Supporting Information

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SI Methods

Fluorescent Microscopy and Slide Preparation. Cells were collected, washed twice by centrifugation at 7,000 × *g* for 5 min with 0.5 mL 0.9% NaCl and immobilized on 10-well poly-L-lysine covered diagnostic slides (Thermo Scientific). Fluorescence bleaching was reduced by applying the antifading agent SlowFade (Invitrogen). The *Bacillus subtilis* BD2833 [quorum-sensing (QS) proficient] and *B. subtilis* BD2876 (signal deficient) strains, which do not carry respective fluorescence markers, were used to determine autofluorescence.

Purification of QS Signal and Surfactin. Expression and purification of ComX from the *Escherichia coli* ED367 producer strain was carried out according to the procedure of Ansaldi et al. (1), except that primary purification was performed using HPLC with gradient elution on a chromatographic column (NUCLEODUR C_{18} Gravity, Macherey-Nagel) in two mobile phases (0.1% TFA and acetonitrile with 0.1% TFA) (1). The approximate concentration of ComX was calculated from the area under the chromatogram (two ComX corresponding peaks) using an estimated extinction coefficient. This coefficient was calculated as a sum of absorbance (λ = 214 nm) of amino acids of ComX pheromone 168, peptide bonds, and squalene (similar to an isoprenoid tail) dissolved in acetonitril and 0.1% TFA. Surfactin was isolated from the spent media according to the protocol described by Cooper et al. (2) and was dissolved in pure methanol. The HPLC system was operated on the NUCLEODUR C_{18} Gravity column at a flow rate of 1.0 mL/min with 80% (vol/vol) acetonitrile and 0.1% TFA in water. Eluting fractions were detected by their absorbance at 214 nm. The concentrations of surfactin in the spent media were estimated from the integrated area under representative peaks.

β-Galactosidase Assay. Responsiveness of the wild type and signal-deficient strain to exogenous ComX was determined by growing the wild-type BD2833 (*srfA-lacZ*) and the QS signal-deficient BD2876 (*srfA-lacZ*) strains with shaking for 16 h at 28 °C in 100 μL fresh CM medium and 100 μL of ComX (purified from *E. coli*) 0.005% to 0.05% (vol/vol) suspensions diluted in sterile MiliQ. Cells were centrifuged (4 °C; 1,800 × *g*) and resuspended in 200 μL Z-buffer with 5.6% (vol/vol) β-mercaptoethanol before adding 10 μL toluene and incubating the cultures on ice for 30 min. The plate was then warmed to 30 °C, 50 μL *ortho*-nitrophenyl-β-galactoside substrate was added and the absorbance (420 nm) was immediately determined at 30 °C.

Hemolytic Assay. Bovine red blood cells (RBCs) were washed two times with isotonic buffer [140 mM NaCl and 20 mM Tris (pH 7.4)] and once with 0.9% NaCl. The RBCs were then resuspended in 0.9% NaCl to OD_{650} 0.7 a.u. One hundred microliters of spent media, 30 μL 96% (vol/vol) ethanol, and 100 μL RBCs were mixed on a microtitre plate. OD_{650} was measured immediately after the addition of RBCs and after 15 min of incubation at room temperature. The percentage of hemolysis was calculated as the decrease of OD_{650} divided by OD_{650} obtained for the bacterial cultures before spent media sampling (3).

Dehydrogenase Activity. To measure dehydrogenase activity, 1,200 μL cells were harvested, centrifuged at 7,000 × *g* for 5 min, and washed in 0.1 M Tris·HCl buffer (pH 7.7). Cell pellets were resuspended in 150 μL of the same buffer mixed with 1 mL 1% (vol/vol) 2,3,5-triphenyltetrazolium chloride (TTC), 7.5 μL 1 M glucose, and 7.5 μL 0.5 M monopotassium phosphate. The control samples were prepared in the same manner only without the addition of TTC to the Tris·HCl buffer. All samples were incubated for 1 h shaking at 100 rpm at 37 °C in the dark. After incubation, cells were harvested by centrifugation at 7,000 × *g* for 5 min at room temperature.

