

Dynamics of germinosome formation and FRET-based analysis of interactions between GerD and germinant receptor subunits in *Bacillus cereus* spores

Supplementary Materials

Table S1. Average FRET efficiency in individual spores of various strains normalized by donor channel as described in Methods.

Fusion protein(s) from plasmid*		Names of plasmid	Number of spores	mean	95%CI Mean
negative control					
SASP-SGFP2	GerD-mScarlet-I	pHT315-f13	191	0	0 ~ 0
positive control					
GerD-SGFP2-mScarlet-I		pHT315-f11	237	0.08	0.07 ~ 0.08
interaction					
GerR(A-C-B)-SGFP2	GerD-mScarlet-I	pHT315-f06	489	0.06	0.06 ~ 0.07
GerRB-SGFP2	GerD-mScarlet-I	pHT315-f09	179	0.02	0.02 ~ 0.02
GerRA-SGFP2	GerD-mScarlet-I	pHT315-f07	323	-0.04	-0.05 ~ -0.04
GerRC-SGFP2	GerD-mScarlet-I	pHT315-f08	81	-0.01	-0.01 ~ 0

*A shuttle vector originally for *E. coli* and *B. thuringiensis*.

Table S2. Primers used in this study

primers	sequence	purpose or function
315_YW1	CGGGGTACCCACCTTCCCTATATCCGCT	<i>Kpn</i> I- <i>gerR</i> promoter-Fw
315_YW2	CCGGAATTCACCTTCCCTATATCCGCT	<i>Eco</i> R I- <i>gerR</i> promoter-Fw
315_YW3	CGGGATCCTCTCACCCCTAACATATATC	<i>Bam</i> H I- <i>gerR</i> promoter-Rv
315_YW4	CGGGATCCATGTTTCGGTTTATCATCT	<i>Bam</i> H I- <i>gerRA</i> -Fw
315_YW5	CTCGCCCTTGCTCACCATgctgccgctgccgctgccT TCATTCTCACCATCACGTT	<i>gerRA</i> -(GS) ₃ -SGFP2-Rv
315_YW6	AACGTGATGGTGAGAATGAAGgcagcggcagcg gcagcATGGTGAGCAAGGGCGAG	<i>gerRA</i> -(GS) ₃ -SGFP2-Fw
315_YW7	CGGGATCCGAATGAATGAAGAAAATTTTA	<i>Bam</i> H I- <i>gerRC</i> -Fw
315_YW8	CTCGCCCTTGCTCACCATgctgccgctgccgctgccT TCCCCGATTCCAGATTGT	<i>gerRC</i> -(GS) ₃ -SGFP2-Rv
315_YW9	ACAATCTGGAATCGGGGAAGgcagcggcagcggc agcATGGTGAGCAAGGGCGAG	<i>gerRC</i> -(GS) ₃ -SGFP2-Fw
315_YW10	CGGGATCCTGAGGTGAAATGAGCAATGA	<i>Bam</i> H I- <i>gerRB</i> -Fw
315_YW11	CTCGCCCTTGCTCACCATgctgccgctgccgctgccA GGTGTATCGGTTGAAGA	<i>gerRB</i> -(GS) ₃ -SGFP2-Rv
315_YW12	TCTTCAACCGATACACCTggcagcggcagcggcagc ATGGTGAGCAAGGGCGAG	<i>gerRB</i> -(GS) ₃ -SGFP2-Fw
315_YW13	CCCAAGCTTTTACTTGTACAGCTCGTCCAT	SGFP2- <i>Hind</i> III-Rv

