Isolation and characterization of a *Pseudomonas aeruginosa* bacteriophage

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Introduction

Pseudomonas aeruginosa (PA) is a common causative agent of canine pyoderma and otitis (2, 5). Because *P. aeruginosa* is naturally resistant to several classes of antibiotics, the consequent skin and ear infections may become chronic, discomforting and challenging to treat (1, 5). In addition, the human-companion animal bond contributes to the transmission of the zoonotic and antibiotic-resistant bacteria through direct or indirect contact (3). Therefore, there is a need to investigate alternative options to traditional chemical antibiotics for the treatment of *P. aeruginosa* skin infections (1, 2, 4).

Bacteriophages (or phages, Φ) are virus that occur naturally in the environment and infect bacteria in a host-specific manner. Among these, the lytic phages are a promising treatment option since these not only infect bacteria to propagate themselves, but also have the ability to lyse the host bacterium upon the release of their progeny virions (Fig. 1). In this study, an anti-*P. aeruginosa* phage was characterized, and a second phage was harvested from wastewater.

Hypothesis and objective

This study consisted of two hypotheses:

1. Phages are common in areas where bacteria thrive, and therefore can be isolated from sewage wastewater.
2. Lytic phages can destroy *P. aeruginosa* by the action of their lytic enzymes on the bacterial cell wall (Fig. 1).

Thus, the objectives of this study were to:

1. Isolate *P. aeruginosa* phage(s) from sewage wastewater.
2. Examine the lytic efficacy of the phage(s) by observing its phenotypic manifestation (lytic zones).
3. Determine PA host-phage growth kinetics.

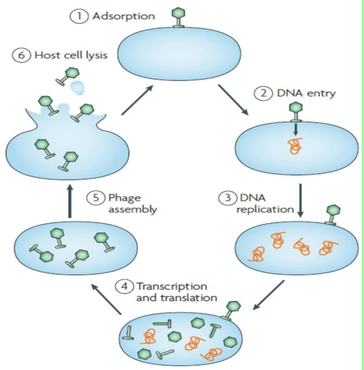
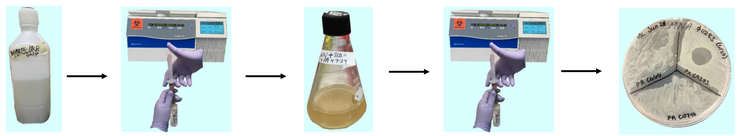


Figure 1. The lytic phage replication cycle. The progeny virions continue the infective cycle if the bacterial host is available in the milieu. Picture taken directly from Brives et al. (1).

Materials and Methods

1. Isolation of Phage



Sewage wastewater sample | Centrifuge and filter (0.45 µm) to remove debris and bacteria | Feed filtrate with trypticase soy broth and PA to select for PA-specific phage | Centrifuge and filter the supernatant to remove bacteria and retrieve the phage | Confirm the presence of lytic phage with spot assay on *P. aeruginosa*

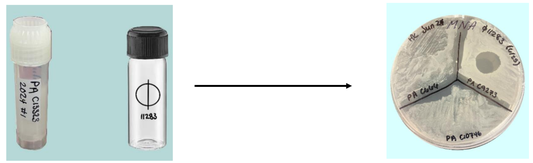
Figure 2. Isolation of anti-PA bacteriophage from Charlottetown sewage wastewater by phage enrichment and purification.

2. Examination of lytic efficacy of phage in spot assays

- Clinical isolates of PA were lawn plated on soft bottom agar and incubated for 4 hours.
- A volume of 10 µL of phage was spotted on the PA lawn and incubated at 37°C for 24 h.
- Next day, plates were examined for lytic zones (Fig. 3).

3. Determination of host-phage growth dynamics

- *P. aeruginosa* culture was infected with phage at MOI of 1.0 in trypticase soy broth. The culture was incubated at 37°C, shaking at 125 rpm.
- At hourly intervals over a 5 hour period:
  - Optical density of mixture was measured at 600 nm wavelength.
  - Ten-fold dilutions of mixture were plated on double agar overlays and blood agar and incubated at 37°C for 24 h.
- Counts of colony-forming units (CFUs) of *P. aeruginosa* were obtained from blood agar plates and counts of plaque-forming units (PFUs) of phage were obtained from double-agar overlays. These data were used to generate a growth curve.



Spotting 10 µL of *P. aeruginosa* phage onto lawns of *P. aeruginosa* (clinical isolates). Incubation at 37°C for 24 hours.

Figure 3. Examination of lytic efficacy of Φ 11283 vs. 3 PA clinical isolates in spot assays. A clear lytic zone is observed on one of the isolates above. Two isolates above were phage-resistant. In total, 46 isolates were tested, and 23 were lysed by Φ 11283.

