Fig S1

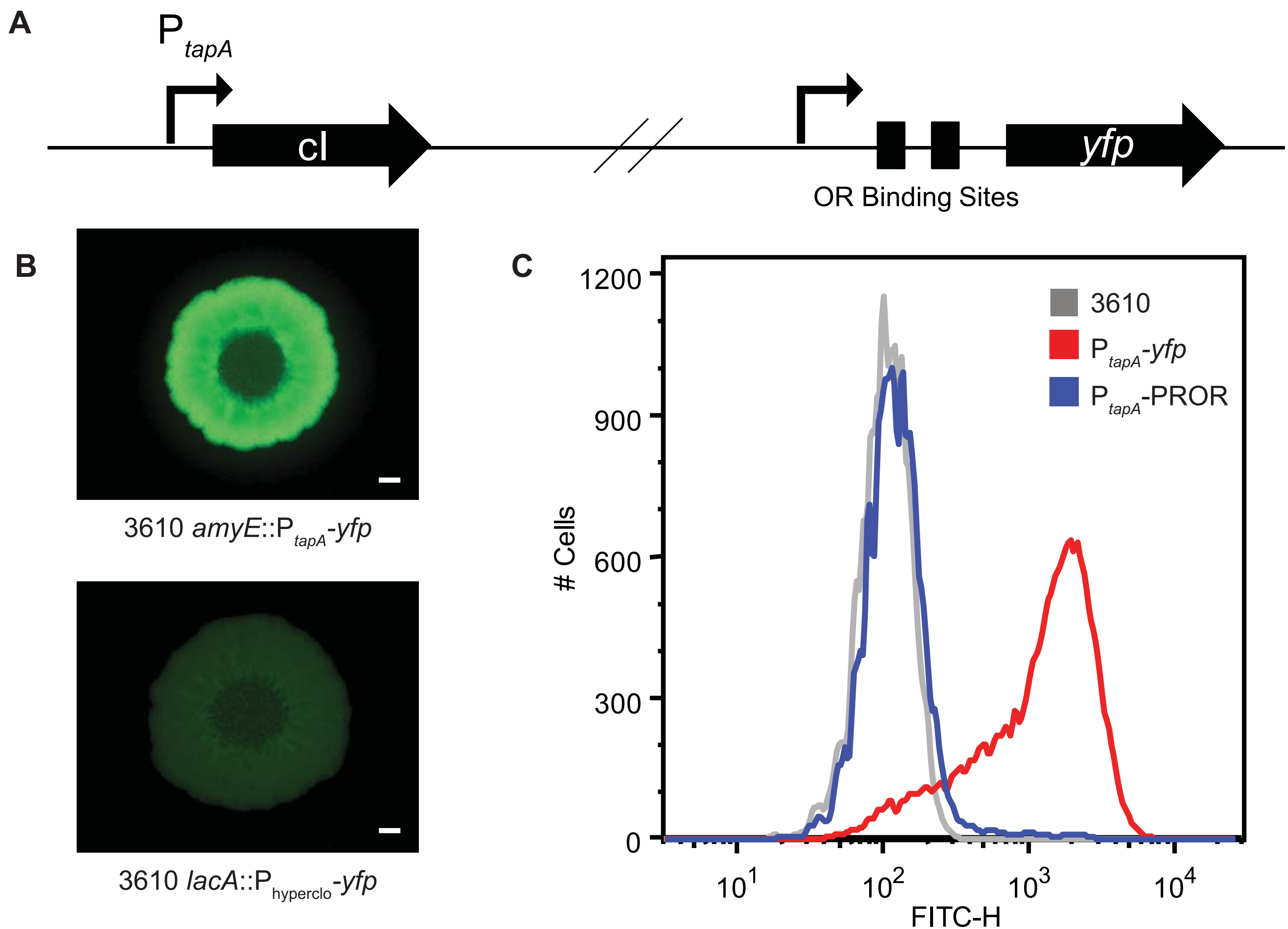


Figure S1: Design and characterization of the *B. subtilis* P_{tapA}-PROR strain, which fluoresces when biofilm gene expression is repressed. A) Schematic of the two-part P_{tapA}-PROR construct. In one part, the native promoter for the biofilm-specific *tapA* gene drives expression of the cI inhibitor protein (*amyE*::P_{tapA}-cI), while the second part contains the OR binding sites (the target of the cI protein) positioned in front of the *yfp* gene (lacA::$P_{hyperclo2}$-*yfp*). B) *B. subtilis* P_{tapA}-*yfp* colony (top) and $P_{hyperclo2}$-*yfp* (bottom) grown on the biofilm-inducing medium MSgg, showing that there is minimal background fluorescence due to the $P_{hyperclo}$-*yfp* construct in B. subtilis colonies. Scale bars are 1 mm. C) Flow cytometry histograms of cells harvested from MSgg colonies after 48 hr of growth of *B. subtilis* strains containing: (grey) no reporter, (red) P_{tapA}-*yfp*, or (blue) P_{tapA}-PROR, demonstrating that the P_{tapA}-PROR construct displays almost no fluorescence at the single-cell level when cells are grown on the biofilm-inducing medium MSgg.

Fig S2

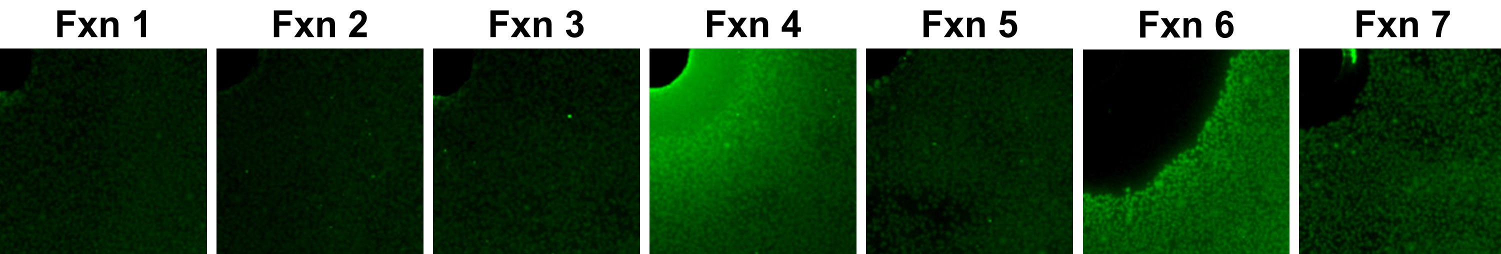


Figure S2: HPLC fractions of *P. protegens* conditioned medium tested for an effect on biofilm gene expression. *P. protegens* conditioned medium was loaded onto XAD16N resin, washed, and then eluted using a gradient of acetonitrile between 45 and 60%. Seven fractions with UV activity were obtained and tested in the microcolony lawn assay with the *B. subtilis* P_{tapA}-PROR strain. Wells containing the HPLC fractions are in the upper left of each panel. Fraction 6 shows both antibiotic activity (and therefore a region of black around the well where no colonies were growing) as well as fluorescence induction of the *B. subtilis* reporter (and therefore fluorescent colonies). Fraction 4 also shows antibiotic activity and fluorescence, but the fluorescence is non-specific (i.e. is not associated with the colonies), suggesting the presense of an auto-fluorescent molecule in this fraction. 1 day of growth

