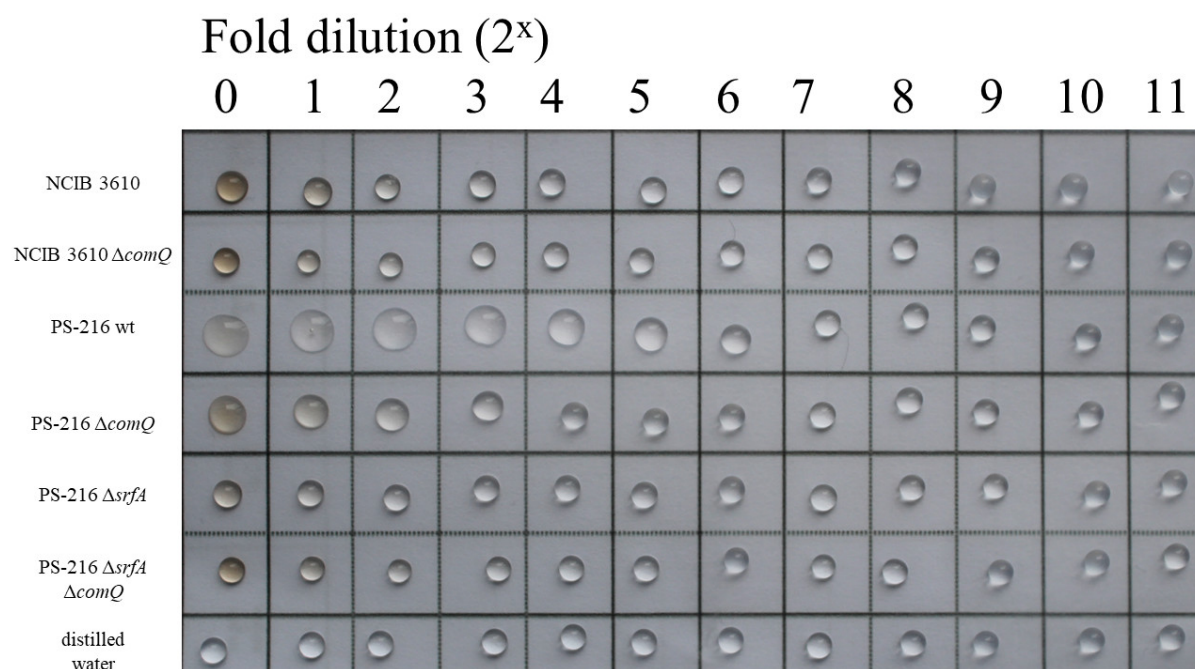
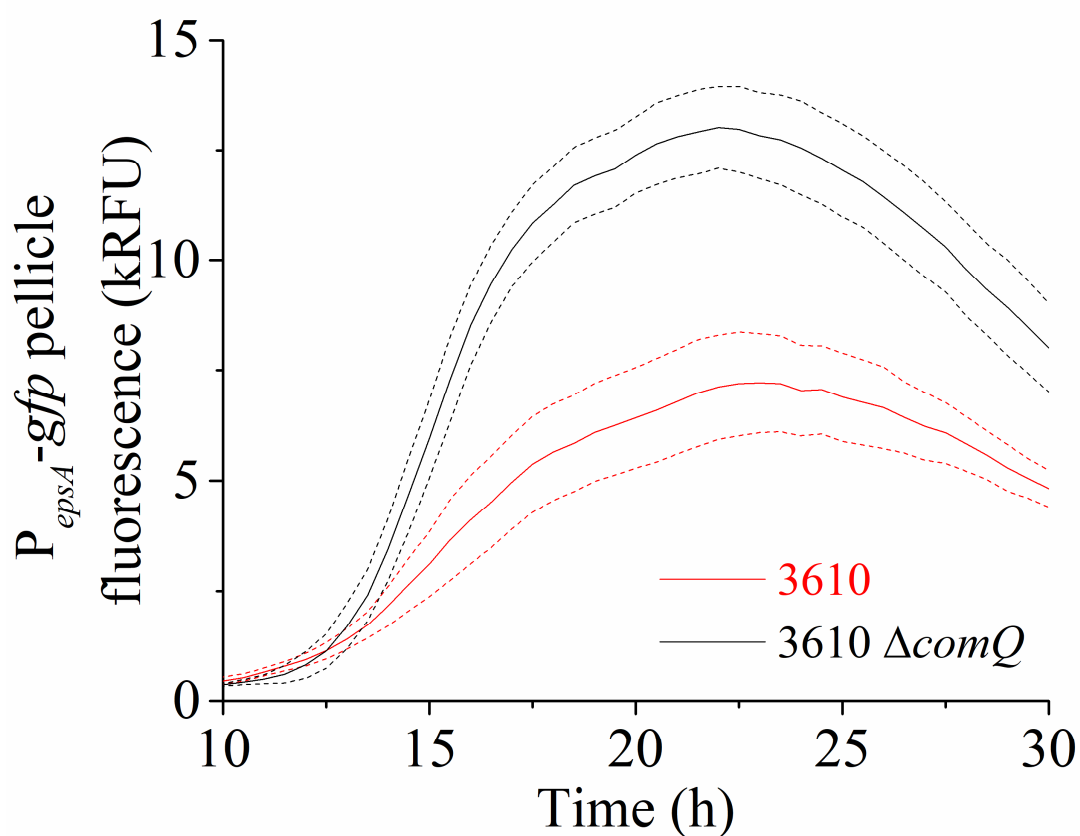




