Supplementary materials

A sporulation factor is involved in morphological change of *Clostridium perfringens* biofilm in response to temperature

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Supplementary materials and methods

Plasmids. The *spo0A*-expression vector, pSpo0A was constructed as indicated below. The DNA fragment containing the *spo0A* promoter, *spo0A* ORF and the intrinsic transcriptional terminator was amplified through polymerase chain reaction (PCR) using the primers NOB-0426/NOB-0427 from the genomic DNA of *C. perfringens*. The amplified DNA was digested with EcoRI and BamHI and cloned into the same restriction enzyme site of pJIR418. We attempted to construct an *abrB*-expression vector using the same manner, but these attempts were unsuccessful. We used a lactose-inducible *bgaL* promoter for *abrB* expression (1). The DNA fragments containing the *abrB* ORF and the intrinsic transcriptional terminator or *bgaR* gene and *bgaL* promoter region were PCR amplified using the primers NOB-0478/NOB-0479 or NOB-0488/NOB-0489 from the genomic DNA of *C. perfringens*. These fragments were digested with BamHI and SalI or SacI and BamHI, respectively, and cloned into the SacI/SalI site of pJIR418. The resulting plasmid was named pCPO0281.

REFFERENCE

1. **Hartman AH, Liu H, Melville SB**. 2011. Construction and characterization of a lactose-inducible promoter system for controlled gene expression in Clostridium perfringens. Applied and environmental microbiology **77**:471–8.

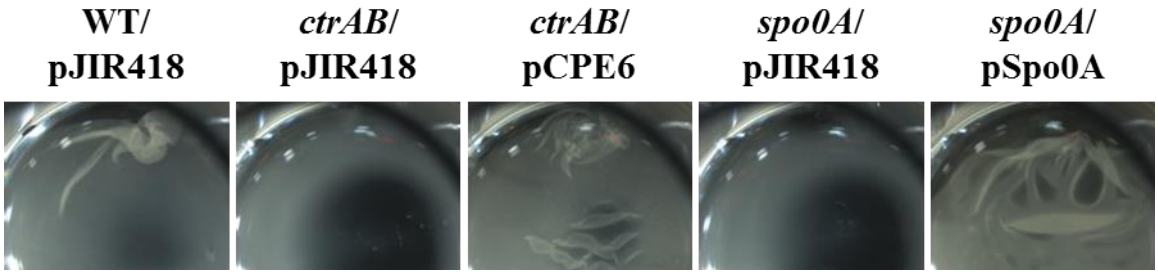
Supplementary table 1. Oligonucleotides used in this study

Name	Sequence 5' to 3'	Used for
NOB-0428	GGCCtctagaGTCCAGCGCAAAATGGTGGA	*spo0A* mutant construction
NOB-0429	GCGCgaattcTCCTTAACCAACTTTATATATCCA	*spo0A* mutant construction
NOB-0495	GGCCggatccTCATGAAATAGGAGTTCCAG	*spo0A* mutant construction
NOB-0496	GCGCgtcgacTTTAAGTTCTCGTAAGGCTG	*spo0A* mutant construction
NOB-0436	GGCCgtcgacGCAAATACTTTTTTGATCTCATCGG	*abrB* mutant construction
NOB-0437	GCGCgaattcTCCTCCTTGCATTTTACAACTTTCG	*abrB* mutant construction
NOB-0438	GGCCggatccTTTAACACTATATCCCCTTATAGTG	*abrB* mutant construction
NOB-0439	GCGCtctagaGAGGATACAAGACAAACACTTAAG	*abrB* mutant construction
NOB-0576	GGCCgtcgacTAGGGAAGCCTTAATTGGAG	*pilA2* mutant construction
NOB-0577	GCGCgaattcCATTGGTTTTCCTCCTTAAA	*pilA2* mutant construction
NOB-0578	GGCCgaattcAGAAATAATCAATAGTATTAAAAG	*pilA2* mutant construction
NOB-0579	GCGCggatccAGGCCCTTCTTCTCTATTCA	*pilA2* mutant construction
NOB-0538	GAAAGGGTTCACGCTAATTG	*pilA2* probe
NOB-0539	ACTGATGCTGATGTGTTTGA	*pilA2* probe
NOB-0426	GGCCGAATTCAGAGTGGATGTTAAAAGATG	*spo0A* probe
NOB-0427	GCGCGGATCCCAAATTATCTCACCTCTCTA	*spo0A* probe
NOB-0434	GGCCCCATGGAATCAACAGGTGTAGTAAGAAGAG	*abrB* probe
NOB-0435	GCGCGGATCCTTTTTTTTAATTCATCTAAACA	*abrB* probe
NOB-0234	GGCCGAATTCCAGGAAACAGCTATGACATG	*ermBP* fragment amplification
NOB-0240	GCGCggatccTTTCAACTTGCCCACTTCGA	*ermBP* fragment amplification
NOB-0478	GGCCggatccAAATAATTTTCTATACGACA	*abrB* ORF amplification
NOB-0479	GCGCgtcgacTTAGCTAAAGGGAAATTTAG	*abrB* ORF amplification
NOB-0488	GGCCgagctcAAGTCTAATTAAGACTTTAG	*bgaL* promoter amplification
NOB-0489	GCGCggatccCATTTTACCCTCCCAATACA	*bgaL* promoter amplification

