A σ^W-dependent stress response in *Bacillus subtilis* that reduces membrane fluidity

Anthony W. Kingston, Chitra Subramanian, Charles O. Rock, and John D. Helmann

Table S1: Consensus P_5 nucleotide sequences in *B. subtilis* and related species

Strain(s)	Consensus P_5 nucleotide sequence[a]	# of *fabH* genes	# of σ^{ECF}
Bacillus subtilis	GTTGAAACTTCTGACGAGTGGATTCGTACAAGAACA	2	7
Consensus from 6 species closely related to B. subtilis[b]		2	7-10
Consensus from 4 *Bacillus ACT* species[c]		2	7-10
Consensus from 17 *Listeria* species[d]		1	1
Consensus from 17 *Staphylococcus* species[d]		1	1
Amino acid consensus from all analyzed strains		NA	NA

[a] For each sequence, the region corresponding to the P5 -35 and -10 elements is underlined.
[b] *Bacillus subtilis subsp. subtilis str. 168, Bacillus subtilis subsp. spizizenii str. W23, Bacillus licheniformis ATCC 14580, Bacillus atrophaeus 1942, Bacillus pumilus SAFR-032,* and *Bacillus amyloliquefaciens DSM7*
[c] *Bacillus anthracis str. Sterne, Bacillus thuringiensis str. Al Hakam, Bacillus cereus ATCC 14579,* and *Bacillus cereus subsp. cytotoxis NVH 391-98*
[d] Includes all 17 *listeria/Staphylococcus* genomes on the U Chicago SEED server (http://www.theseed.org)

Note: Residues W38, R42, and T43 are part of the FabH substrate binding site. W38 and T43 (last residue shown) are highly conserved with R42 showing some variability (Gajiwala *et al.*, 2009).

Table S2: Bacterial strains, plasmids, and oligos used in this study.

