Current Biology

Supplemental Information

Phenotypic Diversity as a Mechanism to Exit Cellular Dormancy

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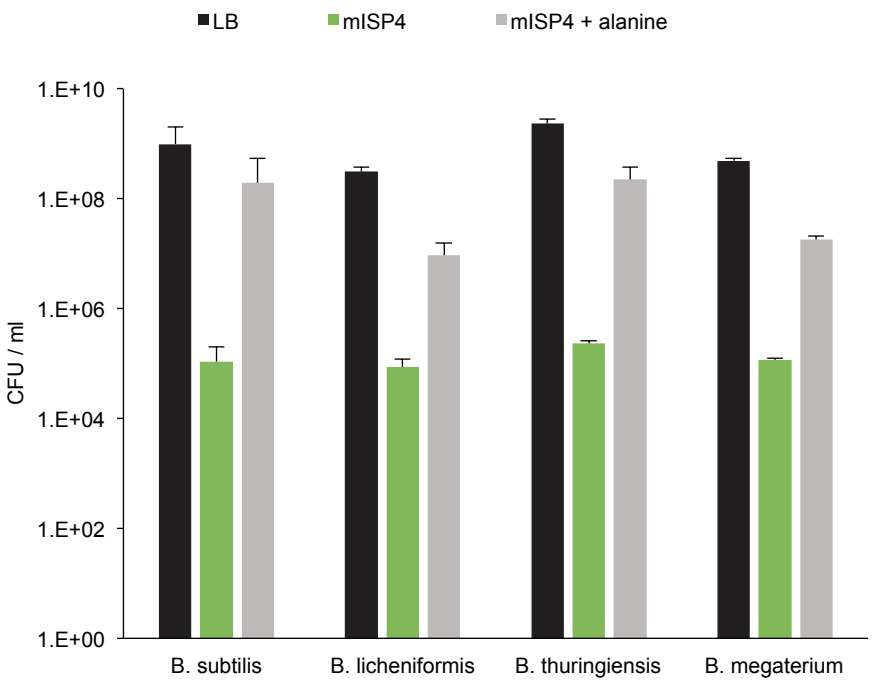
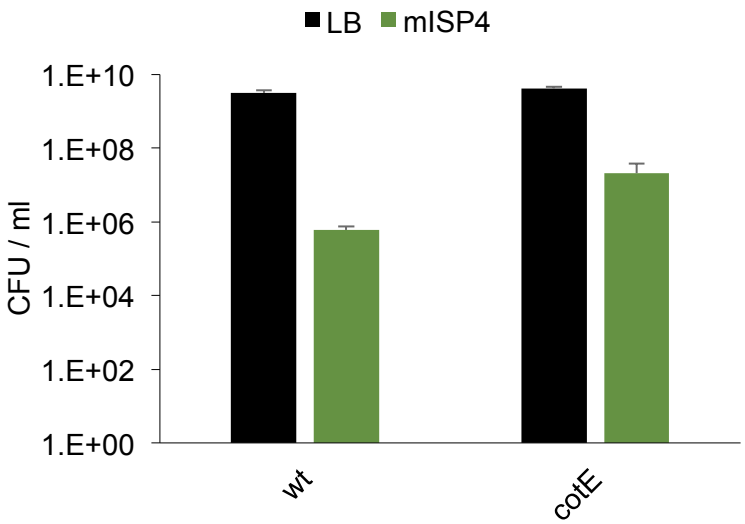
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

