

Fig. S1.

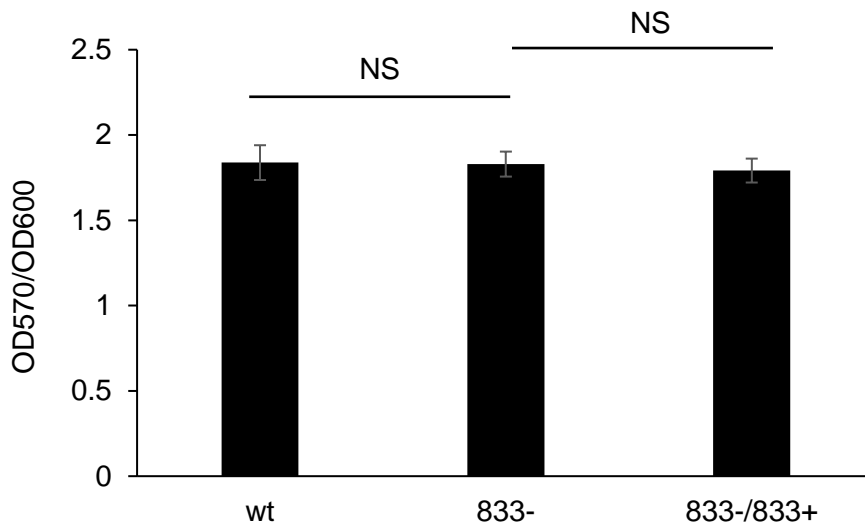
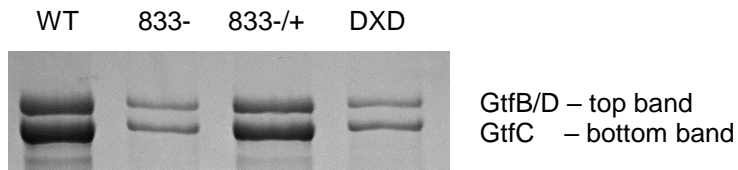


Fig. S1. Comparison of biofilm metabolic activity of the wild type, *smu_833* mutant, and complementation strains. Biofilms were grown on 96-well polystyrene plates in TYE medium supplemented with 1% sucrose for 18h. Biofilms were washed with PBS then alamarBlue® reagent (ThermoFisher Scientific) was added to the wells and the OD570/600 was measured. Data is representative of at least three independent experiments with $p > 0.05$ or N.S. Statistical analysis was done using Student's *t*-test.

Fig. S2.

A



B

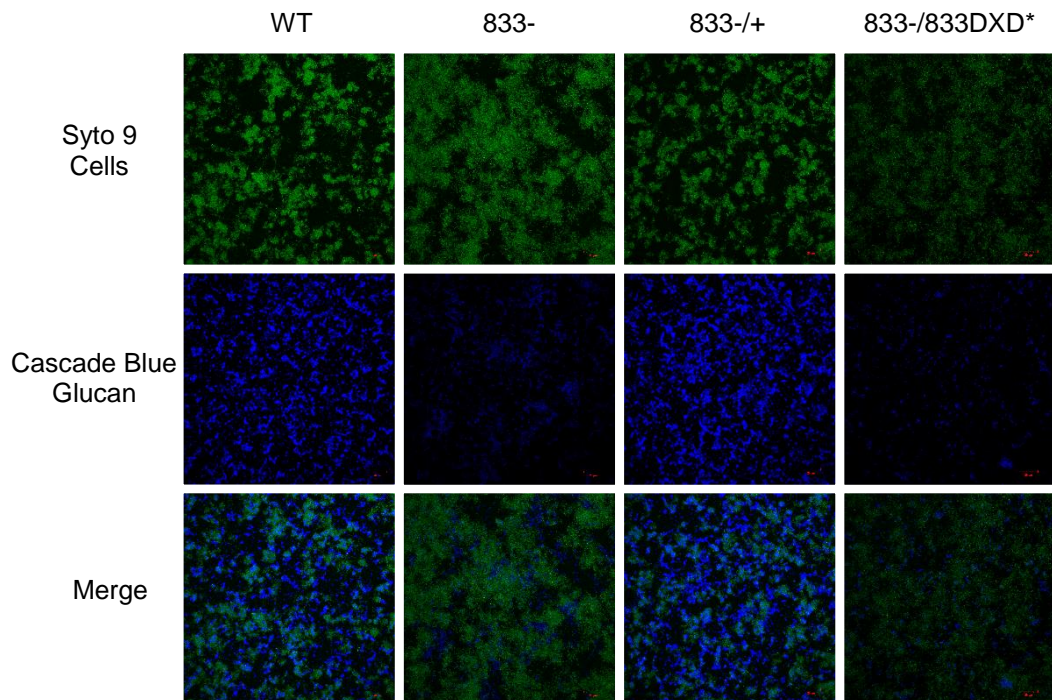


Fig. S2. Gtf protein levels and glucan matrix formation of the *smu_833-DXD** mutational strain. Point mutation in conserved catalytic DXD motif of SMU_833 exhibits the same phenotype as *smu_833* deletion. (A) Coomassie stained SDS-PAGE of Gtf proteins from culture supernatants. (B) Biofilms were grown on 8-well ibiTreat slides in TYE medium supplemented with 1% sucrose and cascade blue conjugated to dextran for 18h. Biofilms were washed with PBS then stained with SYTO9. The stained samples were examined by CLSM (Zeiss LSM 710 laser confocal microscope) with a 63x oil immersion objective.

