Successful elimination of *Pseudomonas aeruginosa* biofilms with a novel bacteriophage

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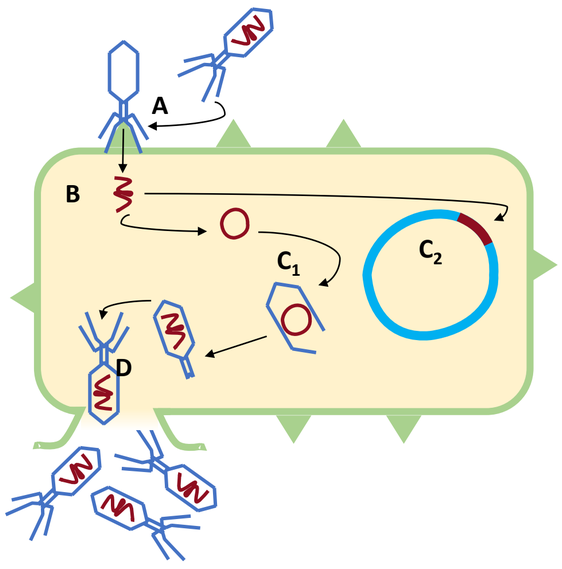
Introduction

- ***Pseudomonas aeruginosa*** is a Gram-negative bacterium commonly found in almost any environment including the human microflora.
- It is an opportunistic pathogen capable of causing severe, and sometimes, life-threatening acute and chronic infections. A member of the "ESKAPE" group of pathogens, it is **one of the leading causes of nosocomial infections worldwide**.
- Known to be resistant to many common antibiotics with an **increasing number of multidrug resistant isolates** appearing in clinical settings. In addition, their **ability to form biofilms increases the complexity of treatment** as biofilm formation increases resistance to antibiotics.
- We must therefore consider new approaches and strategies for treatment including the use of **bacteriophages as a potential therapeutic agent**.
- Bacteriophages are viruses which can recognise specific bacteria and subsequently infect them then use host machinery to replicate and ultimately lyse the bacterial cell.



A – Phage binds to a target site on the surface membrane of bacterial cell.

B – Phage DNA is released into the bacterial cell.

C_1 – Host machinery replicate phage DNA and viral proteins are produced.

C_2 – DNA inserted into bacterial chromosome and enters lysogenic cycle.

D – Phage is fully formed and lyses bacterial cell, killing the bacteria.

Fig 1. Diagram demonstrating the phage life cycle.

Aims

- To isolate novel bacteriophages for use against *P. aeruginosa*
- Test the obtained phages for activity against *P. aeruginosa*
- Characterise each phage to determine its novelty
- Assess the ability of each phage against bacterial biofilms of *P. aeruginosa*

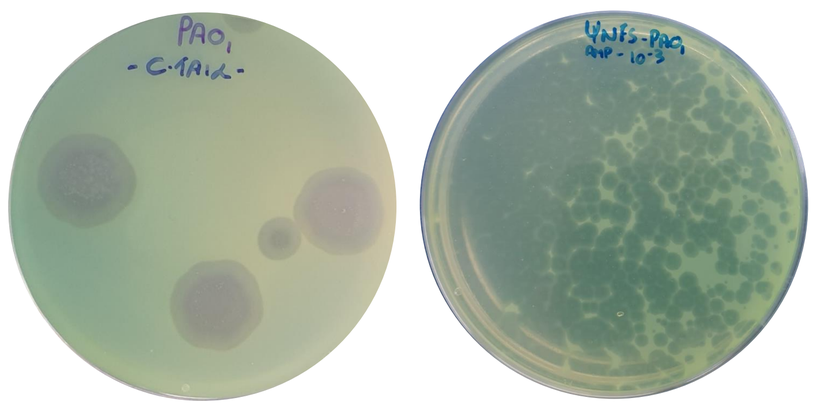
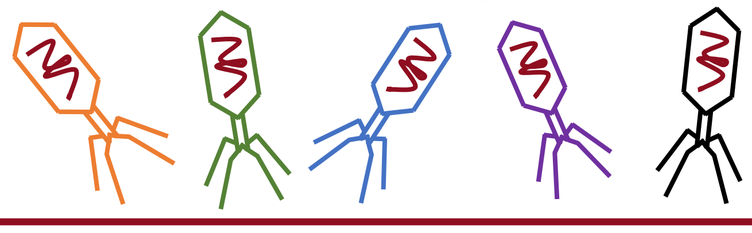


Fig 2. Cocktail phage (RusC) activity using a spot test against *P. aeruginosa* PAO_1 (*left*). Activity of φNFS phage against *P. aeruginosa* PAO_1 demonstrated by a plaque assay (*right*).