Strain, plasmid, or oligo	Genotype or description	Source, reference, or construction[1]
B. subtilis strains		
W168	_trpC2_	Lab Stock
HB0020	_CU1065 sigW::MLS_	(Cao _et al._, 2001)
HB6208	_W168 sigW::spec_	(Butcher and Helmann, 2006)
HB13001	_W168 amyE::P$_{fabHaF}$-P$_5$-lacZ_	pTK001 --> W168
HB13042	_W168 amyE::P$_{xyl}$-sigW_	pVG003 --> W168
HB13054	_W168 thrC::P$_{fabHaF}$-fabHa-FLAG_	pTK013 --> W168
HB13056	_W168 thrC::P$_{fabHaF}$-fabHa-fabF-FLAG_	pTK015 --> W168
HB13058	_W168 thrC::P$_{fabHaF}$-fabHa-FLAG amyE::P$_{xyl}$-sigW_	HB13042 chrDNA --> HB13054
HB13060	_W168 thrC::P$_{fabHaF}$-fabHa-fabF-FLAG amyE::P$_{xyl}$-sigW_	HB13042 chrDNA --> HB13056
HB13069	_W168 amyE::P$_5$-lacZ_	pTK022 --> W168
HB13077	_W168 thrC::P$_{fabHaF}$-fabHa-FLAG sigW::kan_	HB0020 chrDNA --> HB13054
HB13078	_W168 thrC::P$_{fabHaF}$-fabHa-fabF-FLAG sigW::kan_	HB0020 chrDNA --> HB13056
HB13080	_W168 amyE::P$_5$*-lacZ_	pTK031 --> W168
HB13082	_W168 P$_{fabHaF}$-P$_5$*-lacZ_	pTK033 --> W168
HB13099	_W168 amyE::P$_5$-lacZ sigW::MLS_	HB0020 chrDNA --> HB13069
HB13115	_W168 fabHb::kan_	LFH-PCR --->168
HB13117	_W168 P$_{fabHaF}$-fabHa(P$_5$)fabF_	pTK043 --> W168
HB13118	_W168 P$_{fabHaF}$-fabHa(P$_5$*)fabF_	pTK044 --> W168
HB13121	_W168 P$_{fabHaF}$-fabHa(P$_5$)fabF amyE::P$_{xyl}$-sigW_	HB13042 chrDNA --> HB13117
HB13122	_W168 P$_{fabHaF}$-fabHa(P$_5$*)fabF amyE::P$_{xyl}$-sigW_	HB13042 chrDNA --> HB13118
HB13123	_W168 thrC::P$_{fabHaF}$-fabHa(P$_5$*)-FLAG_	pTK045 --> W168
HB13124	_W168 thrC::P$_{fabHaF}$-fabHa(P$_5$*)-fabF-FLAG_	pTK046 --> W168
HB13125	_W168 thrC::P$_{fabHaF}$-fabHa(P$_5$*)-FLAG amyE::P$_{xyl}$-sigW_	HB13042 chrDNA --> HB13123
HB13126	_W168 thrC::P$_{fabHaF}$-fabHa(P$_5$*)-fabF-FLAG amyE::P$_{xyl}$-sigW_	HB13042 chrDNA --> HB13124
HB13127	_W168 fabHa::MLS_	LFH-PCR --->168
HB13128	_W168 amyE::P$_{xyl}$-fabF_	pTK047 --> W168
HB13132	_W168 fabHa::MLS amyE::P$_{xyl}$-fabF_	HB13128 chrDNA --> HB13127
BSU2006	_W168 ΔsigM ΔsigY ΔsigZ ΔsigV ΔylaC ΔsigX_	(Asai _et al._, 2008)
HB13151	_W168 ΔsigM ΔsigY ΔsigZ ΔsigV ΔylaC ΔsigX P$_5$-lacZ_	HB13069 chrDNA --> BSU2006
Plasmids		
pdg1661	Vector for integration of _lacZ_ fusions at _amyE_	(Guerout-Fleury _et al._, 1996)
pdg1664	Vector for integration at _thrC_ locus	(Guerout-Fleury _et al._, 1996)
pMUTIN	Vector for allelic replacement	(Vagner _et al._, 1998)
pVG003	_pSWEET-sigW (cat)_	Veronica Guariglia-Oropeza (unpublished results)
pTK001	_pDG1661-P$_{fabHaF}$-P$_5$-lacZ (cat)_	This work
pTK013	_pDG1664-P$_{fabHaF}$-fabHa-FLAG (MLS)_	This work
pTK015	_pDG1664-P$_{fabHaF}$-fabHa-fabF-FLAG (MLS)_	This work
pTK022	_pDG1661-P$_5$-lacZ (cat)_	This work
pTK031	_pDG1661-P$_5$*-lacZ (cat)_	This work
pTK033	_pDG1661-P$_{fabHaF}$-P$_5$*-lacZ (cat)_	This work
pTK043	_pMUTIN- P$_{fabHaF}$-P$_5$ (MLS)_	This work

pTK044	*pMUTIN- P$_{fabHaF}$-P$_5$* (MLS)*	This work
pTK045	*pDG1664-P$_{fabHaF}$-fabHa(P$_5$*)-FLAG (MLS)*	This work
pTK046	*pDG1664-P$_{fabHaF}$-fabHa(P$_5$*)-fabF-FLAG (MLS)*	This work
pTK047	*pSWEET-fabF (cat)*	This work

Oligos	Name	Sequence
4520	P$_5$ GSP1	CGTACATGAAGCCCGCACA
4521	P$_5$ GSP2	CCGAGTTGTTCTTGAATCATA
4549	AAP	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG
4778	FabHa UP BamHI	AGCTGGATCCCAACTGCATACGCCTCCTT
4780	FabHa Flag DO EcoRI	AGCTGAATTCTTATTATTTATCATCATCATCTTTATAATCCGGCCGAACGACCACATCGCCGTCT
4781	FabF Flag DO EcoRI	AGCTGAATTCTTATTATTTATCATCATCATCTTTATAATCCGGCCGGAATCCTAATGAGTTGCTGA
4883	P$_5$ Fwd cgGa -10 element	TCTGACGAGTGGATTCGGACAAGAACAGGAATAGAAG
4884	P$_5$ Rev cgGa -10 element	TCTATTCCTGTTCTTGTCCGAATCCACTCGTCAGAAG
4576	P$_{fabHaF}$-P$_5$ UP EcoRI	AGTCGAATTCCAACTGCATACGCCTCCT
4577	P$_5$ DO BamHI	AGTCGGATCCCGTACATGAAGCCCGCACA
4852	P$_5$ UP EcoRI	AGTCGAATTCAGGATAGAATTAGTACCTGATA
5059	fabHb up-fwd	CGTACAAAACAAGCAGAGACA
5060	fabHb up-rev (kan)	CCTATCACCTCAAATGGTTCGCTGTCACTCCTTATGGTCAGATTA
5061	fabHb do-fwd (kan)	CGAGCGCCTACGAGGAATTTGTATCGGATTAACCTATACAGGATTGCT
5062	fabHb do-rev	GACAGGAAGCAGCCAAGTAT
5164	P$_{fabHaF}$-up-rev (MLS)	GAGGGTTGCCAGAGTTAAAGGATCGATGTGTTTTCAAGAATGATGT
5165	P$_{fabHaF}$-do-fwd (MLS)	CGATTATGTCTTTTGCGCAGTCGGCCAACTGCATACGCCTCCTT
5166	P$_{fabHaF}$-do-rev	TTAGTCATTAGGGAAGACTCCTTTATATCT
5167	fabF-do-fwd	AGATATAAAGGAGTCTTCCCTAATGACTAAAAAAAGAGTAGTTGT
5168	fabF UP RBS PacI	AGTCTTAATTAA*AGGAGG*ACACAAGATGACTAAAAAAAGA
5169	fabF DO BamHI	AGTCGGATCCTCATGTGATCGCCTCCTCT

