AGG CAG AAA AAG TGA GC	<i>ytaF</i>		Detect
CPP1195	GGG CTA AGG ATA AGG CTA GGG	<i>ytaF</i>		Detect
CPP1196	TCT CAT ATG GAA CTC ACT TCG TT	<i>ytxC</i>		Detect
CPP1197	TCC TAC TTC AGC AGC ATC TGT	<i>ytxC</i>		Detect
CPP1182	GCA GCG TCG ACT TTT GAT GAT TCC TCC TCA GC	<i>yyaC1</i>		Detect
CPP1183	GAC GCT GCA GGG TAC CAA CTA ATG GAC CTA AGC A	<i>yyaC1</i>		Detect
CPP1202	TCT GAT CGC TCC ACA GGA GA	<i>yyaC2</i>		Detect
CPP1203	GCT TAA GTC TCC TAC TTC AGG CA	<i>yyaC2</i>		Detect
CPP1198	TCT GGA TGA CCA TCT ATA GCA	<i>ytxC</i>		CP
CPP1199	GCA GCG TCG ACG CTG TAA GGT GAG AAA CGG C	<i>ytxC</i>		CP
CPP1236	GCG GGT ACC CCC CTG TGC TAG GTA TGC CCT T	<i>yyaC2</i>		CP
CPP1237	GCA GCG TCG ACA GCT TTC GAA GAC GTT GGA AGT	<i>yyaC2</i>		CP

^aRestriction sites are underlined.

^bNucleotide numbering begins at the first base of the translation codon of the relevant gene.

^c GUS, construction of plasmid for GUS assay; MP, construction of mutator plasmid; Detect, primers used for the detection of mutant isolates; CP, construction of complemented plasmid

Supplementary Table S3. List of plasmids used in this study.

Plasmids	Relevant characteristic(s)	Reference
pJIR3566	<i>C. perfringens</i> targetron plasmid derived from pJIR750ai, contains <i>ermB</i> ::RAM and <i>lacZ</i> α ; Cm ^R	[5]
pMRS127	<i>C. perfringens</i> / <i>E. coli</i> shuttle vector carrying a promoterless <i>gusA</i> ; Em ^R	[6]
pJIR750	<i>C. perfringens</i> / <i>E. coli</i> shuttle vector; Cm ^R	[7]
pPKT1	a 459-bp of putative promoter of <i>ylmC</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT2	a 459-bp of putative promoter of <i>ylmC</i> from pPKT1 cloned into pMRS127; Em ^R	This study
pPKT5	a 389-bp of putative promoter of <i>bkdR</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT6	a 389-bp of putative promoter of <i>bkdR</i> from pPKT5 cloned into pMRS127; Em ^R	This study
pPKT9	a 435-bp of putative promoter of <i>ytaF</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT10	a 435-bp of putative promoter of <i>ytaF</i> from pPKT3 cloned into pMRS127; Em ^R	This study
pPKT11	a 321-bp of putative promoter of <i>ylxY</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT12	a 321-bp of putative promoter of <i>ylxY</i> from pPKT3 cloned into pMRS127; Em ^R	This study
pPKT13	a 523-bp of putative promoter of <i>ylzA</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT14	a 523-bp of putative promoter of <i>ylzA</i> from pPKT3 cloned into pMRS127; Em ^R	This study
pPKT15	a 478-bp of putative promoter of <i>ytxC</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT16	a 478-bp of putative promoter of <i>ytxC</i> from pPKT3 cloned into pMRS127; Em ^R	This study
pPKT17	a 456-bp of putative promoter of <i>yyaC1</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT18	a 456-bp of putative promoter of <i>yyaC1</i> from pPKT3 cloned into pMRS127; Em ^R	This study
pPKT19	a 321-bp of putative promoter of <i>yyaC2</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT20	a 321-bp of putative promoter of <i>yyaC2</i> from pPKT3 cloned into pMRS127; Em ^R	This study
pPKT121	a 350-bp group-II intron retargeted to <i>ytaF</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT122	a 350-bp group-II intron retargeted to <i>ytxC</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT131	a 350-bp group-II intron retargeted to <i>ylmC</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT132	a 350-bp group-II intron retargeted to <i>bkdR</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT134	a 350-bp group-II intron retargeted to <i>yyaC1</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT135	a 350-bp group-II intron retargeted to <i>yyaC2</i> cloned into pCR-XL-TOPO; Kan ^R	This study
pPKT123	<i>ytaF</i> intron product from pPKT121 cloned into pJIR3566 (<i>ytaF</i> mutator plasmid); Cm ^R	This study
pPKT124	<i>ytxC</i> intron product from pPKT122 cloned into pJIR3566 (<i>ytxC</i> mutator plasmid); Cm ^R	This study
pPKT136	<i>ylmC</i> intron product from pPKT131 cloned into pJIR3566 (<i>ylmC</i> mutator plasmid); Cm ^R	This study
pPKT137	<i>bkdR</i> intron product from pPKT132 cloned into pJIR3566 (<i>bkdR</i> mutator plasmid); Cm ^R	This study
pPKT139	<i>yyaC1</i> intron product from pPKT134 cloned into pJIR3566 (<i>yyaC1</i> mutator plasmid); Cm ^R	This study
pPKT140	<i>yyaC2</i> intron product from pPKT135 cloned into pJIR3566 (<i>yyaC2</i> mutator plasmid); Cm ^R	This study

References

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