A Multi-prong Approach to Eliminating *S. aureus* Biofilms Using Recombinant Bacteriophages and Biofilm-Degrading Enzymes

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**Background**
- Biofilms are microbial communities held together by a matrix and often cause medical problems.
- DispersinB (DspB), an enzyme that breaks a critical bond in biofilm matrix molecules, facilitates biofilm dispersal.
- Quorum-sensing, the process used by biofilms for communication and coordinated behavior, can be exploited for the regulation of gene expression.
- Phage can be used as a vector for delivering genetic material and propagating it via host cell machinery.
- We propose engineering a phage that eradicates *Staphylococcus aureus* biofilms by exploiting host machinery to produce DspB and more phages under the regulation of the quorum-sensing system.

**The Concept**
- Inserted into phage genome
- Quorum-sensing promoter regulates expression of phage genome
- Gene encoding biofilm matrix degrading enzyme
- Infection
- Dispersed *S. aureus*
- *S. aureus* biofilm

**S. aureus** biofilm exhibits log growth

![Growth curve of two strains (RN4220, green; B325-4, red) of the biofilm culture. The biofilm exhibits exponential growth before attaining dynamic equilibrium at 18h.](image)

**Quorum-sensing system allows control**

![Schematic diagram of the quorum-sensing system. The P2 promoter regulates the activity of the Agr operon which encodes proteins that are involved in the quorum-sensing system. We are exploiting the P2 promoter to regulate phage expression.](image)

**DspB cleaves PNAG analog**

![Structure of the biofilm matrix molecule (left) and its analog (right). DspB cleaves the bond (red) found in both poly-β-(1,6)-linked N-acetylglucosamine (PNAG) and 4-Nitrophenyl N-acetyl-β-D-glucosaminide (NP).](image)

**Model predicts biofilm dispersal**

![Simulation indicates the destruction of the biofilm population (blue). The elevated level of the phage population (orange) and DspB concentration (green) coincides with the sharp decline in the biofilm population at 500 min.](image)

**Conclusions**
- Our DspB part breaks a critical bond in the analog of the biofilm matrix molecule.
- We submitted our report construct part for the quorum-sensing P2 promoter to the Registry.
- We developed a novel phage standard that facilitates the design of BioBrick parts that can be incorporated into phage genomes.
- Our math model can predict the outcome of the introduction of phage to biofilms under different conditions

**References**