

**Title:** Isolation and characterization of a *Pseudomonas aeruginosa* bacteriophage

**Authors:** Hannah Kennedy, Charlie Pye, Shivani Ojha

**Affiliations:** Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE (HK, SO)

Department of Companion Animal Medicine, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE (CP)

### **Abstract**

*Pseudomonas aeruginosa* is a common bacterial cause of severe, chronic otitis in canine species. Since *P. aeruginosa* is naturally resistant to many antimicrobials, infections can be challenging to treat. Therefore, the objective of this study was to investigate an anti- *P. aeruginosa* bacteriophage as an alternative antimicrobial option to target the pathogen. Bacteriophages (or phages, literally “bacteria-eaters”) are viruses that infect bacteria in a host-specific manner. The phage replicates inside the complementary host bacterium, eventually lysing it to release the progeny virions into the environment and begin the next infection cycle. This study examined bacteriophage-11283, which was previously isolated from Charlottetown sewage wastewater. The phage was tested for its lytic efficacy against clinical isolates of *P. aeruginosa* in spot assays performed on soft agar. Clear zones of *P. aeruginosa* growth inhibition (plaques) confirm bacterial cell lysis. A total of 46 isolates of *P. aeruginosa* were tested, and the phage showed lytic activity against 23 isolates, indicating an efficacy rate of 50%. To understand the phage-host growth dynamics, the phage was co-incubated with the propagating *P. aeruginosa* host in trypticase soy broth (liquid phase) over a 5-hour period. The optical density at 600nm (OD<sub>600</sub>) was measured at hourly intervals, and at the same time points colony-forming units and plaque-forming units were counted. The data showed a steep decline in bacterial concentration 3-4 hours after exposure to phage. OD<sub>600</sub> increased initially with bacterial growth, but unexpectedly remained high even though bacterial concentrations declined. This was presumably caused by debris from the lysed bacterial cells. Finally, another *P. aeruginosa* phage was isolated from a composite wastewater sample during the course of this study. Phage-11283 and the newly harvested phage will be further characterized in future studies.

**Financial support:** Zoetis Investment in Innovation Fund, Companion Animal Trust Fund (AVC)

**Student Support:** VetSRA, Sir James Dunn Animal Welfare Centre