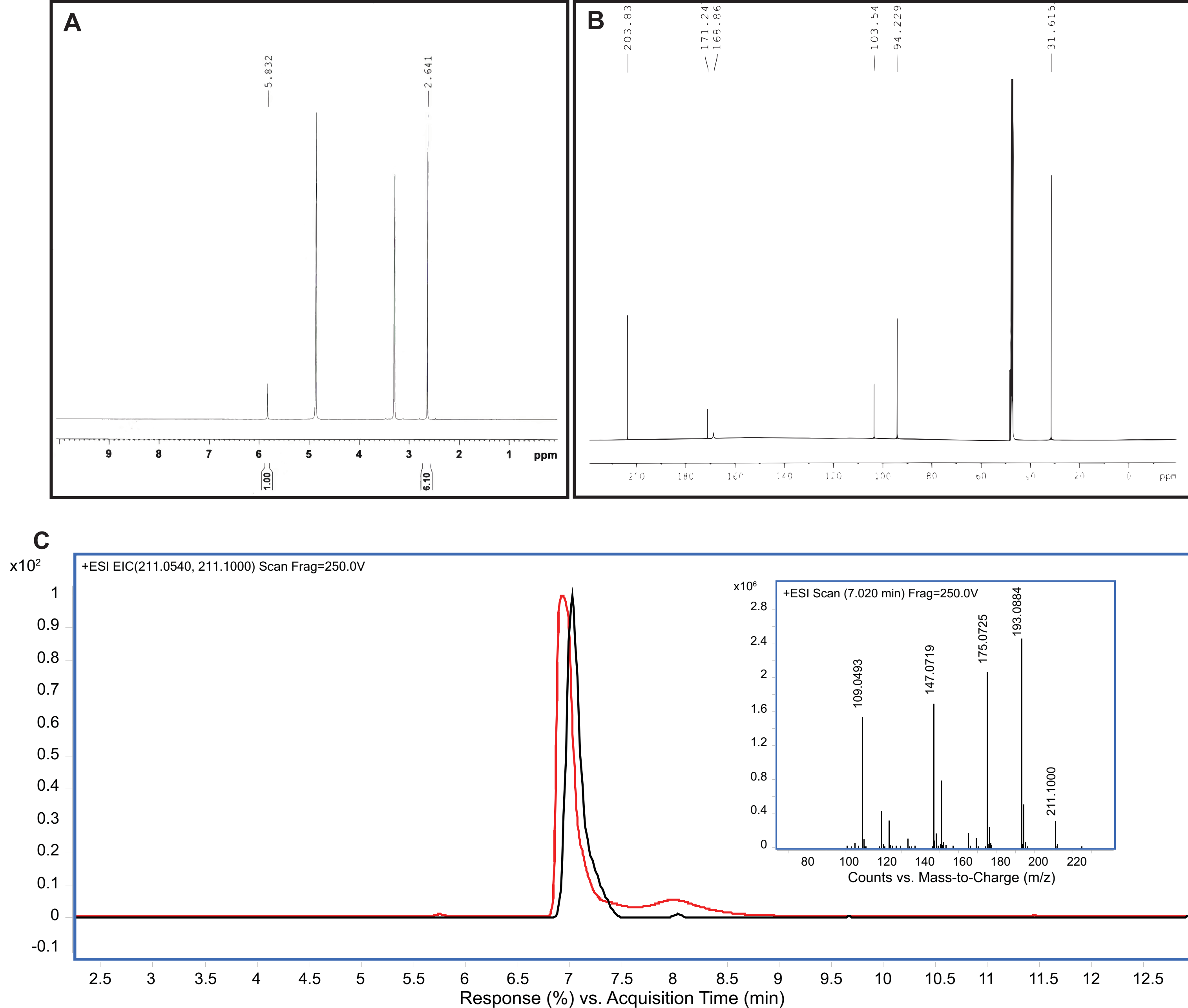
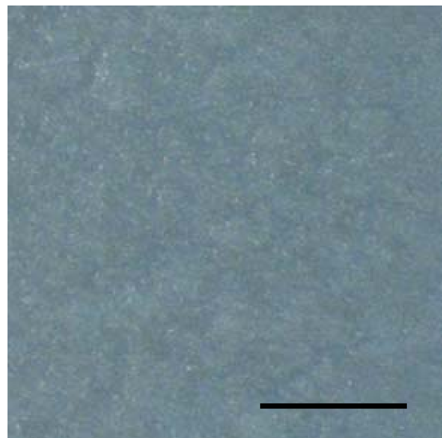
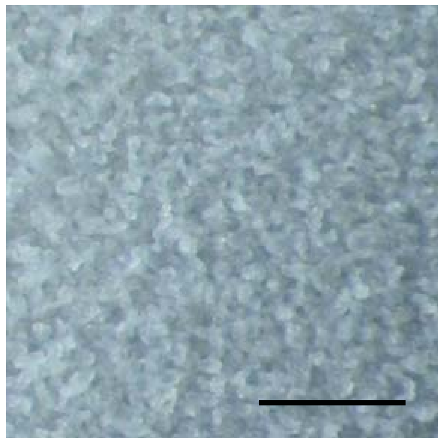


