CApEsid biOfilm: A Suggested Pipel;ine for Clinical Phage Microbiology for Biofilm Infections

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INTRODUCTION

The rise of antibiotic-resistant infections poses a severe threat to global health. This challenge is further intensified by bacterial biofilms, resilient communities of bacteria that exhibit formidable resistance to conventional treatments due to their complex, protective structures. Bacteriophages, or phages, emerge as a viable solution, targeting and destroying bacteria with precision, minimizing harm to the host's microbiota, and displaying self-amplifying properties. Their natural origin reduces toxicity risks, and they can be easily modified and isolated. Crucially, phages equipped with depolymerase enzymes can break down biofilms, offering a potent strategy against these formidable bacterial fortresses.

AIM

Identifying the right phage for biofilm infections poses a challenge, as current methods primarily evaluate phage efficacy on planktonic bacteria, not on biofilms. Without standardized techniques for testing phages on biofilms, selection relies on less representative methods. In this study, we addressed this critical gap by assessing the activity of five phages against *Pseudomonas aeruginosa* biofilms using a comprehensive array of methods. Each phage was tested at two titers to evaluate their ability to discern between high and low phage activity, providing insights into the efficacy of different phages against biofilm-associated infections.

METHODS & RESULTS

1 Calorimetry (calScreenerTM)

Measures heat generated by bacteria

Symcel.com

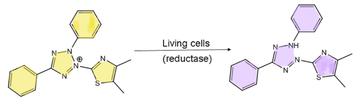
2 pH Change (Seahorse XFe96 Analyzer)

Measures the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) near biofilm cells.

agilent.com

3 Tetrazolium dye Reactions

Measuring metabolic enzyme activity



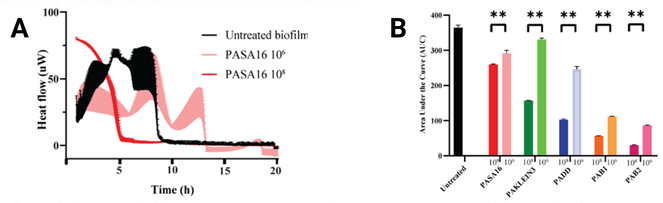


Figure 1. Calorimetric Heat flow-based phage biofilm effect comparison. A) Comparing *P. aeruginosa* biofilm heat generation treated with the phage PASA16 phage at 10^8 and 10^6 PFU/ml. **B)** The AUC of five phages' activity (at a PFU/mL of 10^8 and 10^6) was calculated at different concentrations: a consistent pattern of lower metabolic activity was observed across all five phages when a higher phage titer was applied.

Pros and Cons:

- ✓ Differentiation between all tested phages
- ✓ Differentiation between phage concentrations
- ✓ Detection of bacterial regrow
- ! Labor: ~1 hour
- ✗ Requires professional training
- ✗ Very expansive

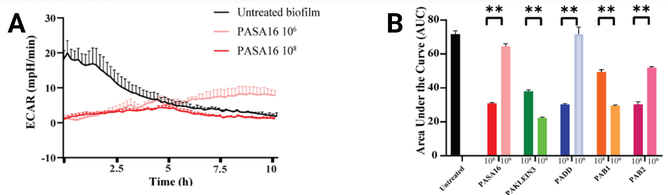


Figure 2. Extracellular acidification rate (ECAR) based phage biofilm effect comparison. **A)** Comparing H+ flux of a *P. aeruginosa* biofilm, treated with the phage PASA16 at a PFU/mL of 10^8 and 10^6. ECAR was measured every 12 minutes, for 10 hours. **B)** The AUC of phage activity was calculated at different concentrations of phages. Notably, a consistent pattern of lower metabolic activity was observed in three out of five phages when a higher phage titer was applied.

Pros and Cons:

- ✓ Differentiation between phage concentrations
- ✓ Labor: ~2 hours
- ! Differentiation between 3/5 tested phages
- ! Expansive
- ✗ Test biofilm on target material
- ✗ Limited to 10 hours of incubation

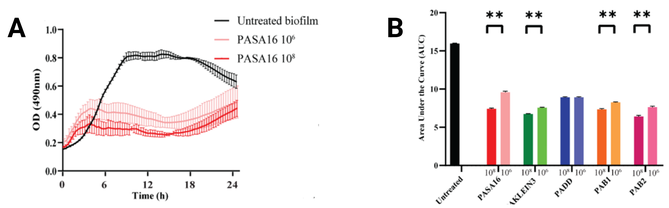
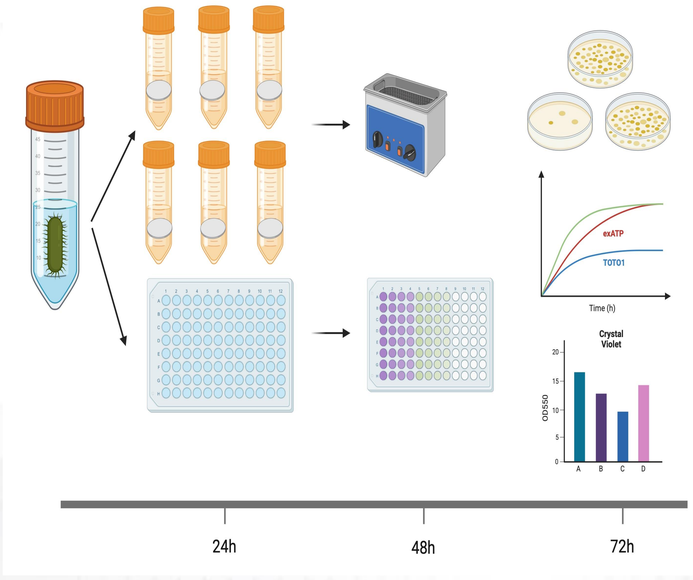


Figure 3. Tetrazolium dye-based phage biofilm effect comparison. MTS-based assay was used, which is converted to formazan by metabolic enzymes and therefore changes OD490. **A)** *P. aeruginosa* biofilm, treated with the phage PASA16 phages at a PFU of 10^8 and 10^6. **B)** The Area Under the Curve of phage activity was computed at different concentrations, as detailed earlier. lower metabolic activity in four out of five phages when exposed to a higher phage titer.

Pros and Cons:

- ✓ Differentiation between phage concentrations
- ✓ Labor: ~½ hours
- ✓ Differentiation between 4/5 tested phages
- ✗ Inexpensive
- ✗ Test biofilm on target material
- ✗ Detection of bacterial regrow

CApEsid biOfilm: a suggested protocol



CApEsid biOfilm – a protocol that asseses phage efficacy against mature biofilms combining:

1. **C**rystal violet staining
2. Extracellular **A**TP measurements
3. **E**xtracellular DNA quantification using a dye
4. Modified colony-forming unit (CFU) assays using stainless steel washers (**O**)

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