Thesis progression

Thesis title: Isolation and characterization of bacteriophage against carbapenem resistant *Pseudomonas aeruginosa*

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Date: 11th March 2026

1. Introduction

Pseudomonas aeruginosa is recognized by the World Health Organization (WHO) as one of the critical priority pathogens, representing a global health emergency due to its drug resistance. In particular, the emergence of Carbapenem-resistant *P. aeruginosa* (CRPA) has significantly restricted therapeutic options, leading to an upsurge in morbidity, mortality, and healthcare expenditures. This pathogen is a leading cause of nosocomial infections, especially among immunocompromised individuals and patients with implanted medical devices. The clinical challenge is further exacerbated by its potent biofilm-forming capability, which facilitates resistance to multiple classes of antibiotics and renders traditional treatments frequently ineffective.

In Thailand, CRPA infections are becoming increasingly prevalent in both hospital settings and local communities, often resulting in extended hospitalizations and unfavorable patient outcomes. Given the limitations of conventional antibiotics against these resistant strains, there is an urgent need to explore alternative therapeutic approaches.

Bacteriophages (phages) are viruses that specifically infect and lyse bacteria offer a promising alternative to traditional antimicrobial therapy. Phage therapy offers several theoretical advantages over conventional antibiotics: high specificity for target bacteria (potentially preserving the beneficial microbiome), self-replicating nature at the site of infection, ability to evolve alongside their bacterial hosts, and capacity to disrupt biofilms through the production of depolymerase enzymes. Furthermore, recent studies have demonstrated promising synergistic effects when phages are combined with antibiotics—a strategy known as phage-antibiotic synergy (PAS)—which may simultaneously reduce bacterial loads while suppressing the emergence of resistant mutants.

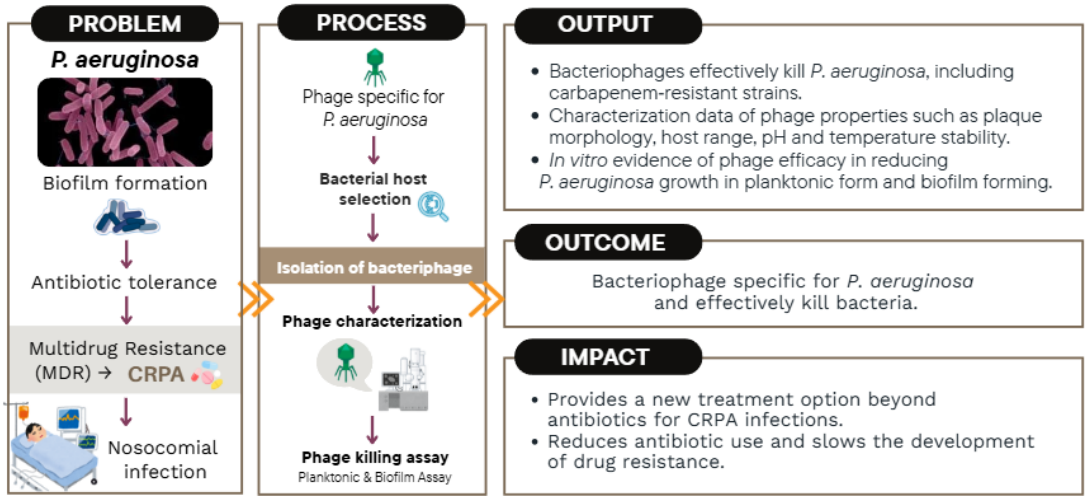
Despite these promising attributes, significant challenges remain before phage therapy can be widely implemented against antibiotic-resistant *P. aeruginosa*. These include the narrow host range of individual phages, potential development of phage resistance, concerns regarding

phage stability and pharmacokinetics in human tissues, regulatory hurdles, and the need for personalized phage cocktails tailored to specific clinical isolates. Additionally, comprehensive genomic characterization of therapeutic phages is essential to ensure they do not carry virulence or antibiotic resistance genes that could be transferred to bacterial hosts.

This study aims to investigate the therapeutic potential of bacteriophages against clinically isolated antibiotic-resistant *P. aeruginosa* strains. Specifically, we sought to isolate and characterize lytic phages with broad host range activity against MDR *P. aeruginosa*, evaluate their ability to disrupt established biofilms, assess potential synergistic interactions with conventional antibiotics, and analyze phage genomic characteristics to ensure safety for potential therapeutic application. The potential phages can be serving as both clinical therapeutic agents and tools for environmental biocontrol.

2. Hypothesis and objectives

2.1 Conceptual framework



2.2 Hypothesis

The identified phages, isolated from environmental sources and bacterial hosts, will specifically infect, and lyse *P. aeruginosa*, including carbapenem-resistant strains.

2.3 Objectives

1. To isolate bacteriophages specific to *Pseudomonas aeruginosa*, including carbapenem-resistant strains, from environmental samples

2. To characterizes the biological properties of the isolated phages with respect to host range, plaque morphology, and stability

3.To evaluates the in vitro efficacy of selected phages in reducing *P. aeruginosa* growth in both planktonic cultures and biofilm forms.

