Examination of Host Range of *Pseudomonas aeruginosa* phages UT1, SN-T, and PEV2 for Treatment of Bacterial Biofilms in Fuels

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**Background**

- Biofilm: community of microorganisms attached to a surface
- Biofilms are found in the human body, natural settings (streams), and man-made environments
- Specifically in fuel systems, biofilms can degrade fuel, clog fuel lines and fuel filters, and contribute to corrosion
- Biofilms excrete extracellular polymeric substance (EPS), comprised of DNA, carbohydrates, and proteins → Confers protection from environmental stressors and antimicrobial agents

**Problem**

The natural resistance of biofilms to antimicrobial agents like antibiotics necessitates the investigation of alternative mitigation strategies

**Proposal**

- Phage therapy: use of bacteriophage (bacterial viruses) for the treatment of bacterial infections
- Use known phages UT1, SN-T, and PEV2 to treat biofilms formed from bacteria isolated from fuel

**Methods**

- **Fuel Isolate Screening**  
  - Bacterial strains spotted with concentrated phage and observed for clearing
- **Biofilm Assay**  
  - Biofilms of PAO1 in 96 well plates in the presence of phage for 16h (inhibition) or in the absence of phage for 10h and then subjected to phage for 24 hours (remediation)
- **Estimation of Biofilm Biomass**  
  - Biofilms stained with 0.25% crystal violet, crystal violet extracted with ethanol, absorbance measured at 590nm
- **Viable Cell Counts**  
  - Biofilm cells mechanically removed from wells, CFU/cm² determined by plating

**Results**

**Table 1.** List of bacterial isolates from aviation jet fuel that show some degree of susceptibility to phages UT1, SN-T, and PEV2 in test clearing and/or inhibitory plaques throughout the cleared zone.  
- Includes biofilms isolated from the injected zone, in clearing throughout with a key background, or complete clearing

**Figure 1.** Efficiency of phage results for UT1, Bacteriophage compared to the PAO1 control. Absence of individual plates indicates that clearing in the spot lysis assay was not due to true infection. Similar results were found for all fuel isolates tested.

**Figure 2.** A. Crystal Violet-stained biofilm assay plate after inhibition treatment of phages UT1, SN-T, and PEV2; B. Absorbance values (as fold change) for crystal violet extracted from biofilms subjected to inhibition assay; C. Absorbance values (as fold change) for crystal violet extracted from biofilms subjected to remediation assay. Error bars represent SEM. Different letters indicate statistical significance.

**Figure 3.** A. Measure (as log CFU/cm²) of viable cells attached to biofilm in remediation biofilm assays; B. Measure (as log CFU/cm²) of viable cells attached to well surface in remediation biofilm assays. Error bars represent SEM.

**Conclusions**

- Phage did not exhibit broad host range against the bacterial species tested
- Results suggest phage cocktails are most effective
- Under conditions tested, phage therapy was more effective in preventing biofilm formation than remediation
  - EPS may have provided greater protection against phage in established biofilms

**Future Directions**

- Incubate phage longer with established biofilms  
  - 48 hours rather than 24
- Use phage in combination with chemical compounds that break down EPS and allow phage greater access to bacterial cells
- Test best performing phage cocktails in fuel model system

**References**


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