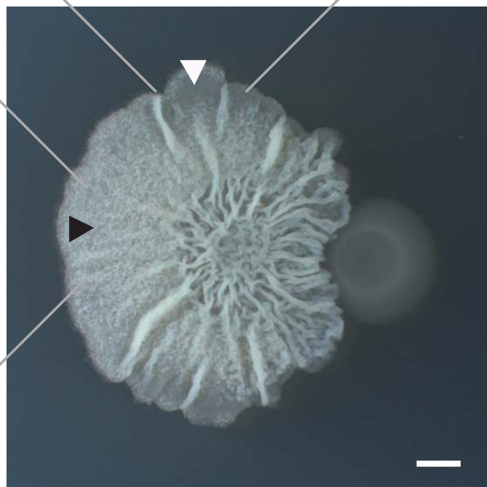
Figure S3: NMR and LC-MS data confirm that DAPG is the active compound. **A)** ^{1}H NMR spectrum of the active HPLC fraction containing two distinct proton shifts. **B)** ^{13}C NMR spectrum of the active HPLC fraction. **C)** Overlaid UV (red) and extracted ion (black) chromatograms of DAPG isolated from *P. protegens* conditioned medium. Inset shows compound mass spectrum. Expected mass $[M+H^+]$: 211.0611. Observed mass $[M+H^+]$: 211.1000. Additional masses shown are from loss of water and facile fragmentation of the low molecular weight ion.

Fig S4

Figure S4: Fruiting body formation varies across *B. subtilis* colony co-cultured with *P. protegens*. Black scale bars are 0.5 mm, white scale bar is 2 mm.







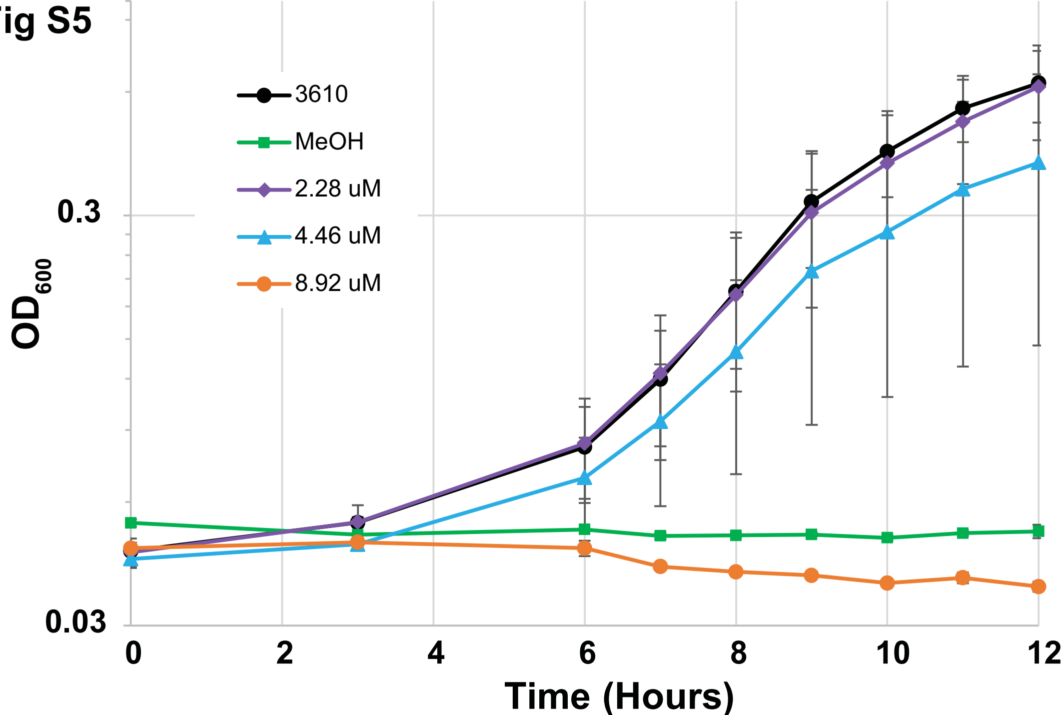


Figure S5: Determination of MIC of DAPG for *B. subtilis*. *B. subtilis* was grown in liquid shaking culture with different concentrations of DAPG (as indicated) to determine the minimal inhibitory concentration.