3. Materials and method

3.1 Bacterial strains

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa* isolates were used as bacterial hosts for the detection and isolation of bacteriophages.

3.2 Preparation of bacterial host cultures and phage detection

3.2.1 Preparation of bacterial host cultures

A single colony of P. aeruginosa was inoculated into Luria-Bertani (LB) broth and incubated at 37°C for 16 hours to obtain an overnight culture. For sub-culturing, the overnight culture was diluted 1:100 into fresh media and further incubated for 4 hours to reach the mid-logarithmic growth phase. The mid-log phase culture was subsequently utilized for phage induction via UV exposure and spontaneous release.

3.2.2 Prophage induction and isolation

Two methods were employed to induce bacteriophages from the host isolates.

- **UV induction**

The overnight culture was harvested by centrifugation at 10,000 rpm for 5 minutes at 4°C. The supernatant was discarded, and the bacterial pellet was resuspended in SM buffer. This washing step was repeated for three cycles, first wash at 10,000 rpm for 5 minutes, second and third washes at 8,000 rpm for 4 minutes at 4°C. The final suspension was transferred to a 24-well plate and expose to UV light (254 nm) for 2 minutes. The UV-exposed suspension was then centrifuged at 10,000 rpm for 5 minutes at 4°C, and the resulting supernatant, containing induced phages, was collected for further analysis.

- **Spontaneous release**

The culture was incubated at 37°C for 24 hours to allow for the natural release of phages. After incubation, the liquid culture was centrifuged at 10,000 rpm for 5 minutes at 4°C. The supernatant was carefully collected to obtain the naturally released phage lysate.

3.3 Phage detection by spot assay

The presence of bacteriophages in the collected lysates was determined using a spot assay with the flush method.

3.3.1 Preparation of growth media for bacterial host

Nutrient Agar (NA) plates supplemented with $CaCl_2$ were prepared to enhance phage adsorption. The bacterial suspension was adjusted to 0.5 McFarland standard using PBS buffer, with turbidity verified against a Wickerham card. Approximately 1.5 mL of the adjusted suspension was flushed on surface of NA plates. The plates were tilted and rotated to ensure uniform coverage, after which excess to dry in a laminar cabinet for 15-30 minutes.

3.3.2 Phage spotting

Once dry, 5 µL of the phage lysates obtained from Method A and Method B were spotted onto the bacterial lawn. SM buffer was used as a negative control. The plates were kept undisturbed until the droplets were completely absorbed and subsequently incubated at 37°C for 18-24 hours. The formation of clear zones of lysis (spot) was observed and recorded as and evidence of phage activity.

3.4 Phage isolation

Bacteriophages were isolated from environmental water samples collected from three distinct locations: hospital sewage, community wastewater, and animal farms. The collected samples were processed to extract potential phages capable of infecting *P. aeruginosa*. Following successful isolation and purification, the Multiplicity of Infection (MOI) was optimized to determine the most efficient ratio of phages to host bacterial cells for maximal viral replication.

4. Results

Phages were induced from bacterial isolates no. 2, 4, 7, 8, 9, 10 by UV exposure. Phages were spontaneously produced from bacterial no. 1, 4, 7, 8, 9, 10 as shown in table 1. Bacterial isolates which produced phage from both methods were not good for propagating host. Therefore, bacterial isolates no. 3, 5, 6 are suitable bacterial host for phage isolation from environment and phage propagation.

Table 1: Representative Spot Assay results. Clear zones (plaques) indicate susceptibility, while the SM Buffer spot (negative control) remains opaque

Phages	Host (Detect), Induce Phage (UV)									
	1	2	3	4	5	6	7	8	9	10
1)70-A-1.4-1										
2)70-B-1.3-1									+	
3)70-B-1.3-2										
4)70-B-1.3-3									+	
5)70-B-1.3-4										
6)70-B-1.4-1										
7)70-B-1.4-2										+
8)70-B-1.4-4									+	+
9)70-C-1.3-1										+
10)70-D-1.3-1									+	

Phages	Host (Detect), Spontaneous (24 hr.)									
	1	2	3	4	5	6	7	8	9	10
1)70-A-1.4-1		+								+
2)70-B-1.3-1										
3)70-B-1.3-2										
4)70-B-1.3-3		+							+	+
5)70-B-1.3-4					ND					
6)70-B-1.4-1										
7)70-B-1.4-2	+	+							+	+
8)70-B-1.4-4		+							+	
9)70-C-1.3-1		+								
10)70-D-1.3-1	+	+						+	+	

No plaque(–): resistant /non-host

Plaque (+) = Phage can infect the bacteria/susceptible host

5. Conclusion

Bacterial isolates no. 3, 5, and 6 were definitively identified as the optimal host strains for the purpose of primary phage isolation and large-scale phage stock propagation.

6. References

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