**Supplemental Materials and Methods**

**Strain construction.** For construction of the insertional deletion mutations of *ackA*, *pta*, and *acsA* in 3610. The above three mutants in the *B. subtilis* strain 168 background were obtained from the *Bacillus* Genetic Stock Center (BGSC, www.bgsc.org) at the Iowa State University. Genomic DNAs were prepared from the above three strains and introduced into 3610 background by genetic transformation.

To construct the transcriptional reporter fusion of $P_{ywbHG}$-*lacZ*, we first amplified the regulatory region of *ywbHG* by PCR and using primers $P_{ywbHG}$-F1 and $P_{ywbHG}$-R1 and the 3610 genomic DNA as the template. PCR products were purified and digested with EcoRI and BamHI. After digestion and gel-purification, PCR products were ligated with the plasmid vector pDR268 (1) also digested with the same restriction enzymes. Ligation product was used to transform competent *E. coli* DH5α. The recombinant plasmid pYC161 was prepared from *E. coli* cells, verified by DNA sequencing, and introduced into *B. subtilis* strain PY79 by genetic transformation. Integration of the transcriptional reporter fusion at the *amyE* locus (encoding a starch-degrading amylase) on the PY79 chromosome was verified on LB plus starch plate for loss of amylase activities. The reporter fusion was then introduced into other strain backgrounds by either SSP1-phage transduction or genetic transformation. The transcriptional reporter fusions of $P_{ysbAB}$-*lacZ* and $P_{yxaKC}$-*lacZ* were similarly constructed by using primers $P_{ysbAB}$-F1 and $P_{ysbAB}$-R1, and $P_{yxaKC}$-F1 and $P_{yxaKC}$-R1, respectively.

To complement the Δ*pta* mutation, the regulatory and the coding sequences of the *pta* gene were amplified by PCR using primers *pta*-F1 and *pta*-R1. The PCR product was digested and cloned into the integration vector pDG1662 (2). The recombinant plasmid was prepared from *E. coli*, verified by DNA sequencing, and introduced into *B. subtilis* strain PY79 by genetic transformation. The DNA fragment containing *amyE*::*pta* for complementation was then introduced into the Δ*pta* mutant by SPP1 phage mediated transduction, resulting in the strain FY255. Complementation of the Δ*pta* mutation was conducted similarly, resulting in strain FY256.

**Assays of β-galactosidase activities.** Cells were cultured in LB (or LBGM) medium at 37°C in a water bath with shaking. One milliliter of culture was collected at each time point. Cells were spun down and pellets were suspended in 1 ml Z buffer (40 mM $NaH_2PO_4$, 60 mM $Na_2HPO_4$, 1 mM $MgSO_4$, 10 mM KCl, and 38 mM β-mercaptoethanol) supplemented with 200 μg $ml^{-1}$ freshly made lysozyme. Cell resuspensions were incubated at 30°C for 15 min. Reactions were started by adding 200 μl of 4 mg $ml^{-1}$ ONPG (2-nitrophenyl β-D-galactopyranoside) and stopped by adding 500 μl of 1 M $Na_2CO_3$. Samples were briefly spun down. The soluble fractions were transferred to cuvettes (VWR), and $OD_{420}$ values of the samples were recorded using a Bio-Rad Smartspec 3000. The β-galactosidase specific activity was calculated according to the equation ($OD_{420}$/time x $OD_{600}$) x dilution factor x 1000. Assays were conducted at least in duplicate.

**Biofilm assays for *S. aureus*.** *S aureus* HG003 cells (kindly provided by Kim Lewis, Northeastern University) were grown in BHI media overnight at 37°C. Next morning, cells were diluted 100 fold into TSB supplemented with 1% glucose (g/v). Cells continued to grow until reaching mid-log phase. Cells were then diluted 100 fold into fresh TSB supplemented with 1% glucose. 1.5-ml diluted culture was added to each well of the 24-well plate (VWR). Various amounts of acetate, when needed, were added to the TSB media at the beginning of cell inoculation. Plates were incubated at 37°C for 24 hours. Culture supernatants in each well were gently pipetted out and discarded. Wells were gently washed with 2-ml PBS buffer three times. The liquids in the wells were thoroughly removed and the plates were air-dried for 10 min at the room temperature. 1-ml of 0.2% crystal violet solution (in distilled water) was added to each well of the 24-well plate and the plate was left on bench for staining for about 10 min. After staining, crystal violet solution was removed, the wells were washed with PBS buffer three times, and the plate was air-dried for 10 min. After air-dry, pictures of the crystal violet-stained, bottom surface-attached biofilms were taken using a Nikon CoolPix S9200 digital camera. For semi-quantitative analysis of the biofilm biomass, the surface-attached, crystal violet-stained biofilms were dissolved in 1-ml of 100% ethanol. The dissolved solutions were serial-diluted, transferred to plastic cuvette (VWR), and measured for absorbance at 570 nm on a Bio-Rad SmartSpec 3000. The arbitrary units from the measurement were used to compare relative biofilm robustness among different samples.

