S1 Appendix

**Construction of B. subtilis mutants**

Because strain NCIB3610 has low transformation ability, mutant alleles were first introduced into the domesticated strain 168 and then transferred to strain NCIB3610 via transformation using genomic DNA [1]. Strain 168 has several mutations that affect biofilm formation. The possible introduction of these unwanted mutations into strain NCIB3610 via transformation using genomic DNA was removed by examining colony morphology of transformants. Strain NCIB3610 was directly transformed with pHYG2 and its derivative plasmids. Primers used in the strain construction are shown in S2 Table. The construction of the ΔsigW::cat, Δspo0A::cat, ΔdegU::cat, Δeps::spc, ΔbslA::spc, ΔsinR::cat, ΔnprB::cat, and amyE::PaprE-gfp strains was described previously [1-4]. The cat gene of the ΔsigW::cat mutant was replaced with neo using pCm::Neo [5]. The ΔdegU::kan mutant was constructed using the same primer sets with the ΔdegU::cat mutant [1]. The construction of the other strains is described below.

(1) **Pspac-hy-yitPOM, Pspac-hy-sdpABC, Pspac-hy-yitQ, and Pspac-hy-sdpI strains**

The entire region of the yitPOM locus, including the SD sequence of yitP, was amplified via PCR using the primers YI-yitP-F1 and YI-yitP-R3. The PCR products were digested with HindIII and BamHI and then ligated with HindIII and BamHI-digested pDLT3-Hy, an amyE integration vector that contains cat, lacI, and the spac-hy promoter between the amyE upstream and downstream sequences. The ligation mixture was used to transform B. subtilis strain 168, and Cm⁻ colonies were selected. Chromosomal DNA samples were prepared from these Cm⁻ colonies, and transformants carrying Pspac-hy-yitPOM were screened by PCR. One verified transformant was used as the Pspac-hy-yitPOM strain. The Cm⁻ marker of the Pspac-hy-yitPOM strain was replaced with Em⁺ using the marker exchange plasmid pCm::Em [5].
P_{spac-hy}yitPOM construct was then transferred into NCIB3610. The P_{spac-hy}sdpABC, P_{spac-hy}yitQ, and P_{spac-hy}sdpI strains were constructed via the same procedure using different primers. The entire regions of the sdpABC, yitQ, and sdpI loci were amplified via PCR using the primer pairs Y1-yitP-F1/Y1-yitP-R3, yitQ-F1/yitQ-R1, and sdpI-F1/sdpI-R1, respectively.

(2) Deletion mutants
Deletion of yitR-yitM was carried out using an overlap-extension PCR technique. A cat cassette was amplified from pCBB31 [1] via PCR using the pUC-R and pUC-F primers (S2 Table). The upstream region of yitR and the downstream region of yitM were amplified via PCR using the primer pairs yitR-F1/yitR-R1 and yitPM-F2/yitPM-R2, respectively. The 5’ sequences of yitR-R1 and yitPM-F2 were complementary to the sequences of pUC-R and pUC-F, respectively. To connect the three PCR fragments, all three were mixed and used as a template for a second round of PCR using primers spx-F1 and spx-R2. The resultant PCR products were used for transformation of strain 168. Deletion mutants of nprB-yitM, yitQ, and sdpA-sdpR were constructed via the same procedure using different primer sets, i.e., ΔyitR-yitM, yitR-F1/yitR-R1 and yitPM-F2/yitPM-R2; ΔnprB-yitM, nprB-F3-2/nprB-R3-2 and yitPM-F2/yitPM-R2; ΔyitQ, yitQ-F3-2/yitQ-R3 and yitQ-F4/yitR-F1; and ΔsdpA-sdpR, Y1-sdpA-F4/Y1-sdpA-R4 and Y1-sdpA-F5/Y1-sdpA-R5. These deletions were then transferred to NCIB3610 and other strains.

(3) P_{yit-gfp} and P_{nprB-gfp}
yitP and nprB promoter regions were amplified via PCR using primer sets, the yitP-P-F1/yitP-P-R1 and nprB-P-F1/nprB-P-R1 primers, respectively. The PCR products were digested with EcoRI and HindIII and ligated with EcoRI and HindIII-digested plasmid pHYG2, which is a derivative of the E. coli and B. subtilis shuttle plasmid pHY300 PLK [6] containing a promoterless gfp gene. The ligation mixture was used to transform E. coli JM105 to obtain pHYG2-yitP and pHYG2-nprB. These plasmids were used to transform strain
S1 Appendix References