Other Bacillus Strains	Bacillus Genetic Stock Center (BGSC) #
B. subtilis ssp. spizizenii NRRL B-23049	2A8^T
B. subtilis ssp. spizizenii NRRL B-14472	2A9
B. licheniformis ATCC14580	5A36T
B. amyloliquefaciens FZB42	NA
B. atrophaeus NRS-213	11A2T
B. atrophaeus ESM rplV str	12A1

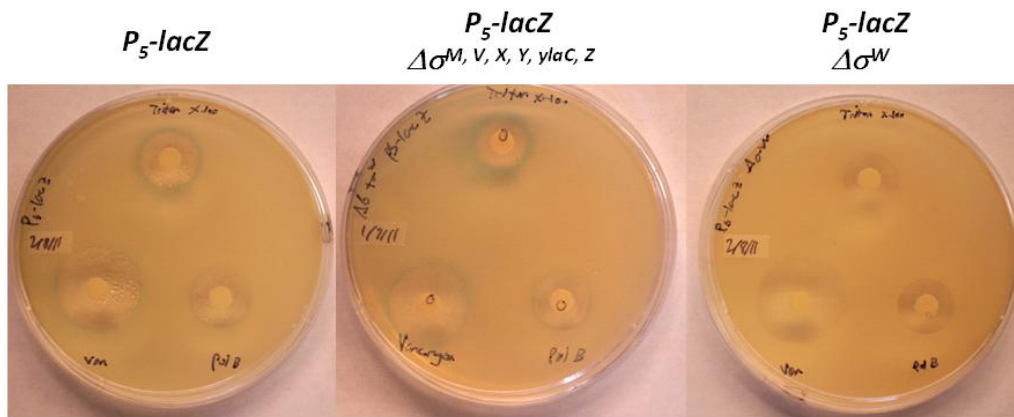[1] Abbreviations used: --> indicates transformation; chrDNA indicates chromosomal DNA.