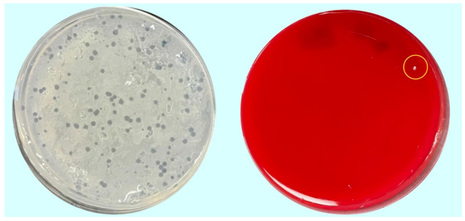


Figure 4a. PFUs vs. CFUs at 3 h time-point. Lytic zones (PFUs) on double agar overlay (left) and bacterial colony growth on blood agar (right, circled). Phage plaques outnumber the bacterial colonies at 3 h.

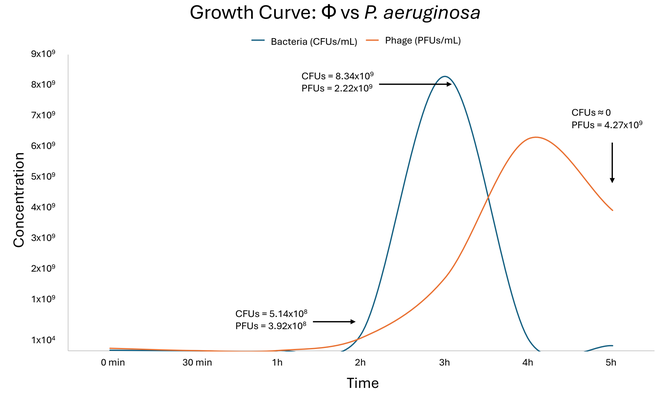


Figure 4b. Growth kinetics of Φ 11283 vs. PA. PA grew exponentially for the first 3 hours of co-incubation with phage. At the 3-to-4 hour mark, bacterial concentration sharply declined as phage concentration increased. By 5 hours, bacterial concentration was near zero, while phage concentration was at 5 x 10^9 PFUs/ml. The data are average of four replicates of growth kinetic experiments.

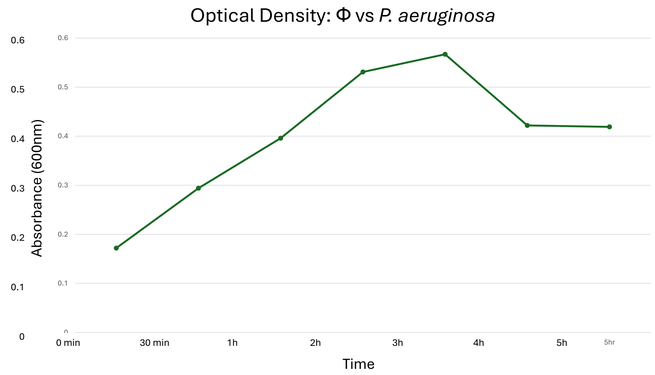


Figure 4c. Optical density of the liquid phase in growth kinetics of phage vs. PA. OD_{600} increased initially corresponding to an increase in bacterial concentration, peaking at 3 hours. At 4 hours, optical density decreased and remained constant.

Results

- One phage (Φ 11283) was characterized in this study. This phage was isolated previously using the described method (Fig. 2).
- The phage was tested against 46 clinical *P. aeruginosa* isolates. Clear inhibition zones of bacterial lysis were observed on 23 isolates, indicating a lytic efficacy of 50% (Fig. 3).
- Growth kinetics data of phage versus PA host showed phage concentration overtaking bacterial concentration at around 3 - 4 hours (Fig. 4a, 4b).
- A second anti-PA phage was harvested from the sewage wastewater as described (Fig. 2).

Conclusions

- A lytic bacteriophage, Φ 11283, complementary to *P. aeruginosa* was characterized.
- The lytic activity of the phage was highly pronounced at 3 and 4 hours causing a sharp decline in PA concentration.
- While CFU counts were negligible at 4 and 5 hours, the optical density was high. It is assumed that lysed bacterial cell debris contributed to a high optical density through the 5 hour end point.
- The phage was effective in lysing *P. aeruginosa* isolates implicated in skin and ear infections in dogs in vitro.
- Φ 11283 can be a potential candidate in future therapeutic multi-phage formulation.

Future studies

- Genetic sequencing and analyses of Φ 11283 .
- Characterization of the newly isolated anti-PA phage.
- In vitro testing of phage efficacy in curbing PA in combination with chemical antibiotics.
- Continued harvesting of PA phages from human wastewater and canine fecal samples.
- Phenotypic and genomic characterization of potential therapeutic phages for inclusion in a multi-phage cocktail which may be ultimately used to treat an infected patient.

References

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