Phage therapy for chronic *Pseudomonas aeruginosa* infections in Cystic Fibrosis patients

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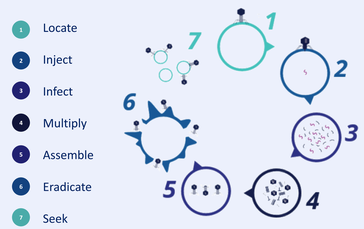
Abstract

Background: Chronic respiratory infections with *Pseudomonas aeruginosa* (*P. aeruginosa*) are a major cause of morbidity and mortality in Cystic Fibrosis (CF) patients[1]. Due to extensive antibiotic use, there are increasing rates of colonization with multi-drug resistant (MDR) *P. aeruginosa* isolates[2]. In addition, biofilm-forming propensity of many *P. aeruginosa* isolates contributes to treatment failure with antibiotics[3].

Phages are naturally occurring viruses that kill specific bacteria. Unlike antibiotics, phages are specific to the strain level and therefore have unique advantages in terms of minimizing perturbation of the microbiome. They have no capability to infect mammalian cells and so are considered safe.

Phages are self-amplifying therapeutic agents



Phages are also unique in that they are self-amplifying and, once their target bacteria is eliminated, they are passively cleared of the clinical system. Combinations of phages are utilized to prevent the appearance of resistant mutant bacteria.

Aim: To develop a "cocktail" of phages that could efficiently infect a broad range of *P. aeruginosa* isolates cultured from sputum samples of CF patients.

Methods: Hundreds of environmental samples were screened for natural phages which can infect clinical *P. aeruginosa* isolates. The phages were sequenced, analyzed for the absence of undesirable genes as stipulated by the regulatory authorities, and characterized with respect to activity on multiple bacterial target isolates and on bacteria within biofilms.

Results: Phages active against *P. aeruginosa* isolates from CF patients' sputum samples from the US (n=122) and Europe (n=24) were identified. *In vitro* host range analysis of a selected subset of 3 phages against 146 *P. aeruginosa* isolates, revealed ~80% coverage. Phage cocktail was efficacious in significantly reducing target bacteria encased in biofilm relative to treatment with antibiotic or vehicle (p-value < 0.0001).

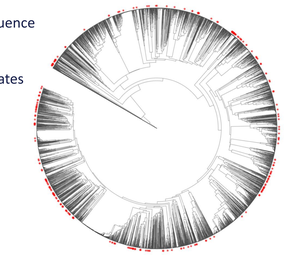
Results

1 MDR *P. aeruginosa* isolates are highly variable

P. aeruginosa isolates were cultured from sputum samples of Cystic Fibrosis patients (n=122 US, n=24 EU). Bioinformatic analysis revealed that 97% (141/146) are MDR isolates, including large number of genes that confer beta lactam and chloramphenicol and streptomycin resistance.

To evaluate the similarity of the *P. aeruginosa* isolates, a phylogenetic tree based on the sequences of these, and other public *P. aeruginosa* isolates was generated. This revealed that the *P. aeruginosa* isolates are highly variable and dispersed across the entire *P. aeruginosa* phylogenetic tree, guaranteeing the genomic diversity of *P. aeruginosa* isolates that are used for isolation and characterization of novel phages.

Phylogenetic tree of *P. aeruginosa* isolates

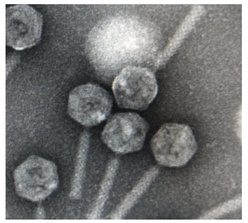


Phylogenetic distribution of all available *P. aeruginosa* public sequences (4,819), and of the 146 *P. aeruginosa* isolates identified by BiomX (marked with red *)

2 Identification of novel phages for *P. aeruginosa*

Screening of a large library of environmental samples resulted in the isolation of phages recognizing drug resistant *P. aeruginosa* isolates. Sequence analysis of the phages revealed that they belong to different genera in the Caudovirales order, contain linear dsDNA genomes between ~40-180 kbp in length and are devoid of undesirable genes. Scanning electron micrographs of one type of phage virion (Pbunavirus genus, in the family Myoviridae), demonstrated its isometric heads and long non-contractile tail.