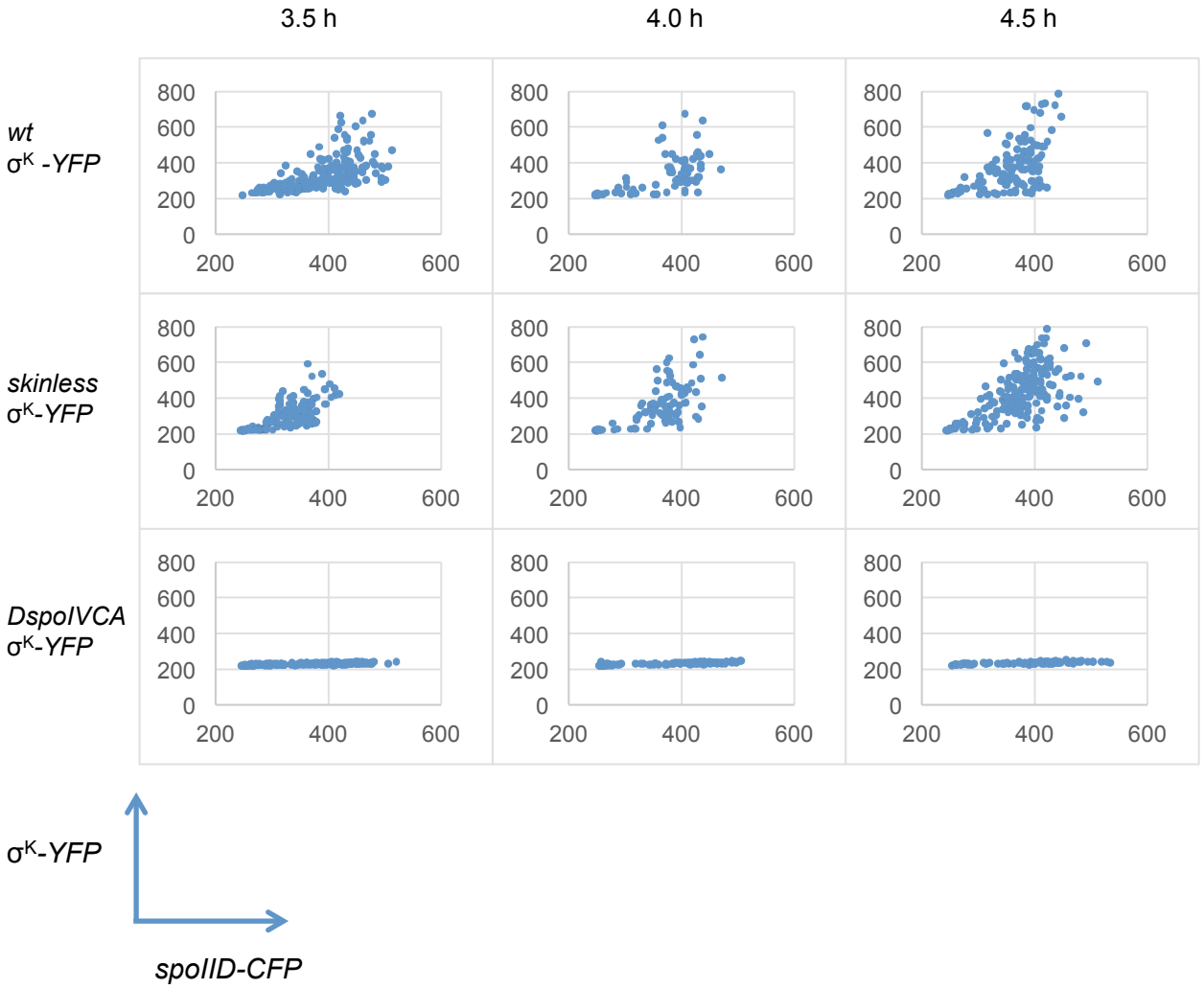


Figure S1 (related to Fig 1). Spontaneous germination of different Bacillus spp.

B. subtilis' - JDB1772 '*B. licheniformis'* – JDB1438; '*B. thuringiensis'* - JDB1169 *and* '*B. megaterium'* – JDB1430. Spores were heat treated and then plated on LB (black), mISP4 (green) and mISP4 supplemented with alanine (10mM, grey) to determine the fraction of germinating spores.

Figure S2 (related to Fig 3). The spore coat protein CotE affects spontaneous germination

Wild type spores (JDB1772) and spores of a strain that lacked the gene for *cotE* (JDB1323), a scaffold protein in the coat, were plated after heat shock on LB and mISP4 to determine the rate of spontaneous germination.

Figure S3 (related to Fig 3). Expression of σ^K and earlier genes (Refers to Fig 3)

spoIIIC that encodes the c-terminal part of σ^K was transcriptionally fused to YFP to monitor excission of the skin element and expression of sigK (also see Figure 3A). The reporter was constructed in a way that only after excission of the *skin* element and the fusion of *spoIVCB* and *spoIIIC* YFP would be transcribed. We tested the reporter in wt (JDB1772), in a *skinless* mutant and a *spoIVCA* mutant that lacks the recombinase to excise the skin element. In addition a marker for expression of earlier genes in sporulation, *spoIID*, was monitored by a transcriptional CFP fusion. We observed that in a *skinless* mutant the ratio σ^K */spoIID* expression drastically differs at t = 3.5 h and 4.5 h suggesting that in a *skinless* mutant, expression of earlier genes in sporulation is less reduced by perturbed σ^K expression. We assume that more perturbations in the regulatory cascade take place through the missing regulatory step in the skinless mutant that might interfere with the spore architecture and thus the spontaneous germination

rate. In a *spoIVCA* mutant the *skin* element was not excised and at no point we could detect σ^K expression with our reporter. In the course of the experiment, those cells that initiated sporulation in the *spoIVCA* mutant eventually died because of the absence of σ^K.