Figure S1: Disk diffusion assays to monitor induction of *P₅-lacZ* by cell envelope antibiotics. Representative results are shown for HB13069 (*P₅-lacZ*), HB13151 (*P₅-lacZ ΔsigM ΔsigY ΔsigZ ΔsigV ΔylaC ΔsigX),* and HB13099 (*P₅-lacZ sigW::MLS*) under triton X-100 (top disk), vancomycin (bottom left disk), and polymyxin B (bottom right disk) treatments. Pictures are representative of at least three independent experiments performed with biological triplicates.
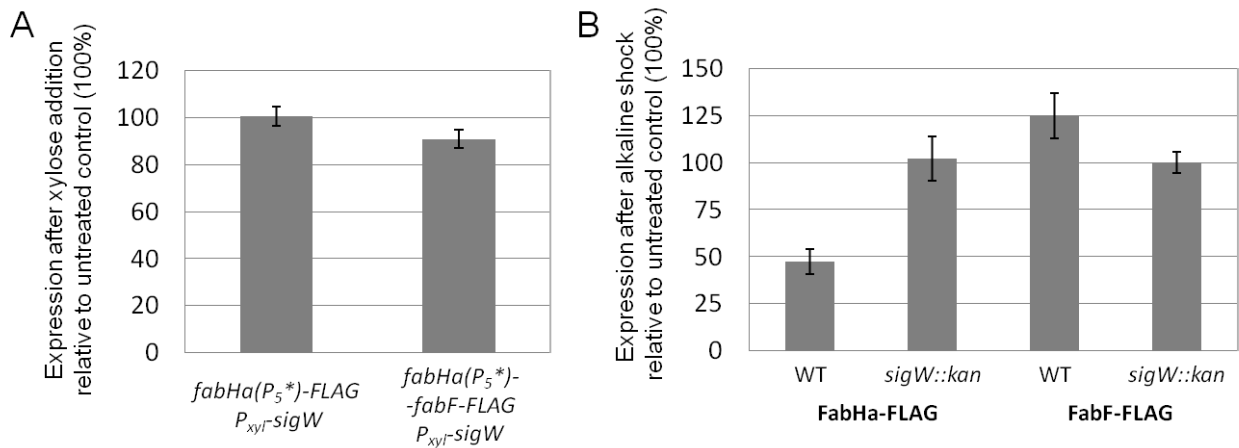
Figure S2: Supplementary Western blot quantification.

A. Change in expression of FabHa-FLAG in *fabHa(P₅*)-FLAG P_{xyl}-sigW* (HB13125) and of FabF-FLAG in *fabHa(P₅*)-fabF-FLAG P_{xyl}-sigW* (HB13126) upon xylose treatment. This experiment was performed in biological triplicate and repeated at least three times. Bars represent the relative levels of FabHa-FLAG or FabF-FLAG compared to an untreated control (error bars indicate standard error). Student's *t* tests showed no statistically significant difference (P value $\geq$ 0.05) between the xylose and non-xylose treated cells for both FabHa-FLAG and FabF-FLAG expression.

B. Change in expression of FabHa-FLAG and FabF-FLAG levels following alkaline shock in strains *fabHa-FLAG* (HB13054), *fabF-FLAG* (HB13056), *fabHa-FLAG sigW::kan* (HB13077), and *fabF-FLAG sigW::kan* (HB13078). Bars represent the relative levels of FabHa-FLAG or FabF-FLAG compared to an untreated control with error bars indicating standard error. Student's *t* tests showed a statistically significant difference (P value $\leq$ 0.05) between the *fabHa-FLAG* and *fabHa-FLAG sigW::kan* strains, but a non-significant difference (P value $\geq$ 0.05) between the *fabF-FLAG* and *fabF-FLAG sigW::kan* strains.
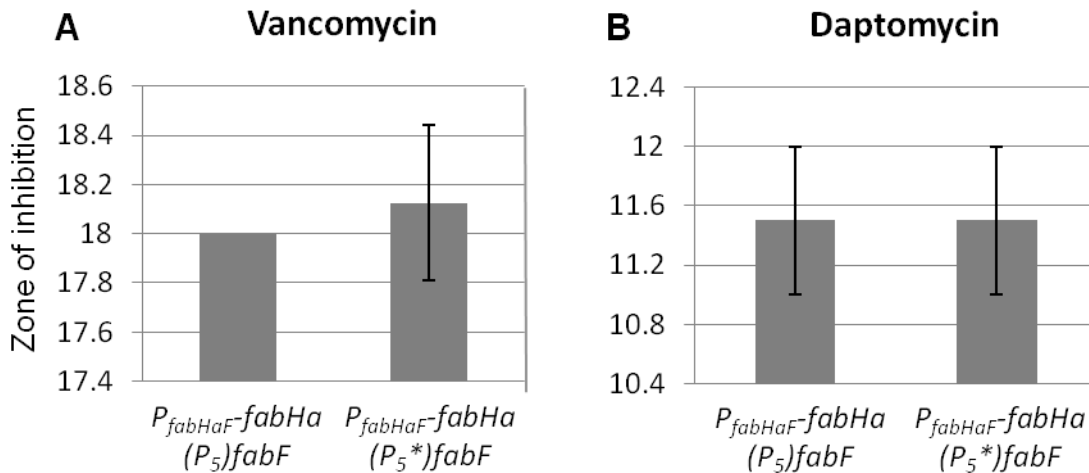
A Vancomycin   **B** Daptomycin