Fig. S3.

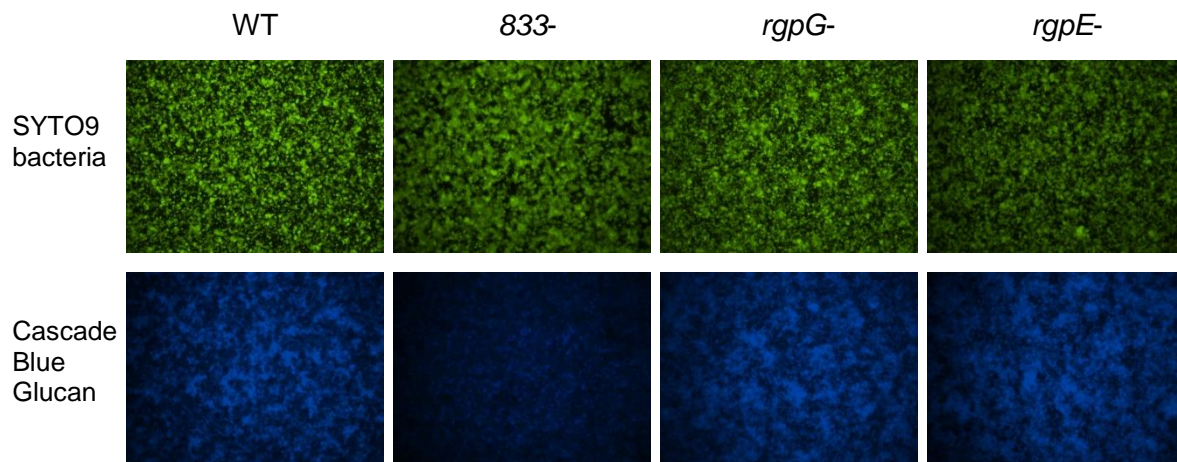


Fig. S3. Visualization of biofilm and glucan matrix. Wild type, *smu_833* mutant, *rgpG* mutant (lacking all RGP), and *rgpE* mutant (rhamnose backbone lacking glucose side chains) biofilms were grown on 96-well polystyrene plates in TYE medium supplemented with 1% sucrose and cascade blue conjugated to dextran for 18h. Biofilms were washed with PBS then stained with SYTO9. Images are representative of at least three independent experiments.

Fig. S4.

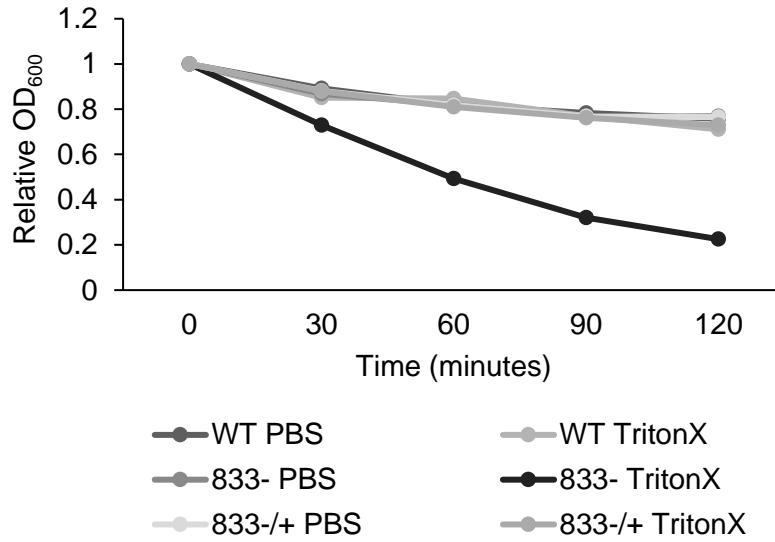


Fig. S4. Measuring susceptibility to autolysis using 0.2% TritonX in PBS. Wild type, *smu_833* mutant, and complementation strains were grown to early log, washed with PBS, then resuspended in either PBS with 0.2% TritonX or PBS alone as a control. OD₆₀₀ was measured every 30 minutes for 2 hours as a measure of cell lysis.