Fig S6

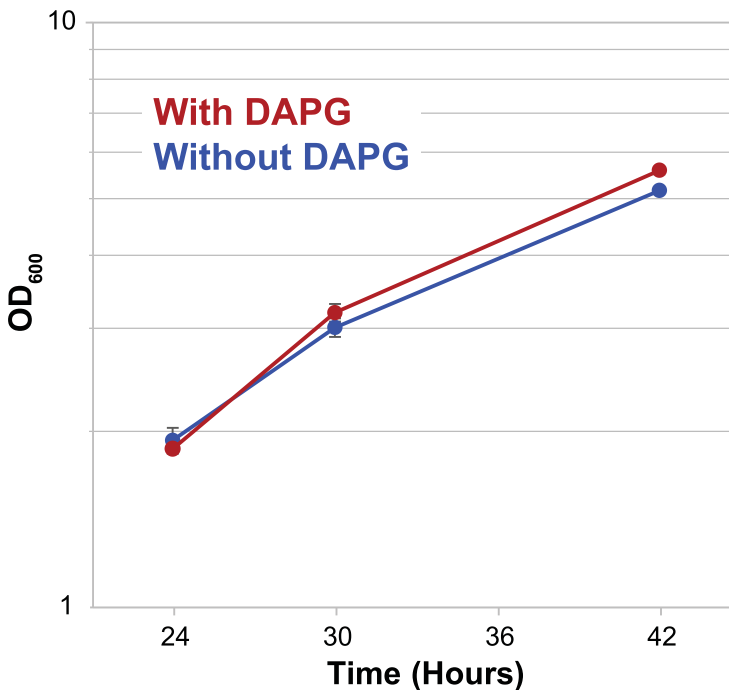


Figure S6: DAPG did not inhibit growth during spore count experiments. *B. subtilis* cultures grown with (orange) or without (blue) sub-inhibitory concentrations (2.23 µM) of purified DAPG. OD_{600} readings were taken at the same time samples were harvested for spore count experiments. DAPG did not inhibit growth at these time points. Error bars are standard deviation from two biological experiments with two technical replicates.

