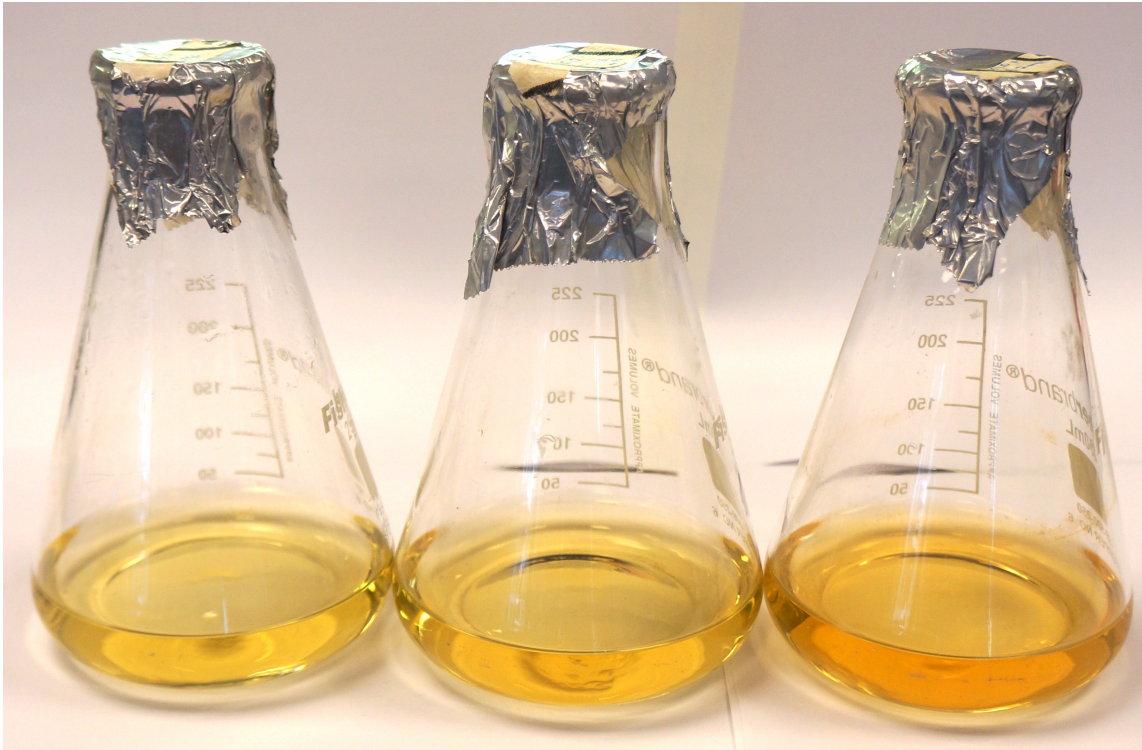


Supplemental Figure 1: Loss or overexpression of pulcherrimin biosynthesis genes does not affect cell growth of *B. subtilis*.

Growth of the wild type, pulcherrimin mutant and complementation strain in LB broth with or without IPTG supplementation at 1mM was accessed in a 96-well plate for 16 hours at 37°C. Experiment was performed with 8 technical replicates and average OD₆₀₀ nm values were plotted using Graphpad Prism 9. Error bars correspond to standard deviation.