Methodology

- Samples were collected and then enriched for amplification.
- Determination for the presence of phage using a spot test assay.
- Isolation of single phage colonies using plaque assays.
- Production of a high titre phage lysate.
- Phenol:chloroform extraction of phage DNA from the high titre lysate.
- Genomic sequencing.



Results

Table 1. Summary of isolated phages to date for *P. aeruginosa* and *MRSA*.

Pathogen	Phages found	Isolation	Host range	Sequencing
P. aeruginosa	11	Completed	Planned	TBC
S. aureus	N/A	In progress	Planned	TBC

Anti-biofilm assays (MBEC)

- Biofilms of *P.aeruginosa* PAO1 were grown on an 96-well MBEC plate (Fig. 3) and subsequently exposed to different phage titres of RusC phage.
- A 24 hour incubation stage followed before assessing the efficacy of the phage to degrade the biofilm.
- Complete eradication of the PAO1 biofilm occurred with the highest phage titre used (10^7 plaque forming units/mL).

Fig 3. 96-well MBEC plate containing crystal violet stained pegs demonstrating activity of the RusC phage. From left to right, a decrease in staining occurs and thus biomass as the phage titre increases in value resulting in an increased degradation of the PAO1 biofilm.

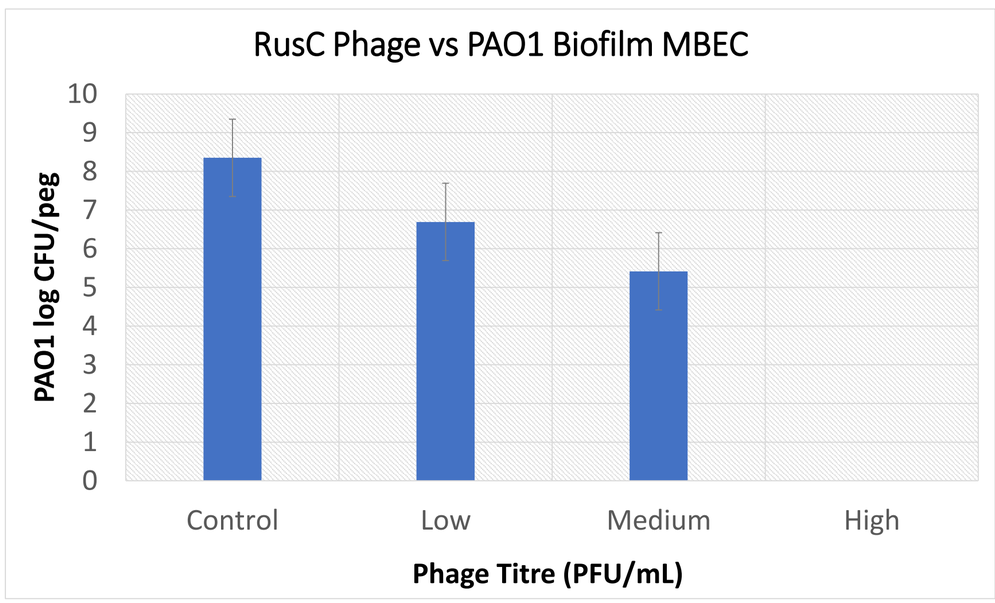


Fig 4. The effect of low (10^1), medium (10^4) and high (10^7) phage titres on PAO1 biofilm eradication upon exposure to the RusC phage.

Conclusions

- To date, 11 bacteriophages have been isolated that is active against strains of *P. aeruginosa*. The lytic zone of each isolated phage varies quite significantly between 1mm to several millimetres in diameter.
- Two phages have been tested against PAO1 biofilms via an MBEC method. Both phages (φNFS and RusC phage) show great promise with the RusC phage showing complete eradication of the biofilm.
- With the success of isolating phages for *P. aeruginosa*, plans have been put forward to attempt isolation of phages for other ESKAPE pathogens including *S. aureus* (MRSA).