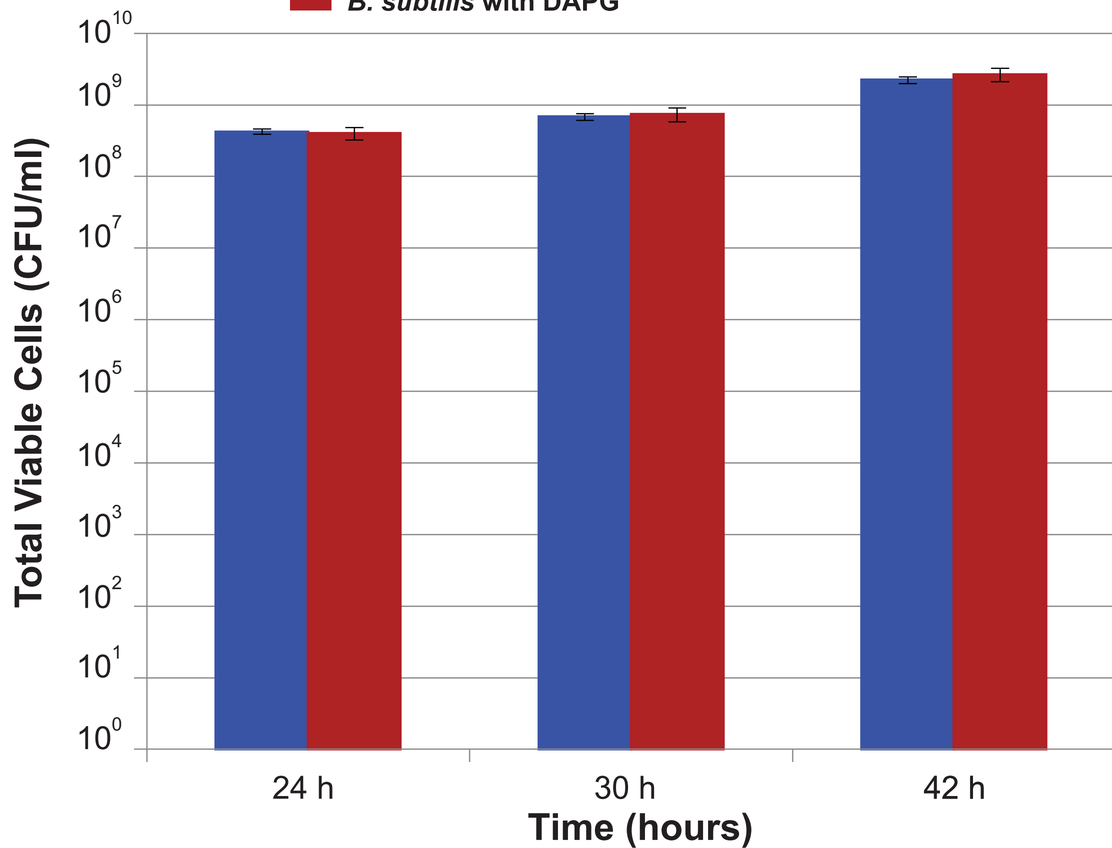
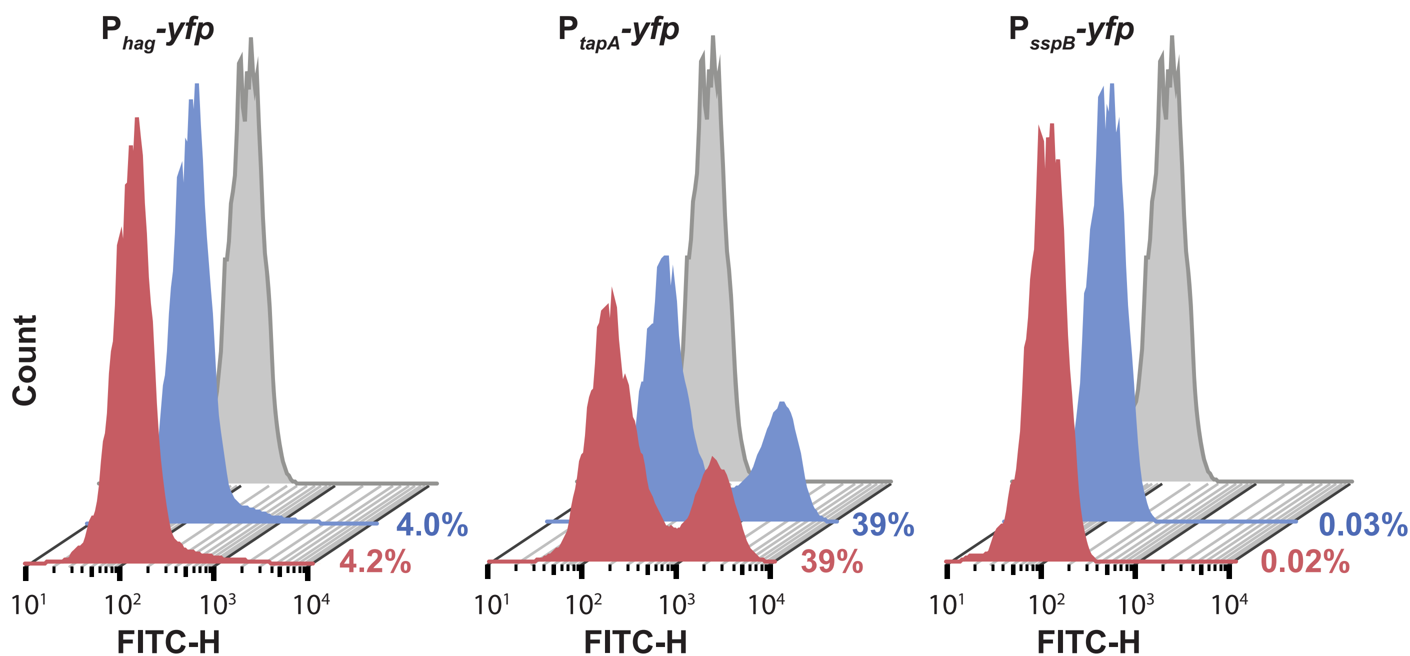
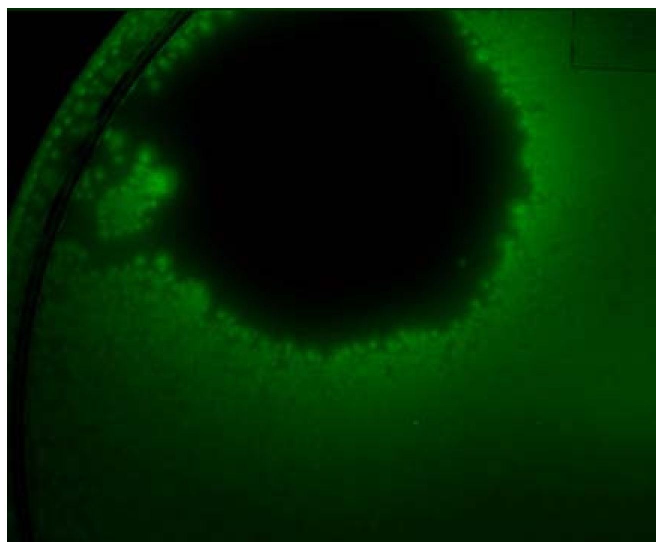