The supernatant was discarded and 2,3,5-triphenyltetrazolium formazan (TTF) was extracted from the cell pellet with 300 μL methanol with continuous vortex mixing for 5 min. Methanol extracts were centrifuged at 7,000 × *g* for 5 min at room temperature to remove cell debris, and supernatant absorbance at 485 nm was measured spectro-photometrically. The concentration of TTF produced was calculated based on a calibration curve of freshly prepared TTF in methanol. The cell dehydrogenase activity was expressed in micrograms of TTF produced per viable cell.

1. Ansaldi M, Marolt D, Stebe T, Mandic-Mulec I, Dubnau D (2002) Specific activation of the *Bacillus* quorum-sensing systems by isoprenylated pheromone variants. *Mol Microbiol* 44(6):1561–1573.
2. Cooper DG, Macdonald CR, Duff SJ, Kosaric N (1981) Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. *Appl Environ Microbiol* 42(3):408–412.
3. Moran AC, Martinez MA, Sineriz F (2002) Quantification of surfactin in culture supernatants by hemolytic activity. *Biotechnol Lett* 24:177–180.

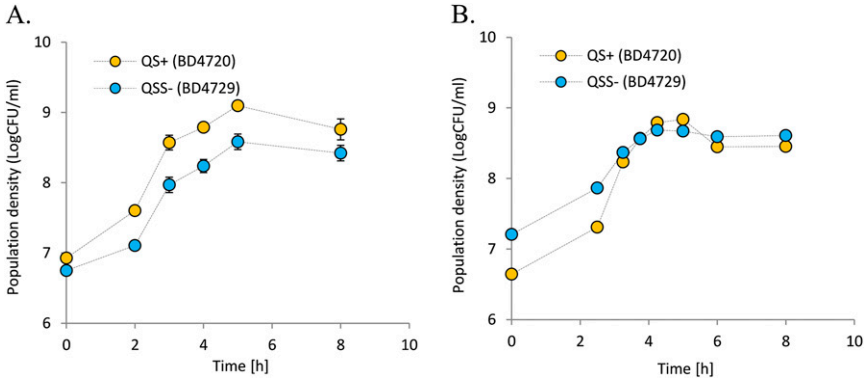

Fig. S1. Bacterial growth was monitored by viable cell counts (CFU) of (*A*) domesticated QS proficient (QS^+) *B. subtilis* BD4720 (*srfA-yfp*) and QS signal deficient (QSS^-) *B. subtilis* BD4729 (*srfA-cfp*) grown alone and (*B*) in 1:1 cocultures of *B. subtilis* BD4720 and *B. subtilis* BD4729. Data are presented as mean values of at least three biological replicates and the SE is indicated for every time point.