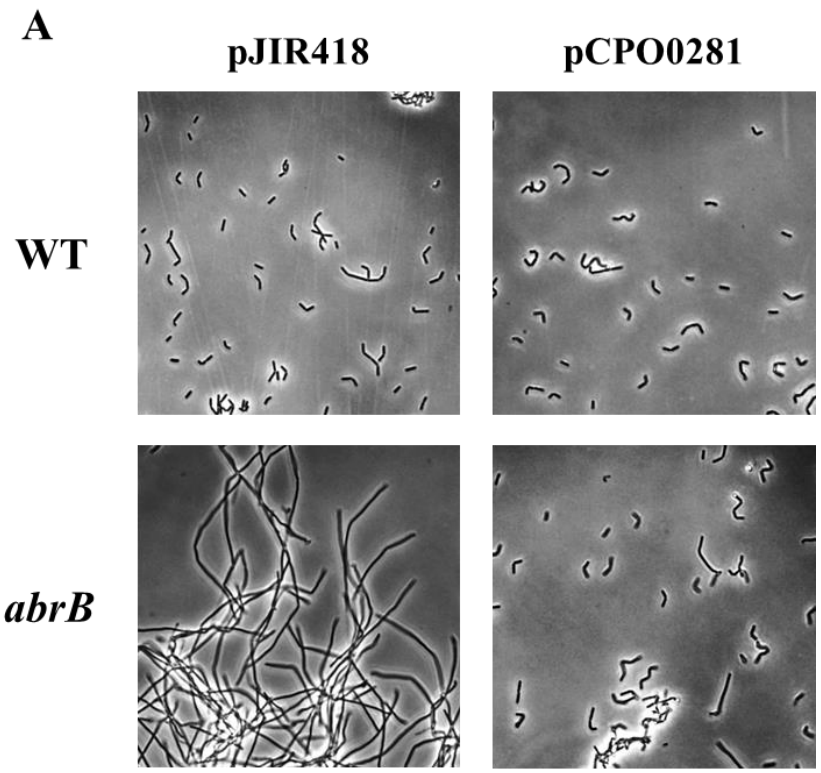
Supplementary fig. 1. Pellicle biofilm formation in *spo0A* and *ctrAB* mutants is complemented using the *spo0A* or *ctrAB*-expression vector. wild-type *C. perfringens* harboring the empty vector, pJIR418, the *spo0A* mutant harboring pJIR418 or the *spo0A*-expression vector, pSpo0A and the *ctrAB* mutant harboring pJIR418 or the *ctrAB*-expression vector, pCPE6 were cultured at 25°C for 2 days. The edge of the pellicle biofilm formed at the bottom of wells was picked and flipped through gentle pipette aspiration.

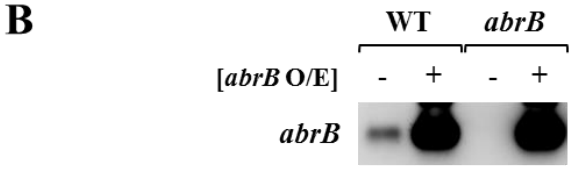
Supplementary fig. 2. The *abrB* mutant cells are elongated and aggregated. (A) Phase contrast microscopy images of *C. perfringens* wild-type and *abrB* mutant cells harboring pJIR418 and the *abrB*-expression vector, pCPO0281. The *abrB* gene in pCPO0281 is expressed under the control of lactose-inducible *bgaL* promoter. The cells were cultured in GAM broth supplemented with 1 mM lactose, which induces the expression of *abrB* in pCPO0281. (B) Northern blot analysis of *abrB* mRNA in wild-type and *abrB* mutant strains harboring pCPO0281. The minus and plus signs indicate that the cells were grown in the absence and presence of 1 mM lactose, respectively. (C) *C. perfringens* wild-type and *abrB* mutant harboring pCPO0281 were cultured with or without 1 mM lactose. The cells were aggregated in the absence of *abrB* expression.

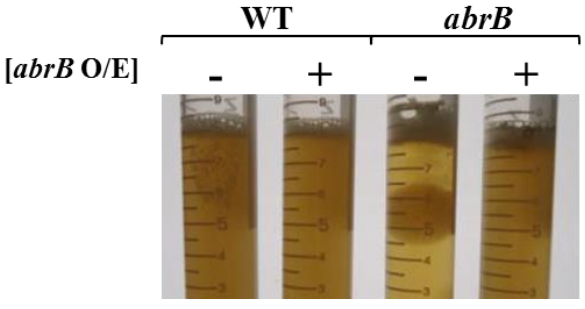
Supplementary fig. 3. Toxin expression in the transcriptional regulator mutants at different temperatures. Northern blot analysis of the genes encoding major toxins in *C. perfringens* using total RNA (2 μg) extracted from the mutant cells grown to the mid-exponential phase at 25 or 37°C. The 23 and 16S rRNAs stained with methylene blue are indicated at the bottom as loading controls.



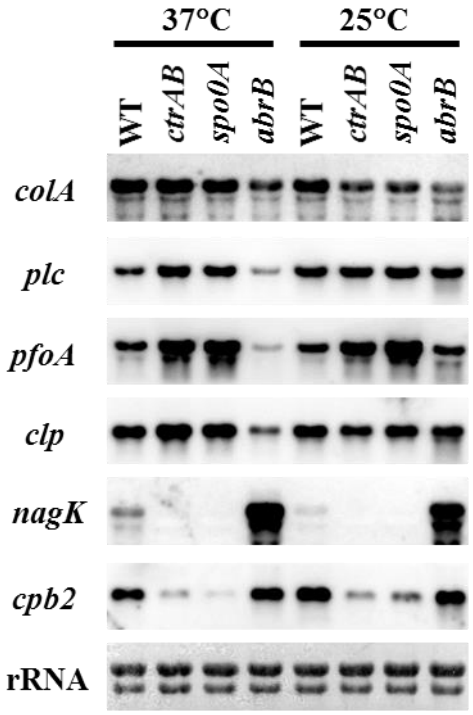
Supplementary fig. 1







Supplementary fig. 2



Supplementary fig. 3