Figure S7: Total number of viable cells was the same at each time point between *B. subtilis* cells grown with or without 2.23 μM DAPG. The total number of viable cells (CFU/ml) obtained from liquid cultures of *B. subtilis* grown with or without 2.23 μM DAPG at 24, 30, and 42 hour time points. n = 3. Error bars are standard deviation.

Fig S8





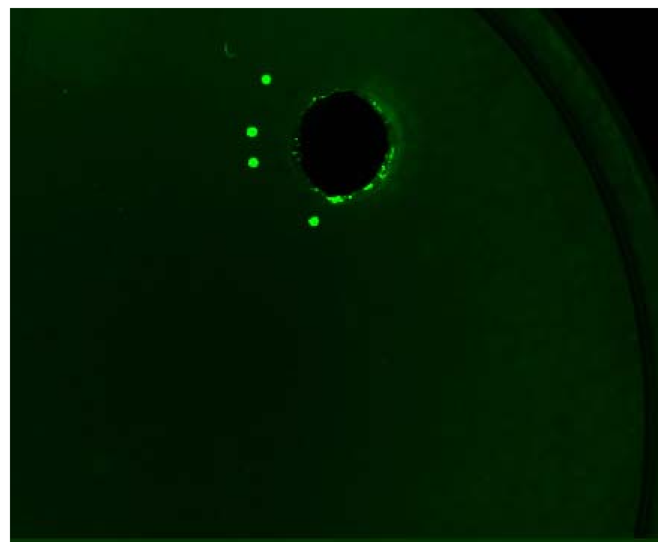


Figure S8: MAPG does not induce biofilm gene expression in *B. subtilis*. A) Flow cytometry analysis of *B. subtilis* cells from pellicles containing cell-type-specific reporters in response to MAPG. *B. subtilis* strains containing the reporters for motility (P_{hag}-*yfp*, left panel), biofilm formation (P_{tapA}-*yfp*, center panel), and sporulation (P_{sspB}-*yfp*, right panel) were grown as pellicles for 18 hr in 1X MSgg, with or without 62.5 µM of MAPG, resuspended, fixed, and analyzed by flow cytometry. Representative histograms of the FITC-H positive (fluorescent) cells are shown; 50,000 cells were analyzed for each sample. Pellicles grown in the presence of MAPG (red, front distributions), or an equivalent volume of methanol as a control (blue, center distributions); the grey histograms in the back are from wild-type control cells containing no reporter construct. The percentage of fluorescent cells in each sample (an average obtained from two independent experiments) is indicated to the right of the histograms. **B)** Microcolony lawns (1 day of growth) of *B. subtilis* P_{tapA}-PROR containing 11.2 ug of either DAPG or MAPG; MAPG showed no induction of fluorescence.