

Supplemental Material

Supplementary Tables

Table S1. Bacterial strains and plasmid used

Strain or plasmid	Relevant characteristic	Source or reference
<i>C. perfringens</i> strains		
SM101	Electroporatable derivative of food poisoning type A isolate NCTC8798; carries a chromosomal <i>cpe</i> gene	(1, 2)
DPS101	<i>gerKA::catP</i>	(3)
DPS119	<i>gerKA::ermB</i>	This study
DPS122	<i>gerKC::ermB</i>	This study
DPS124	<i>gerKB::catP gerAA::intron</i>	This study
DPS122(pSB18)	<i>gerKC</i> mutant expressing wild-type <i>gerKA-KC</i>	This study
Plasmids		
pJIR750ai	<i>C. perfringens/E. coli</i> shuttle vector containing an <i>L1.LtrB</i> intron retargeted to the <i>plc</i> gene	(4)
pJIR3566	<i>C. perfringens/E. coli</i> shuttle vector; Em ^r .	(5)
pJIR750	<i>C. perfringens/E. coli</i> shuttle vector; Cm ^r .	(6)
pDP276	~ 350-bp BsrGI-HinDIII fragment retargeted to <i>gerKC</i> cloned between BsrGI-HinDIII sites in pJIR3566	This study
pDP300	~350-bp BsrGI-HinDIII fragment retargeted to <i>gerKA</i> cloned between BsrGI-HinDIII sites in pJIR3566	This study
pDP10	~ 3.1- kb <i>gerKA-KC</i> operon in pMRS104	(3)
pSB18	~ 3.1-bp KpnI-XhoI fragment carrying <i>gerKA-KC</i> operon from pDP10 cloned between KpnI-SalI sites of pJIR750	This study

Table S2. Primers used

Primer name	Primer sequence ^a	Gene	Position ^b	Use ^c
CPP443	5' GATGAAAATGAAGTGGAAATATAGAC 3'	<i>gerKC</i>	+120 to +137	MD
CPP440	5' GTTGTGCCATTAATTCAACATCAACA 3'	<i>gerKC</i>	+1076 to +1103	MD
CPP209	5' TATA GTGAAAATCCAAGTATCTC 3'	<i>gerKA</i>	-224 to - 201	MD
CPP208	5' ATCATTATTATCACCTCTGCTACTAT 3'	<i>gerKA</i>	+980 to +1006	MD
CPP206	5' CAAGTATTAATCCTCCAATAACAG 3'	<i>gerAA</i>	+1102 to +1126	MD
CPP211	5' CTTAATGGGAATTATAGCA 3'	<i>gerAA</i>	-264 to -244	MD
CPP876	5' AAAAAGCTTATAATTATCCTTATTAGGCCAGCCG TGC GCCCAGATAGGGTG 3'	<i>gerKA</i>	IBS 91s	MP
CPP877	5' CAGATTGTACAAATGTGGTGATAACAGATAAGTCC CAGCCACTAACCTACCTTCTTGT 3'	<i>gerKA</i>	EBS1d 91s	MP
CPP878	5' TGAACGCAAGTTCTAATT CGGT CCTAATCGATA GAGGAAAGTGTCT 3'	<i>gerKA</i>	EBS2 91s	MP
CPP879	5' AAAAAGCTTATAATTATCCTTATTCGGTGT GTGC GCCCAGATAGGGTG 3'	<i>gerKC</i>	IBS 469s	MP
CPP880	5' CAGATTGTACAAATGTGGTGATAACAGATAAGTCG GTGTTTTAACCTACCTTCTTGT 3'	<i>gerKC</i>	EBS1d 469s	MP
CPP881	5' TGAACGCAAGTTCTAATT CGGTAAATCGATA GAGGAAAGTGTCT 3'	<i>gerKC</i>	EBS2 469s	MP

a- The nucleotide numbering begins from the translation start codon and refers to the relevant position within the respective coding sequence.

b- Nucleotide numbering being at the first base of the translation codon of the relevant gene.

c- MD, Mutation detection; MP, Construction of mutator plasmid.

References

1. **Zhao Y, Melville SB.** 1998. Identification and characterization of sporulation-dependent promoters upstream of the enterotoxin gene (*cpe*) of *Clostridium perfringens*. *J. Bacteriol.* **180**:136-142.
2. **Collie RE, McClane BA.** 1998. Evidence that the enterotoxin gene can be episomal in *Clostridium perfringens* isolates associated with non-food-borne human gastrointestinal diseases. *J. Clin. Microbiol.* **36**:30-36.
3. **Paredes-Sabja D, Torres JA, Setlow P, Sarker MR.** 2008. *Clostridium perfringens* spore germination: characterization of germinants and their receptors. *J. Bacteriol.* **190**:1190-1201.
4. **Chen Y, McClane BA, Fisher DJ, Rood JI, Gupta P.** 2005. Construction of an alpha toxin gene knockout mutant of *Clostridium perfringens* type A by use of a mobile group II intron. *Appl. Environ. Microbiol.* **71**:7542-7547.
5. **Cheung JK, Keyburn AL, Carter GP, Lanckriet AL, Van Immerseel F, Moore RJ, Rood JI.** 2010. The VirSR two-component signal transduction system regulates NetB toxin production in *Clostridium perfringens*. *Infect. Immun.* **78**:3064-3072.
6. **Bannam TL, Rood JI.** 1993. *Clostridium perfringens-Escherichia coli* shuttle vectors that carry single antibiotic resistance determinants. *Plasmid* **29**:233-235.