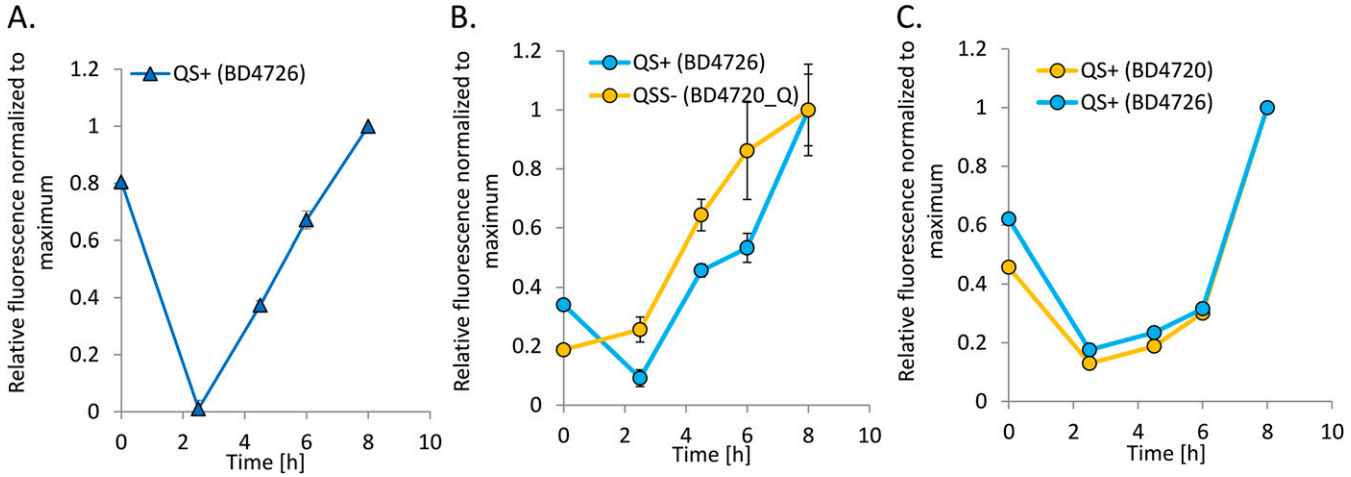

Fig. S2. Changes in relative QS response during growth of (*A*) domesticated QS-proficient (QS^+) *B. subtilis* BD4726 (*srfA-cfp*) and (*B*) domesticated QS^+ *B. subtilis* BD4726 (*srfA-cfp*) and QS signal-deficient (QSS^-) *B. subtilis* BD4720_Q (*ΔcomQ; srfA-yfp*) in coculture. (*C*) Changes in relative QS response during growth of two domesticated QS^+ strains of *B. subtilis* BD4720 (*srfA-yfp*) and *B. subtilis* BD4726 (*srfA-cfp*) in coculture. The fluorescence of the *srfA-yfp* and *srfA-cfp* reporter fusions was normalized to maximal fluorescence for each fluorophore and presented as relative fluorescence (*Methods* and *SI Methods*). Data are presented as the mean of biological triplicates with SEs indicated for each time point.

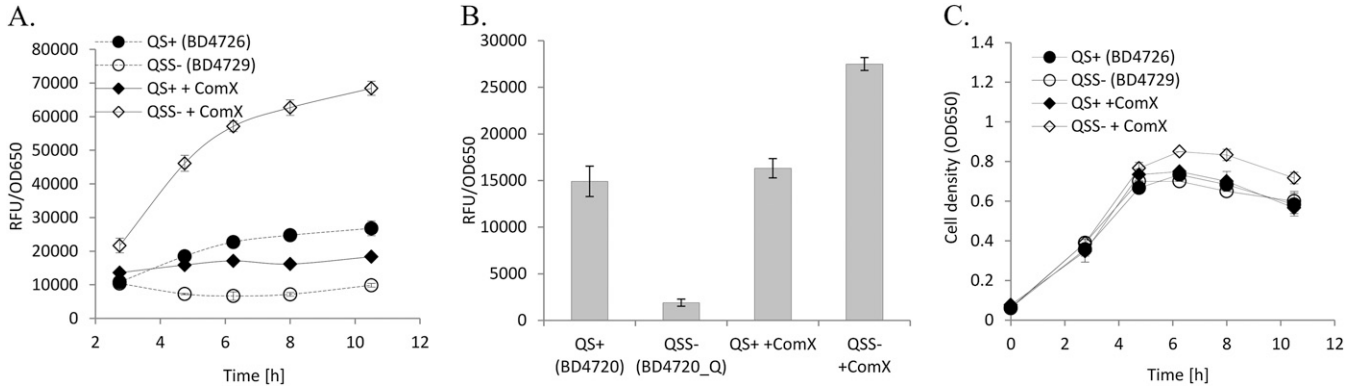

Fig. S3. (*A*) Cultures of domesticated QS-proficient (QS^+) *B. subtilis* BD4726 (*srfA-cfp*) and QS signal-deficient (QSS^-) *B. subtilis* BD4729 (*srfA-cfp*) were incubated without or with 0.05 fraction of ComX pheromone purified from *E. coli* ED386 and the QS response was monitored spectro-fluorimetrically over time. (*B*) Cultures of domesticated (QS^+) *B. subtilis* BD4720 (*srfA-yfp*) and QSS^- *B. subtilis* BD4720_Q (*srfA-yfp*) were incubated without or with 0.05 fraction of ComX pheromone purified from *E. coli* and the QS response was measured by fluorometer after 8 h. (*C*) Growth curves of indicated strains obtained during the experiment presented in Fig. S1*A*. Data are presented as the mean of biological triplicates and SE is indicated. Error bars in panels *A*–*C* represent SEs indicated for each time point. RFU, relative fluorescence units.