Supplementary Materials:



**Figure S1:** Semi quantification of surfactant concentrations in the pellicle biofilm spent media after 40 h of static growth in MSgg medium at 37 °C using a droplet surface wetting assay



**Figure S2.** Bulk pellicle fluorescence measurements of the  $P_{epsA}$ -gfp reporter activity of *Bacillus subtilis* NCIB 3610 wild type (wt) and QS mutant ( $\Delta comQ$ ) phenotypes during static growth in MSgg medium at 37 °C. Averages of three biological replicates are shown. Statistical significance was determined by calculating the 95% confidence interval (dashed line);

Table S1

Transcriptional reporter	Excitation wavelength [nm]	Emission wavelength [nm]	Gain
$P_{epsA}$ -gfp	480	510	100
$P_{lapA}$ -yfp	500	530	80
$P_{spoIIQ}$ -yfp	500	530	80
$P_{43}$ -mKate2	570	620	100

**Table S1:** Emission and excitation wavelengths and gain settings for transcriptional reporters used in this study,

Table S2

Transcriptional reporter	Excitation Laser wavelength [nm]	Emission filter [nm]	Laser power [%]	Master gain [v]	Pinhole [ $\mu\text{m}$ ]
<b>BM1631 and BM1622</b>					
$P_{epsA}\text{-gfp}$	488	400-580	15	650	71
$P_{43}\text{-mKate2}$	561	585-700	2.5	600	84
<b>BM1613 and BM1614</b>					
$P_{tapA}\text{-yfp}$	488	400-590	7	600	72
$P_{43}\text{-mKate2}$	561	590-700	2.5	600	86
<b>BM1625 and BM1626</b>					
$P_{spoIIQ}\text{-yfp}$	488	400-590	7	600	72
$P_{43}\text{-mKate2}$	561	590-700	2.5	600	86

**Table S2:** Confocal laser scanning microscope settings for different transcriptional reporters used in this study;

<https://www.youtube.com/watch?v=1FRfrhZIPKY&feature=youtu.be>

**Video S1:** Fast forward video showing diffusion of the 50  $\mu$ L methylene blue droplet placed on the PS-216 wild type (wt) or QS mutant ( $\Delta comQ$ ) pellicle biofilms grown statically in MSgg medium at 37 °C for the time periods indicated on the video, <https://www.youtube.com/watch?v=1FRfrhZIPKY&feature=youtu.be>. Upload date: 17.9.2018.