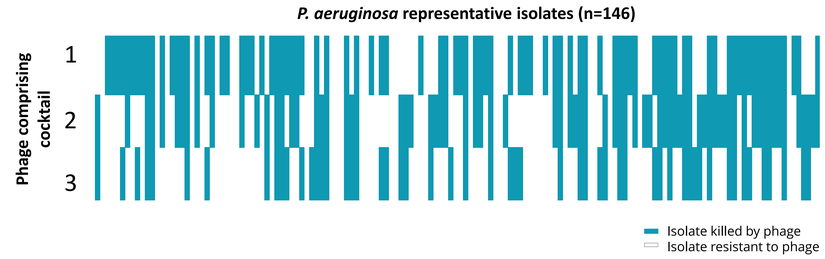
Example of phage morphology



Scanning Transmission Electron Micrographs (STEM) of stained phages infecting *P. aeruginosa* isolate. The head has a diameter of about ~48 nm and the tail length is around ~80 nm

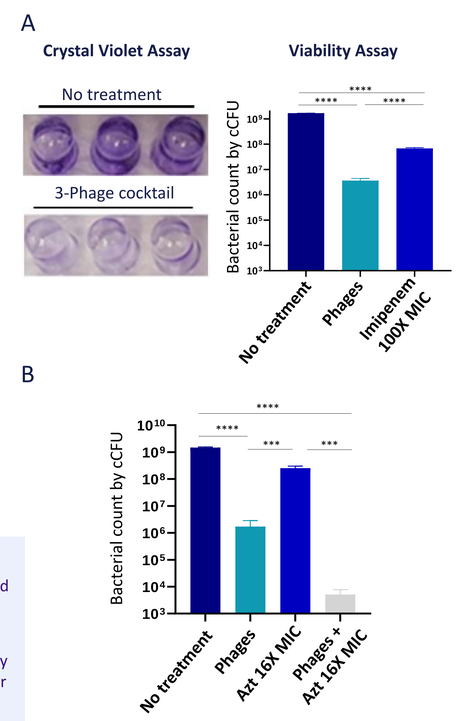
3 Phages infect a broad range of *P. aeruginosa* isolates

High throughput efficiency of plating (EOP) infection assays showed that 3 phages were able to target ~80% of the 146 tested *P. aeruginosa* clinical isolates with most of these isolates being targeted at an efficiency of plating (EOP) of at least 0.01.



4 In vitro activity of phage cocktail against P. aeruginosa in biofilm

CF lungs are colonized with P. aeruginosa in a biofilm layer. We tested the phages capability to reduce bacterial burden in biofilm. Bacteria were grown in conditions allowing biofilm formation, and 24h later the 3-phage cocktail was added for 6 hours. The results revealed that the phages were able to penetrate biofilm and reduce bacterial burden of embedded *P. aeruginosa* (~3 logs). The significant reduction by phage is also corroborated as a visible decrease in biofilm by staining with crystal violet, which stains DNA of dead bacterial cells and the extracellular matrix.
Treatment with the phage cocktail was efficacious in significantly reducing target bacteria encased in biofilm relative to treatment with imipenem (MIC X100) or vehicle (p-value < 0.0001).
Although phage therapy alone achieved a statistically significant reduction in P. aeruginosa levels, phages & aztreonam treatment had the highest bacterial burden reduction (~5 logs), suggesting that a synergistic interaction may exist between the phages and aztreonam.



(A) Visualization of biofilm mass reduction after 3-phage cocktail treatment by crystal violet staining and viability assay (cCFU=calculated CFU by relative luminescence levels)

(B) Activity of 3 phage cocktail on biofilm embedded *P. aeruginosa* clinical isolate as compared to antibiotics (Aztreonam, 'Azt') by viability assay. Statistical analysis was performed using two-way ANOVA test for detection of significant differences. *** p < 0.001, **** p < 0.0001

Discussion and Conclusions

The 3-phage cocktail comprised of natural phages demonstrated broad host range, potent antimicrobial activity even in the presence of biofilms, and synergistic activity with antibiotics against clinical *P. aeruginosa* isolates and should be further explored for the treatment of P. aeruginosa pulmonary infections in patients with CF.

1 Pamukcu, 1995
2 Lipuma, 2010
3 Drenkard, 2002

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