Bacteriophages for Biofilm Eradication

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Introduction

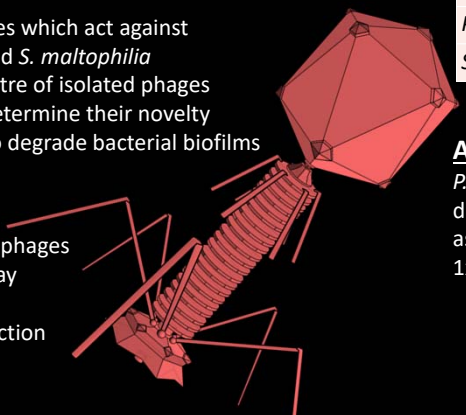
Bacteriophages or phages are a unique type of virus that recognize a specific type of bacteria and then infect, replicate and kill the host via cell lysis. The application of phages and their enzymes for treating bacterial biofilms has recently gained significant interest due to a number of significant advantages compared to traditional antibiotics, including high specificity and efficacy, low immunogenicity and production costs.

Proteus mirabilis, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* are all clinically relevant microorganisms associated with biofilm formation and thus there is great difficulty treating such infections.

With the increasing number of multi-drug resistant strains being reported the time for novel antimicrobial agents is now and thus we propose the use of bacteriophages to treat the said bacterial species' biofilms.

Aims

- To isolate novel bacteriophages which act against *Proteus* spp., *P. aeruginosa* and *S. maltophilia*
- Obtain high and pure phage titre of isolated phages
- Characterize each phage to determine their novelty
- Assess the ability of phages to degrade bacterial biofilms



Methodology

- Environmental enrichment of phages
- Top agar overlay spot test assay
- Top agar plaque assay
- Phenol:chloroform DNA extraction
- Genomic restriction analysis
- MBEC assay

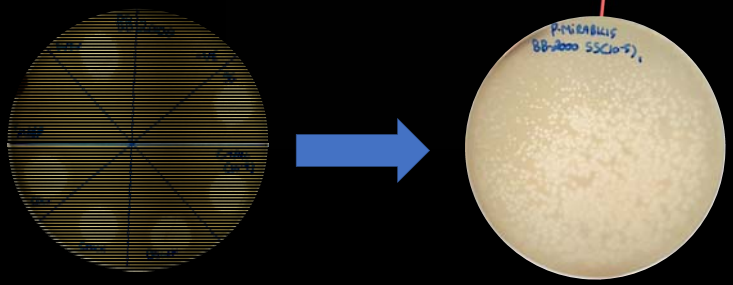


Fig. 1. (Left) Spot tests of phages isolated from various animal faecal sources and waste water effluent. **(Right)** Plaque assay confirming the presence and activity of one of the newly isolated phages (BB2000) against *P. mirabilis*.

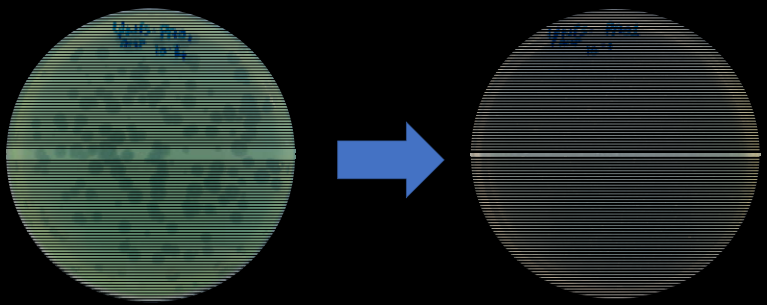


Fig. 2. (Left) Plaque assay results at a 10^{-4} dilution for φNFS phage active against *P. aeruginosa* PA01. **(Right)** Total lysis of PA01 with 10^{-1} diluted φNFS phage demonstrates the potential of this phage as a biofilm biocontrol agent.

Future work

- Isolate *S. maltophilia* phages from various environmental samples
- Optimize our current phage biofilm assay techniques
- Assess the potential for a phage-cold plasma synergistic technique to facilitate total biofilm eradication
- Assess the effect of a cocktail of phages in a biofilm model to see if they enhance the degradation of the biofilm
- Sequence novel phages using MinION nanopore sequencing and identify genes of potential biofilm degradation enzymes
- Obtain phage enzymes and assess their potential as biofilm biocontrol agents

Results

To date we have isolated 6 phages against 6 different Proteus bacteria (Fig. 1), with 2 phages lysing all strains. Furthermore, we have 9 phages which act against *P. aeruginosa*. Phage φNFS (Fig. 2) has shown great preliminary results when attempting to degrade PA01 biofilms and resulted in a clinically significant two log reduction of the biofilm. The Proteus phages are yet to be tested against biofilms, but are expected to have a similar outcome to that of φNFS. These results so far show the extreme potential of phages to act as biocontrol agents against biofilms in an era of increasing multi-drug-resistance.

Pathogen	Phage #	Isolation	Host range	Sequencing	Anti-biofilm assay (MBEC)
P. aeruginosa	9	Completed	Planned	Completed	In Progress
Proteus spp.	50	In progress	In Progress	TBC	In Progress
S. maltophilia	0	In progress	Planned	TBC	Planned

Anti-biofilm assays (MBEC)

P. aeruginosa biofilms grown via the MBEC device (Fig. 3) were exposed to different phage titres of φNFS phage and incubated for 18-24 h before assessing the ability of the phages to degrade the biofilms. A phage titre of $1x10^7$ showed a 100x reduction of the PA01 biofilm (Fig. 4).

Fig. 3. Shows the CV stained pegs of the MBEC device used for the φNFS. As can be seen from left to right an increase in staining and hence biomass as the phage titre decreases in value resulting in less biofilm degradation

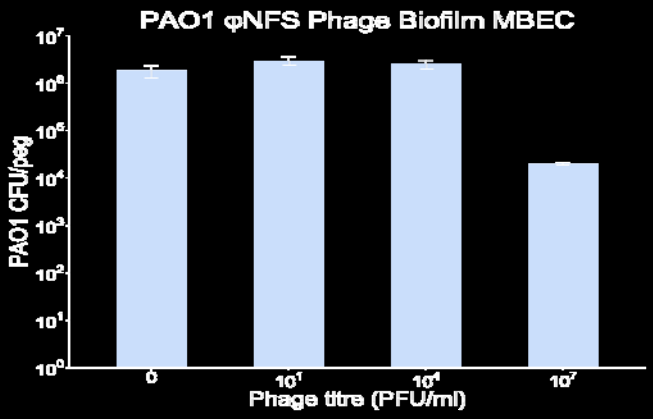


Fig. 4. Shows the effect of various phage titres and the consequent 100x reduction of the PA01 biofilm when exposed to φNFS bacteriophage

Planned MinION nanopore sequencing

- We plan to sequence all our phages using MinION nanopore sequencer
- Test runs with *Escherichia coli* phage λ produced 130k reads in 6 h!