

Title: Enhancing Biofilm Inhibition and Eradication Using Bacteriophages

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Bacteria are single-celled organisms that communicate with each other with small signaling molecules. Some bacteria can secrete molecules that build a protective layer outside the cell called a biofilm. Biofilm formation protects bacteria from both the host's immune defenses and antibiotics, which is why biofilm is labeled as a virulence factor. Since biofilms significantly reduce antibiotic efficiency, alternative therapeutic strategies are urgently needed. Bacteriophages are bacterial viruses that show strong potential for biofilm control because they multiply within bacterial populations. Two different bacteriophages specific for each of five different bacterial species (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans*) have been tested for biofilm eradication with a Crystal Violet Assay. To evaluate eradication, pre-formed biofilms were treated with bacteriophage, and the reduction in biomass was compared to untreated controls. Thus far, the degree of biofilm eradication appears to be affected by the time biofilm is allowed to be established prior to phage addition, as well as whether fresh media is added at the time of the phage addition. Of the ten phages tested, one *E. coli* phage eradicates biofilm by 50 %, and two phages that enhance biofilm formation by 100-400 %, one in *Streptococcus mutans* and one in *Staphylococcus aureus*. Future experiments include maximizing eradication by optimizing phage titers for each phage/species pair and optimizing the timing of phage addition to established biofilms. Once optimization is established, phage in conjunction with inhibitory compounds will be tested to determine if effects are additive or multiplicative. A phage: antimicrobial combination strategy has been the focus of many recent studies. This work contributes to the development of more effective and targeted approaches for biofilm control in clinical settings.