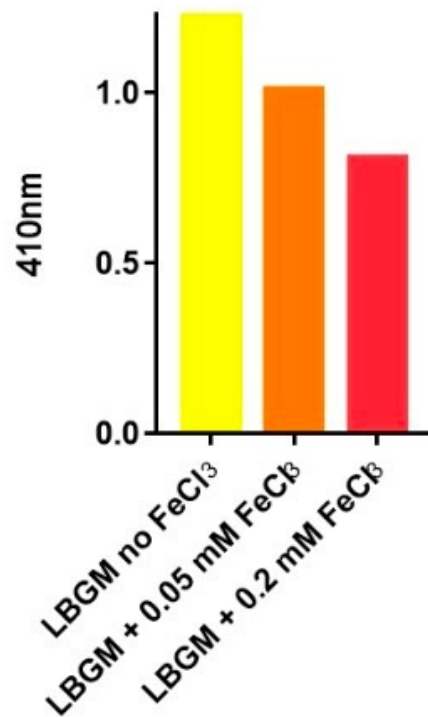
Regular LBGm

+0.05 mM FeCl₃

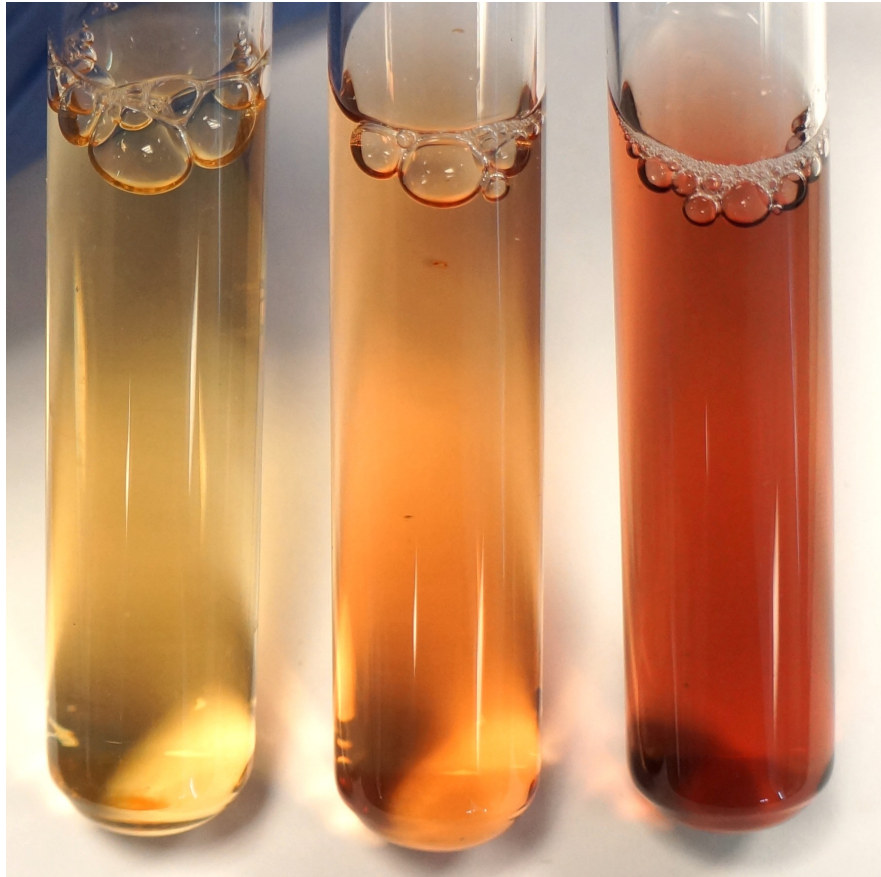
+0.2 mM FeCl₃

Supplemental Figure 2: FeCl₃ supplementation does not alter the color of LBGm broth. LBGm broth was prepared and aliquoted into 3 separate flasks. The first flask (left) did not have FeCl₃ added, the second flask (middle) had FeCl₃ added to a final concentration of 0.05 mM, and the third flask (right), to 0.2 mM. The broth color did not significantly change after FeCl₃ supplementation.

Figure S2



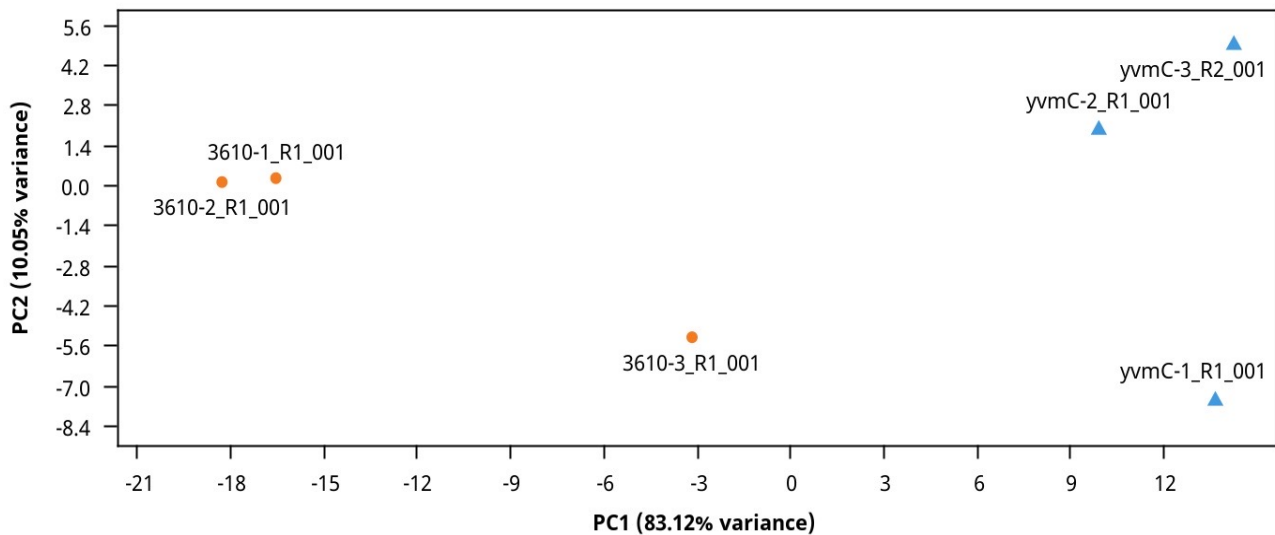
Supplemental Figure 3: The amount of pulcherriminic acid in the supernatant decreases with increasing FeCl₃ supplementation. Bar graphs represent values after quantification of total pulcherriminic acid from spent supernatants from overnight cultures of the wild-type strain with no FeCl₃ supplementation, 0.05 mM, and 0.2 mM FeCl₃. 410 nm values were plotted using Graphpad Prism 9.