**Assays of matrix gene induction and biomass during pellicle formation.** Induction of the matrix genes was assayed by using *B. subtilis* (YC110) cells that contain a promoter reporter for the *epsA-O* operon ($P_{epsA}$-*lacZ*). YC110 cells were inoculated into LBGM for pellicle biofilm formation under two different conditions, one with and the other without influence of strong volatiles. The two conditions are very similar to what was described in Fig. 3C (No. 1 and 2), where No. 1 represents the condition of without the influence of strong volatiles and No. 2 represents with the influence of strong volatiles.

YC110 pellicles were collected at different time points during pellicle biofilm development. Using the media in each well, the pellicles were resuspended, removing any parts stuck to the well via gentle pipetting. The pellicles were centrifuged and washed with Z buffer twice, and resuspended in 1-ml of Z buffer. Mild sonication (3-5 pulses for 5 s, 50% duty) was applied to disrupt the chains of cells (but not individual cells), a unique feature of *B. subtilis* pellicle biofilms (3). Cell separation was confirmed under phase-contrast microscopy. Well-separated cells were then assayed for β-galactosidase-specific activities according to the protocol described in the Materials and Methods.

Biomass of the pellicle biofilms collected at different time points under the two different conditions was accessed by measuring the optical density of the biofilm-associated cells. To do so, pellicle biofilms were similarly collected and treated with mild sonication in order to disrupt chains of cells as described above. Cell samples were diluted appropriately and cell optical density of the samples was measured by using a Bio-Rad Smartspec 3000.

**Quantification of eDNA release by qPCR.**

Quantification of pellicle biofilm-associated eDNA release was conducted by using a qPCR based method following a published protocol (4) with some modifications. Pellicle biofilms by the wild type cells and the triple mutant of holin-antiholin genes (CY886) were collected after 24 hours of incubation at 30°C by brief centrifugation at 4°C and 14,000 rpm. Pellicle biofilms were resuspended in 50 mM Tris·HCl/10 mM ETDA/500 mM NaCl, pH 8.0, and vigorously vortexed for 3 min. Pellicle biofilms were centrifuged again for 5 min at 4°C and 14,000 rpm. 500 µl of the supernatant was transferred to a clean tube, and extracted once with an equal volume of phenol/chloroform/isopropanol (25:24:1) and once with chloroform/isopropanol (24:1). The aqueous phase was then mixed with 3 volumes of ice-cold 100% (v/v) ethanol and 1/10 volume of 3 M Na-acetate (pH 5.2) for DNA precipitation. DNA samples were stored at −20°C for two hours. The ethanol-precipitated DNA was spun down by centrifugation for 25 min at 4°C and 14,000 rpm, washed with ice-cold 70% (v/v) ethanol once, air-dried, and finally dissolved in 20 µl of TE buffer.

The amount of eDNA was quantified by qPCR using the three primer pairs (for *sda*, *sinR* and *codY*) listed in Table S1. The concentrations of the DNA templates and the primers were determined by following the manufacture's instructions. PCR was performed in the Mastercycler® ep realplex by using the following programs: one cycle of 95°C for 3min, 40 cycles of 95°C for 3 s, 53°C for 20 s, and 60°C for 20 s. The results from all quantitative PCR were analyzed using the Mastercycler® ep realplex software.

**Supplemental references**


1. **Antoniewski C, Savelli B, Stragier P.** 1990. The spoIIJ gene, which regulates early developmental steps in *Bacillus subtilis*, belongs to a class of environmentally responsive genes. J. Bacteriol. **172:**86-93.

2. **Guérout-Fleury AM, Frandsen N, Stragier P.** 1996. Plasmids for ectopic integration in *Bacillus subtilis*. Gene **180:**57-61.

3. **Branda SS, Gonzalez-Pastor JE, Ben-Yehuda S, Losick R, Kolter R.** 2001. Fruiting body formation by *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **98:**11621-11626.

4. **Rice KC, Mann EE, Endres JL, Weiss EC, Cassat JE, Smeltzer MS, Bayles KW.** 2007. The cidA murein hydrolase regulator contributes to DNA release and biofilm development in Staphylococcus aureus. Proceedings of the National Academy of Sciences **104:**8113-8118.