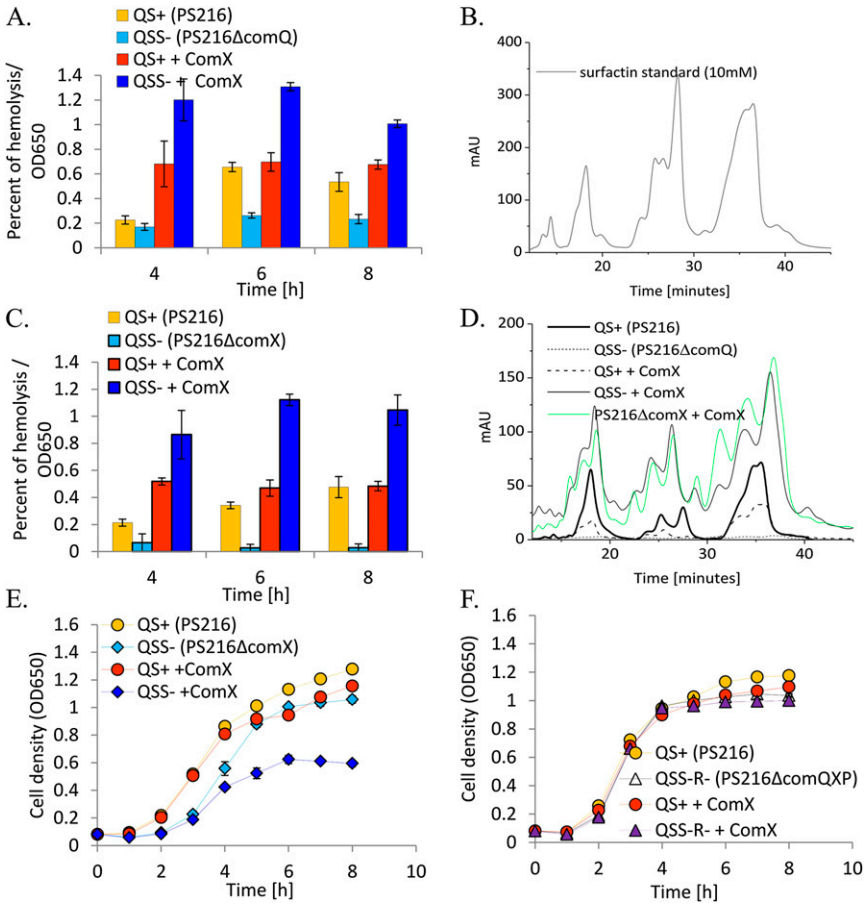

Fig. S4. (*A*) Hemolytic activity of biosurfactants in spent media of undomesticated QS-proficient (QS^+) *B. subtilis* PS216 and signal-deficient (QSS^-) *B. subtilis* PS216ΔcomQ was measured after 4, 6, and 8 h. Both strains were grown with or without 0.05 fraction of ComX. (*B*) The HPLC chromatogram of the commercially available surfactin standard is presented. (*C*) Hemolytic activity of biosurfactants was measured after 4, 6, and 8 h in spent media of undomesticated QS^+ *B. subtilis* PS216 and QSS^- PS 216ΔcomX strains which were incubated without (control) or with 0.05 fraction of purified ComX. Data are presented as mean of biological triplicates with SE indicated for every time point in panels *A*, *C*, *E*, and *F*. (*D*) HPLC chromatogram (green) represents surfactin produced by undomesticated signal-deficient *B. subtilis* PS216ΔcomX mutant grown in the presence of 0.05 fraction of ComX. (*E*) Growth curves of undomesticated QS-proficient (QS^+) *B. subtilis* PS216 and signal-deficient (QSS^-) *B. subtilis* PS216ΔcomX incubated without or with 0.05 fraction of exogenous ComX. (*F*) Growth curves of undomesticated QS-proficient (QS^+) *B. subtilis* PS216 and signal-deficient and receptor-deficient (QSS^-R^-) *B. subtilis* PS216ΔcomQXP incubated without or with 0.05 fraction of exogenous ComX. Data are presented as the mean of biological triplicates with SE indicated.