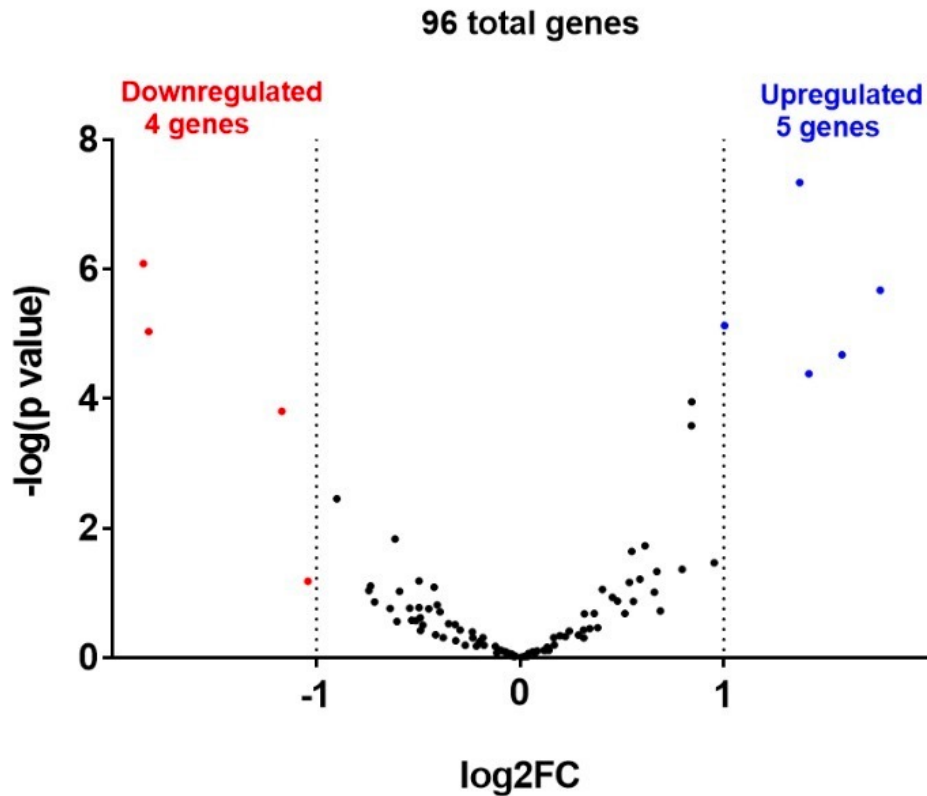
No addition +0.05 mM FeCl₃ +0.2 mM FeCl₃

Wild-type spent supernatants

Supplemental Figure 4: Addition of FeCl₃ to wild-type pellicle spent supernatants leads to conversion of pulcherriminic acid into the reddish-brown pigment pulcherrimin. Images obtained from a 3-days old wild-type pellicle filtered supernatant without FeCl₃ added (left), or supplemented with FeCl₃ to a final concentration of 0.05 mM (middle) or 0.2 mM (right). The supernatant turns from light yellow to a reddish-brown color (corresponding to pulcherrimin) once FeCl₃ is added.



Supplemental Figure 5: Principal Component Analysis carried out with expression of 4,237 genes. Two principal components (PCs) with most of the variation are shown. PC1 (83.12%) separates the pulcherrimin mutant ($\Delta ycmC-cypX$, blue triangles) from the wild type (3610, orange circles) RNA-Seq samples.



Supplemental Figure 6: Volcano plot generated after RNA-seq analysis using pBS32 plasmid from NCIB3610 as reference. Volcano plot depicting differentially expressed genes (blue and red dots) based on RNA-Seq analysis using pBS32 plasmid as reference (96 genes total). Downregulated genes with a log₂FC of -1 or lower are depicted in red (4 total), and upregulated genes with a log₂FC of 1 or higher are depicted in blue (5 total). Black dots represent genes not differentially expressed.