315_YW14	AATCTGGAATCGGGGAATAGTGAGGTGAA ATGAGCAATG	<i>gerRC-gerRB-Fw</i>
315_YW15	CATTGCTCATTTCACCTCACTATTCCCCGAT TCCAGATT	<i>gerRC-gerRB-Rv</i>
315_YW16	<u>GCTCTAG</u> ACAACCATAAAGAACAGAGC	<i>Xba I-gerD promoter-Fw</i>
315_YW17	TTCTCCTTTACTCACCATgctgccgtgccgtgccCT GTTCTTCCTTCTTCTCG	<i>gerD-(GS)₃-mScarlet-I-Rv</i>
315_YW18	CGAGAAGAAGGAAGAACAGggcagcggcagcgg cagcATGGTGAGTAAAGGAGAA	<i>gerD-(GS)₃-mScarlet-I-Fw</i>
315_YW19	<u>GCTCTAG</u> ATTATTTGTATAGTTCATCCAT	<i>mScarlet-I-Xba I-Rv</i>
315_YW20	CCGGAATTCTTATTTGTATAGTTCATCCAT	<i>mScarlet-I-EcoR I-Rv</i>
315_YW21	<u>GCTCTAG</u> ACACCTTCCCTATATCCGCT	<i>Xba I-gerR promoter-Fw</i>
315_YW22	GCTGAAAGGAGTTCCAACCTTATGAAACGGA TGCTATT	<i>gerD promoter-gerD-Fw</i>
315_YW23	AATAGCATCCGTTTCATAAGTTGGAACCTC TTTCAGC	<i>gerD promoter-gerD-Rv</i>
315_YW24	GCATGGACGAGCTGTACAAGggcagcggcagc gcagcATGGTGAGTAAAGGAGAA	<i>SGFP2-(GS)₃-mScarlet-I-Fw</i>
315_YW25	TTCTCCTTTACTCACCATgctgccgtgccgtgccCT TGTACAGCTCGTCCATGC	<i>SGFP2-(GS)₃-mScarlet-I-Rv</i>
315_YW26	<u>CCCAAGC</u> TTTTATTTGTATAGTTCATCCAT	<i>mScarlet-I-Hind III-Rv</i>
315_YW27	<u>GGGGTACC</u> CTTCACCTTGTTCCGTTT	<i>Kpn I-SASP promoter-Fw</i>
315_YW28	CAATTAGGCGGTTACCAAAAaggcagcggcagc ggcagcATGGTGAGCAAGGGCGAG	<i>SASP-(GS)₃-SGFP2-Fw</i>
315_YW29	CTCGCCCTTGCTCACCATgctgccgtgccgtgccT TTTTGGTAACCGCCTAATTG	<i>SASP-(GS)₃-SGFP2-Rv</i>
315_YW30	<u>GGGGTACC</u> ACAACCATAAAGAACAGAGC	<i>Kpn I-gerD promoter-Fw</i>
315_YW31	CTCGCCCTTGCTCACCATgctgccgtgccgtgccC TGTTCTTCCTTCTTCTCG	<i>gerD-(GS)₃-SGFP2-Rv</i>
315_YW32	CGAGAAGAAGGAAGAACAGggcagcggcagcgg cagcATGGTGAGCAAGGGCGAG	<i>gerD-(GS)₃-SGFP2-Fw</i>
315_YW33	CTCTTCGCTATTACGCCA	T vector-sequencing-Fw
315_YW34	TCACACAGGAAACAGCTA	T vector-sequencing-Rv
315_YW35	ATGTTGTGTGGAATTGTGAG	315-sequencing-Fw
315_YW36	AAGGCGATTAAGTTGGGT	315-sequencing-Rv
315_YW37	CAAAGACCCCAACGAGAA	SGFP2-sequencing-Fw
315_YW38	GCTGGGGAAAATAAGTATGA	<i>gerRC</i> -sequencing-Fw
315_YW39	GCGCTACAATCCAAACAAA	<i>gerRA</i> -sequencing-Fw
315_YW40	CCGGAATTCATGAAACGGATGCTATTC	<i>EcoR I-gerD-Fw</i>
315_YW41	CGTTTTGGACTAGGGATG	<i>gerD promoter-sequencing-Rv</i>

a. Restriction cleavage sites are underlined.

b. The flexible linker (GS)₃ coding sequence is written in lower-case letters.

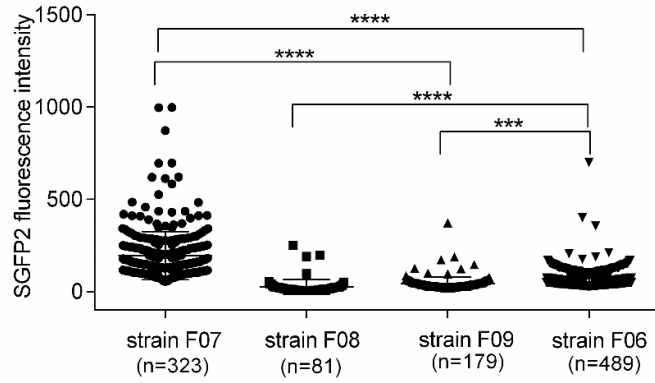


Fig S1. The SGFP2 fluorescence intensities of individual dormant spores of *B. cereus* with wide-field microscopic imaging. The SGFP2 fluorescence intensities of strains F07 (GerRA-SGFP2 and GerD-mScarlet-I), F08 (GerRC-SGFP2 and GerD-mScarlet-I), F09 (GerRB-SGFP2 and GerD-mScarlet-I), and F06 (GerR(A-C-B)-SGFP2 and GerD-mScarlet-I) were measured by the ObjectJ plugin SporeAnalyzer_1c.ojj. ***: $p < 0.001$ and ****: $p < 0.0001$.