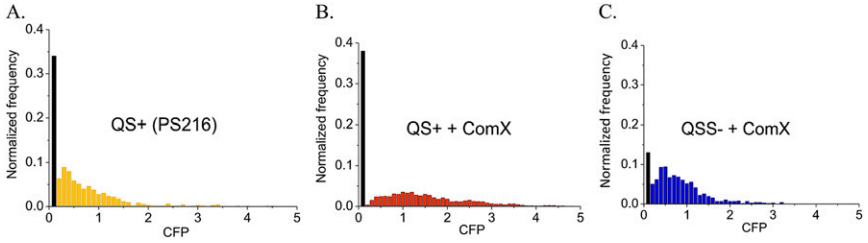

Fig. S5. Histograms representing the distribution of *srfA-cfp* expression in the (*A*) QS^+ *B. subtilis* PS216 (yellow), (*B*) in the same strain incubated with ComX (red), and (*C*) in the QSS^- *B. subtilis* PS216ΔcomQ (blue) incubated with purified ComX. The noninduced population (left-most bar in each panel) is marked in black.

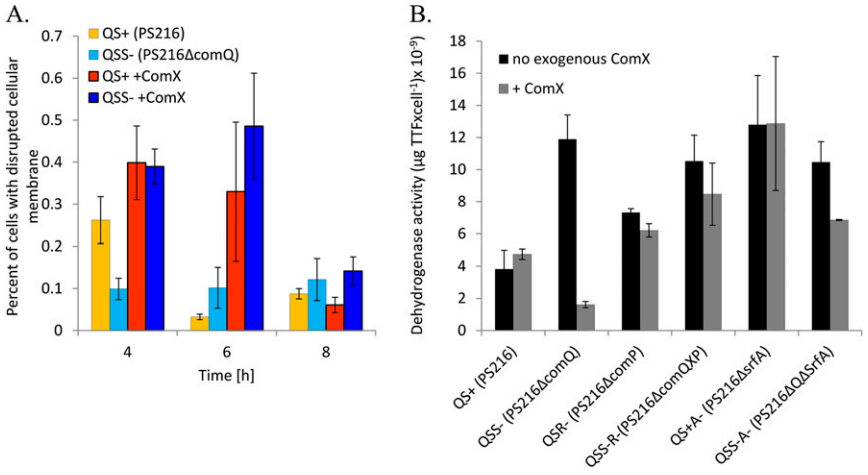

Fig. S6. (*A*) The fraction of cells with disrupted cellular membranes was determined by fluorescent microscopy (LIVE/DEAD *Bac*Light staining) for QS^+ *B. subtilis* PS216 and signal-deficient (QSS^-) PS216ΔcomQ grown without (control) or with 0.05 fraction of exogenous ComX after 4, 6, and 8 h of incubation. (*B*) The dehydrogenase activity *B. subtilis* PS216 and mutant derivatives: signal-deficient PS216ΔcomQ (QSS^-), receptor-deficient PS216ΔcomP (QSR^-), signal-deficient and receptor-deficient PS216ΔcomQXP (QSS^-R^-), surfactin-deficient PS216ΔsrfA (QS^+A^-), and signal-deficient and surfactin-deficient PS216ΔcomQΔsrfA (QSS^-A^-) strains were measured after 6 h of incubation. The same strains were also grown with 0.05 fraction of exogenous ComX. Data are presented as the mean with SE of biological triplicates. SE are indicated for each time point in panels *A* and *B*.