Figure S3: Disk diffusion assays of vancomycin (A) and daptomycin (B) sensitivity in the P_{fabHaF}-fabHa(P_5)fabF (HB13117), and P_{fabHaF}-fabHa(P_5*)fabF (HB13118) strains. Each bar represents the average zone of inhibition of at least three assays performed with three independent clones of each strain. Error bars represent standard error. Student's *t* tests were performed, and the zones of inhibition for the two strains were not significantly different (*P* value $\geq$ 0.05) under vancomycin or daptomycin treatment.
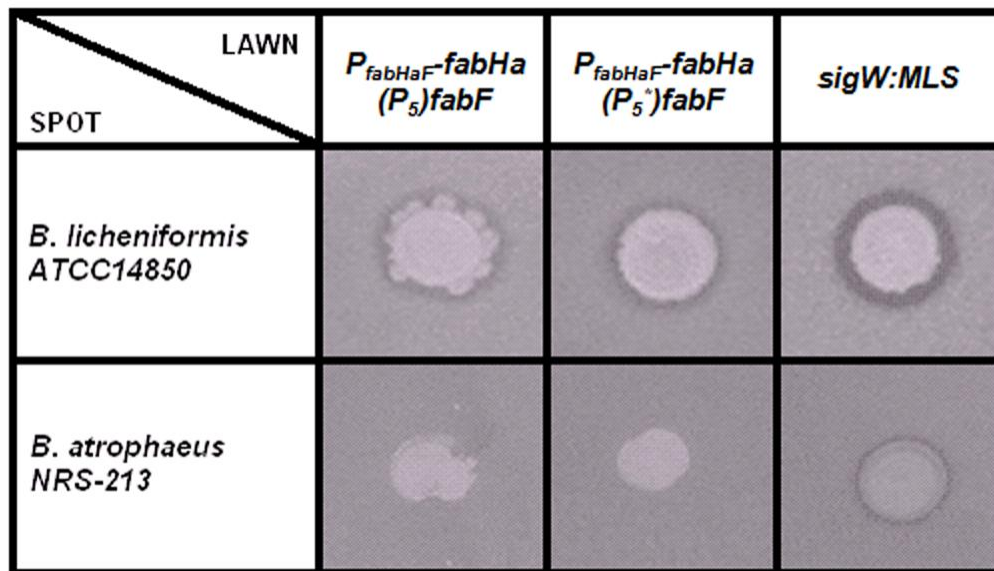

Figure S4: Spot on lawn assays showing the sensitivity of the P_{fabHaF}-fabHa(P_5)fabF (HB13117), P_{fabHaF}-fabHa(P_5*)fabF (HB13118), and *sigW::spec* (HB6208) lawns to *B. licheniformis ATCC14850* and *B. atrophaeus NRS-213*. Pictures are representative of at least three assays performed with three independent clones of each strain.

References

Asai, K., Ishiwata, K., Matsuzaki, K., and Sadaie, Y. (2008) A viable *Bacillus subtilis* strain without functional extracytoplasmic function sigma genes. *J Bacteriol* **190**: 2633-2636.

Butcher, B.G., and Helmann, J.D. (2006) Identification of *Bacillus subtilis* σ^W-dependent genes that provide intrinsic resistance to antimicrobial compounds produced by Bacilli. *Mol Microbiol* **60**: 765-782.

Cao, M., Bernat, B.A., Wang, Z., Armstrong, R.N., and Helmann, J.D. (2001) FosB, a cysteine-dependent fosfomycin resistance protein under the control of σ^W, an extracytoplasmic-function σ factor in *Bacillus subtilis*. *J Bacteriol* **183**: 2380-2383.

Gajiwala, K.S., Margosiak, S., Lu, J., Cortez, J., Su, Y., Nie, Z., and Appelt, K. (2009) Crystal structures of bacterial FabH suggest a molecular basis for the substrate specificity of the enzyme. *FEBS Lett* **583**: 2939-2946.

Guerout-Fleury, A.-M., Frandsen, N., and Stragier, P. (1996) Plasmids for ectopic integration in *Bacillus subtilis*. *Gene* **180**: 57-61.

Vagner, V., Dervyn, E., and Ehrlich, S.D. (1998) A vector for systematic gene inactivation in *Bacillus subtilis*. *Microbiology* **144**: 3097-3104.