Table S1. Strain used.

Strains (JDB3905, JD2919, JDB3118, JDB3615, JDB1366, JDB3614, and JDB1914) were generated by transformation of genomic DNA from FB85 into JDB1772 using established techniques for *B. subtilis* [S1].

Table S1 – Strains

Strain	Genotype of interest	Reference
JDB1772	wt trpC2	Chet Price
JDB3	PY79	Lab collection
JDB1318	*B. cereus*	Bacillus Genetic Stock Center (BGSC)
JDB1438	*B. licheniformis*	BGSC
JDB1139	*B. sphaericus*	BGSC
JDB1430	*B. megaterium*	BGSC
JDB1169	*B. thuringiensis*	USDA
JDB3613	*sacA::PgerE-YFP (cat)*	M. Elowitz
JDB3902	*spoIVCB-spoIIIC fusion (skinless)*	[S2]
JDB3905	*sacA::PgerE-YFP* (*cat*) *spoIVCB-spoIIIC fusion (skinless)*	This work
JDB3577	*ΔgerE::cat*	[S3]
FB85	*ΔgerA::spc ΔgerB::cat ΔgerK::erm ΔyndDEF::tet ΔyfkQRT::neo*	[S4]
JDB2919	*ΔgerA::spc*	This work
JDB3118	*ΔgerB::cat*	This work
JDB3615	*ΔgerK::erm*	This work
JDB1366	*ΔgerA::spc ΔgerB::cat*	This work
JDB3614	*ΔgerA::spc ΔgerB::cat ΔgerK::erm*	This work
JDB1914	*ΔgerA::spc ΔgerB::cat ΔgerK::erm ΔyndDEF::tet ΔyfkQRT::neo*	This work

Supplemental Experimental Procedures

Strains and growth conditions

Plasmid and strain construction followed standard protocols. Strains used are described in Table S1. Spores were produced using Schaeffer's sporulation medium (DSM) and purified as described Spontaneous germination was monitored by plating dilutions of cultures on agar plates containing a modified version of ISP4 (Difco) agar lacking calcium and potassium salts (mISP4) and incubating for ~10 days at 37ºC. Spontaneous germination in liquid was assayed using mISP4 with 0.25% xylose instead of 1% starch to improve image quality.

Fluorescence Microscopy

Fluorescence microscopy was performed to assess *gerE* expression in cells that were sporulated by resuspension [S1]. All samples were taken at hour 5 following initiation of sporulation and applied to 2% agarose pads containing PBS and placed on a glass slide. Samples were covered with a cover slip and visualized with a Nikon Eclipse 90i using a 100x phase objective. >500 cells for each strain were imaged for phase contrast and YFP. ImageJ (NIH) was used for quantitative image analysis of single cell fluorescence intensity: cell boundaries were defined in the phase contrast channel and the corresponding mean YFP intensity recorded in AU. Sporulating and non-sporulating cells were distinguished by eye.

Flow cytometry

To analyze *gerE* expression, a *B. subtilis* strain harboring a P_{gerE}-YFP reporter was sporulated in resuspension medium at 37ºC and >20,000 cells were analyzed using an LSRII flow cytometer (488 nm excitation, 530/30 nm bandpass filter). To assess

spontaneous germination of the highest and lowest *gerE* expressing cells, a culture at hour 5 of sporulation by resuspension was diluted 1:5 in resuspension medium at RT and sorted using a FACS Aria II (488 nm excitation, 525/50 nm bandpass filter) under sterile conditions (~60 min; 37ºC). ~10^6 high (top 5%) and low YFP (bottom 5%) expressing cells were separated and returned to conditions of high aeration in resuspension medium to complete sporulation (>12h, 37ºC). The spores were heat treated for 20 min at 80°C to kill residual vegetative cells and then dilutions were plated on both mISP4 and LB to determine the spontaneous germination frequency.

Supplemental References

[S1] Harwood, C.R., and Cutting, S.M. eds. (1990). Molecular biological methods for Bacillus (New York: Wiley).

[S2] Oke, V., and Losick, R. (1993). Multilevel regulation of the sporulation transcription factor sigma K in Bacillus subtilis. J Bacteriol *175*, 7341-7347.

[S3] Ghosh, S., Setlow, B., Wahome, P.G., Cowan, A.E., Plomp, M., Malkin, A.J., and Setlow, P. (2008). Characterization of spores of Bacillus subtilis that lack most coat layers. J Bacteriol *190*, 6741-6748.

[S4] Paidhungat, M., and Setlow, P. (2000). Role of ger proteins in nutrient and nonnutrient triggering of spore germination in Bacillus subtilis. J Bacteriol *182*, 2513-2519.