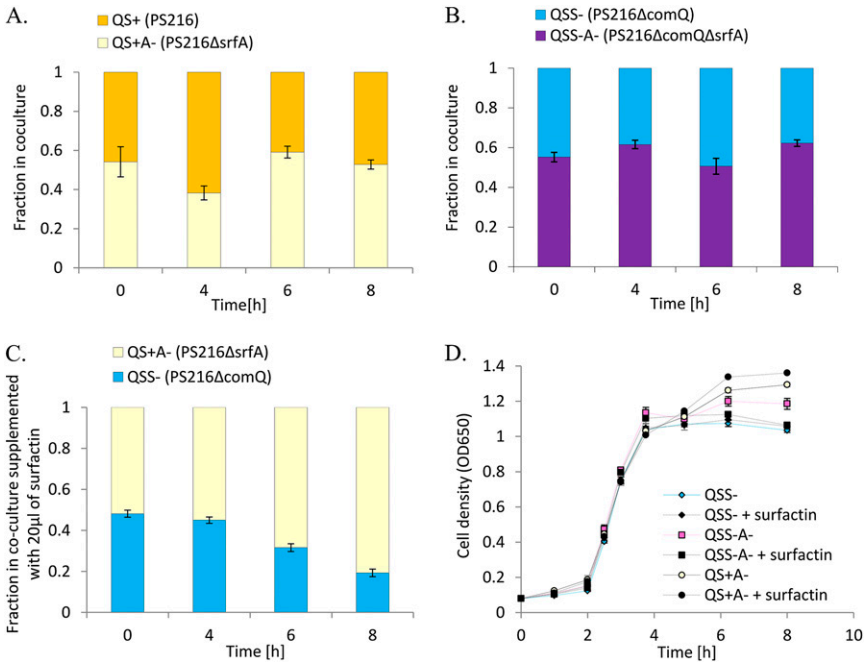

Fig. S7. Fractions of (*A*) PS216 and PS216ΔsrfA, (*B*) PS216ΔcomQ and PS216ΔcomQΔsrfA, and (*C*) PS216ΔsrfA and PS216ΔcomQ supplemented with surfactin (20 µg/mL) in coculture over time were determined by viable cell counts (CFU) using antibiotic selection. (*D*) Growth curves of PS216ΔsrfA (QS^+A^-), PS216ΔcomQ (QSS^-), and PS216ΔcomQΔsrfA (QSS^-A^-) incubated with or without 0.05 fraction of exogenous ComX. Data are presented as the mean of biological triplicates with SE indicated.

Table S1. Engineered *B. subtilis* strains used in this study

B. subtilis strains	Genome description	Source
BD2833	*his leu met srfA-lacZ (tet)*	(1)
BD2876	*his leu met srfA-lacZ (tet) comQ::kan*	(1)
BD4720	*his leu met srfA-yfp (spec)*	This work
BD4729	*his leu met srfA-lacZ (tet) comQ::kan srfA-cfp (cat)*	This work
BD4726	*his leu met srfA-cfp (spec)*	This work
BD4720_Q	*his leu met srfA-yfp (spec) comQ::kan*	This work
PS-216*	Undomesticated wild-type isolate	(2)
PS216	*srfA-cfp (cat)*	This work
PS216ΔcomQ	*comQ::kan srfA-cfp (cat)*	This work
PS216ΔcomP	*comP::cat*	This work
PS216ΔsrfA	*srfA::Tn917(cat, mls) srfA-cfp (cat)*	This work
PS216ΔcomQXP	*comQXP::kan*	This work
PS216ΔcomQΔsrfA	*comQ::kan srfA::Tn917 srfA-cfp (cat)*	This work
PS216ΔcomX	*ΔcomX pKB68 (comXW53A cat mls)*	This work
KTB308	*ΔcomX amyE::(srfA-lacZΩ 682 neo) pKB68 (comXW53A cat mls)*	(3)
OKB120	*pheA1 sfp srfA::Tn917*	(4)
BD1658	*his leu met comP::cat*	(1)

Strain descriptions: The domesticated bacterial strains used in this study were isogenic for all genetic loci except those indicated above. In short, the QS-proficient *B. subtilis* BD4720 was tagged with the *srfA-yfp* fusion, whereas the QS signal-deficient strain *B. subtilis* BD4729 was tagged with *srfA-cfp* and also carried Δ*comQ*, which prevented maturation of the ComX signal. The plasmid pED1093, carrying the *srfA* promoter region, was used to transform *B. subtilis* IS75 to obtain the BD4720 strain. BD4729, carrying the *cfp* gene attached to the promoter region of *srfA*, was constructed by transformation of the BD2876 strain with pED1092. The undomesticated bacterial strains were also isogenic in all loci except those indicated below. Strains PS216 and PS216ΔcomQ were obtained by tagging the PS-216 wild-type isolate (2) with *srfA-cfp* fluorescent fusions according to the protocol described above. Strains PS216ΔsrfA and PS216ΔcomQΔsrfA were obtained by transforming the wild-type (wt)216 and PS216ΔcomQ, respectively with the DNA isolated from the strain OKB120 (4). The genetic competence of PS216ΔcomQ was induced by adding a 0.05 fraction of ComX purified from *E. coli* in the late exponential phase. PS216ΔcomQXP ($QSS^{-}R^{-}$) was obtained by transforming the PS216 strain with the pED302 plasmid (1). PS216ΔcomP was obtained by transforming *B. subtilis* PS216 with the DNA isolated from B. *subtilis* BD1658 (1). To obtain B. *subtilis* PS216ΔcomX, PS216 was transformed with the DNA isolated from the *B. subtilis* KTB308 (3).

*The complete genome sequence of PS-216 has been published recently (5). The authors referred to the isolate as PS216. In our study we use this name for the same strain carrying the *srfA-cfp* fusion.

1. Tortosa P, et al. (2001) Specificity and genetic polymorphism of the *Bacillus* competence quorum-sensing system. *J Bacteriol* 183(2):451–460.
2. Stefanic P, Mandic-Mulec I (2009) Social interactions and distribution of *Bacillus subtilis* pherotypes at microscale. *J Bacteriol* 191(6):1756–1764.
3. Bacon Schneider K, Palmer TM, Grossman AD (2002) Characterization of comQ and comX, two genes required for production of ComX pheromone in *Bacillus subtilis*. *J Bacteriol* 184(2): 410–419.
4. Nakano MM, Marahiel MA, Zuber P (1988) Identification of a genetic locus required for biosynthesis of the lipopeptide antibiotic surfactin in *Bacillus subtilis*. *J Bacteriol* 170(12):5662–5668.
5. Durrett R, et al. (2013) Genome sequence of the *Bacillus subtilis* biofilm-forming transformable strain PS216. *Genome Announc* 1(3), 10.1128/genomeA.00288-13.

Table S2. Engineered *E. coli* strain and plasmids used in this study

E. coli plasmids	Genetic attributes	Source
pED1200	DH5alpa + pMDlibyfp (= pUC19 + YFP + *spec*)	Gift from D. Dubnau (The Public Health Research Institute, Newark, NJ)
pED250	DH5alpha + pUC18cm (= pUC19 + *cat*)	Gift from D. Dubnau
pDR200	XL-gold + pUK19 (= pUC19 + CFP + *kan*)	Gift from D. Rudner (Harvard Medical School, Boston)
pED302	PCR script with kan cassette between *degQ* and *comA*	(1)
pED367	BL21 + pED367 (= pET22 + *comQ* and *comX*)	(2)

The *srfA* promoter region was amplified using primers psrfA3 5′-GCA GCA GGA TCC AAG GAA TTA ACG CTT GCA GTG ATT GGC G-3′ and psrfA4 5′-CGA CGA GGA TCC CAT ATT GTC ATA CCT CCC CTA ATC TTT ATA AGC AGT GAA CAT-3′ and was ligated into the BamHI site of the pED1200 plasmid, resulting in plasmid pED1093. To construct plasmid pED1092, the promoter region of the *srfA* gene was amplified with primers psrfA1 5′-GCA GCA GAA TTC AAG GAA TTA ACG CTT GCA GTG ATT GGC G-3′ and psrfA2 5′-GCA GCA AAG CTT CAT ATT GTC ATA CCT CCC CTA ATC TTT ATA AGC AGT GAA CAT-3′ and the *cfp* gene sequence was obtained from plasmid pDR200 using restriction enzymes HindIII and BamHI before ligation into plasmid pED250, resulting in pED1092. The pED302 plasmid (1) was used for construction of the PS216ΔcomQXP mutant. *E. coli* ED367 (2) was used for heterologous expression and purification of the signaling peptide ComX.

1. Tortosa P, et al. (2001) Specificity and genetic polymorphism of the *Bacillus* competence quorum-sensing system. *J Bacteriol* 183(2):451–460.
2. Ansaldi M, Marolt D, Stebe T, Mandic-Mulec I, Dubnau D (2002) Specific activation of the Bacillus quorum-sensing systems by isoprenylated pheromone variants. *Mol Microbiol* 44(6): 1561–1573.