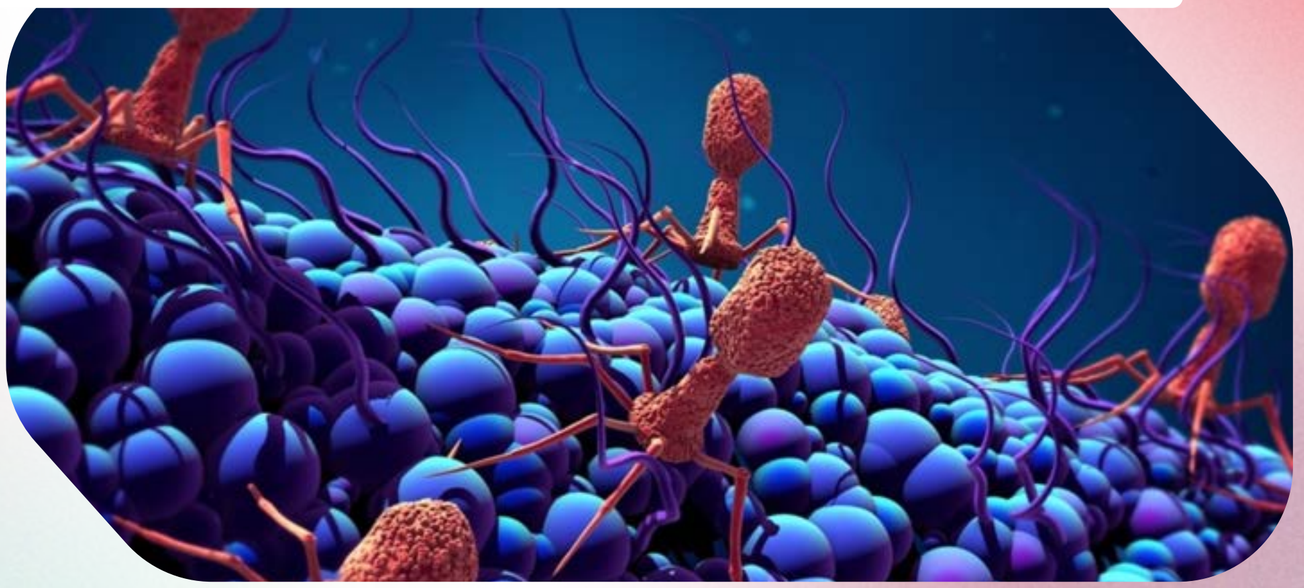
Potential application of bacteriophage therapy as an alternative treatment against *Pseudomonas aeruginosa*



Presented by
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Department of Microbiology, Faculty of Medicine,Khon Kaen university

Introduction

Pseudomonas aeruginosa

- Gram-nagative bacilli
- Widely found in natural environment
- Major cause of nosocomial infections

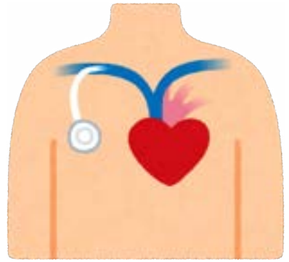
Common infections :

- Pneumonia
- Bloodstream infections
- Urinary tract infections
- Surgical site infections

P. aeruginosa

biofilm formation

formed on medical devices used for treatment, such as catheters, vascular lines, and tracheal tubes



Introduction

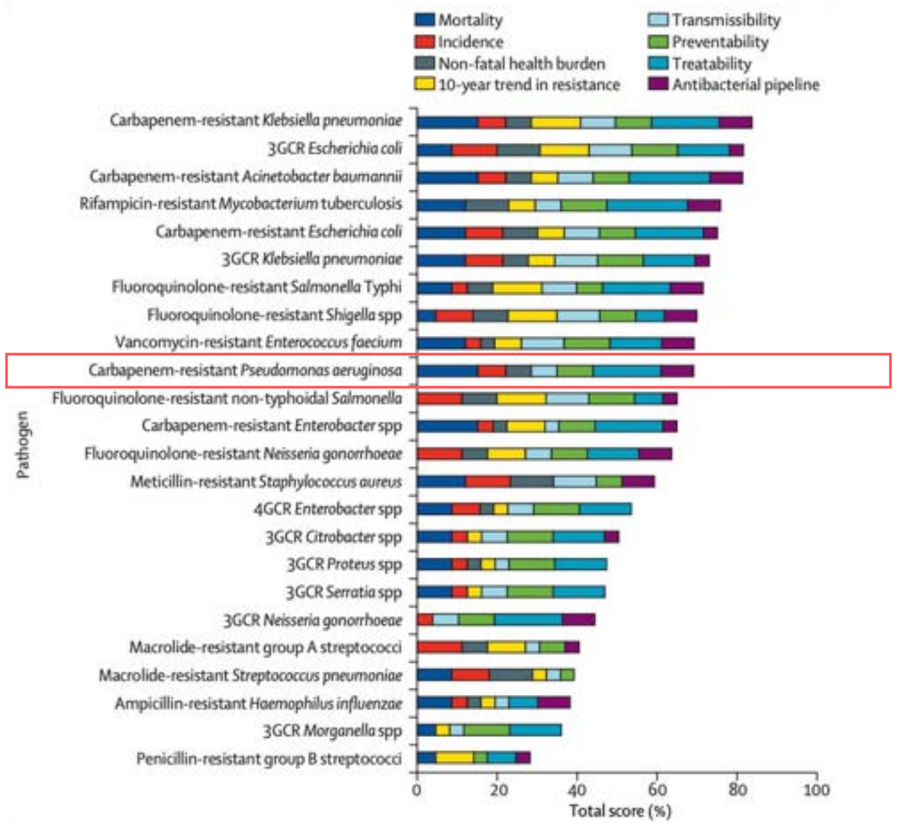
Antibiotic-resistant

- A major global public health threat
- Resistant bacteria are harder to treat
- Increases healthcare costs & mortality

IMPACT

- Longer and more complicated treatments
- Higher medical costs
- Increased morbidity and mortality

Antibiotic-resistant *P. aeruginosa* is a significant global problem because it can form biofilms, exhibit multiple resistance mechanisms, and cause treatment failure in chronically ill or hospitalized patients.



In the 2024 WHO BPPL ranking:

carbapenem-resistant *P. aeruginosa*

P. aeruginosa remains a **critical pathogen** due to high disease burden, rising long-term antibiotic resistance, and limited new treatment options

Introduction

Bacteriophage

“A type of virus that infects and replicates inside bacteria”

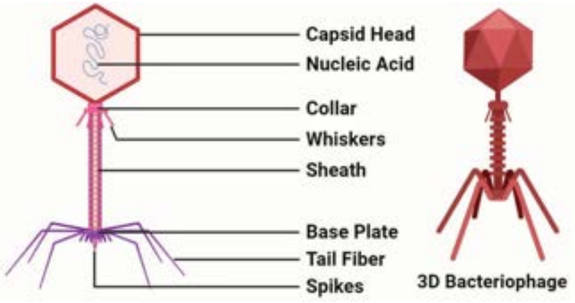
[can be found in soil and seawater, oceanic and terrestrial surfaces and extreme environments]

- Specific to the target bacteria
- Minor side effect
- Abundance in nature
- Harmless to human

FUNCTION

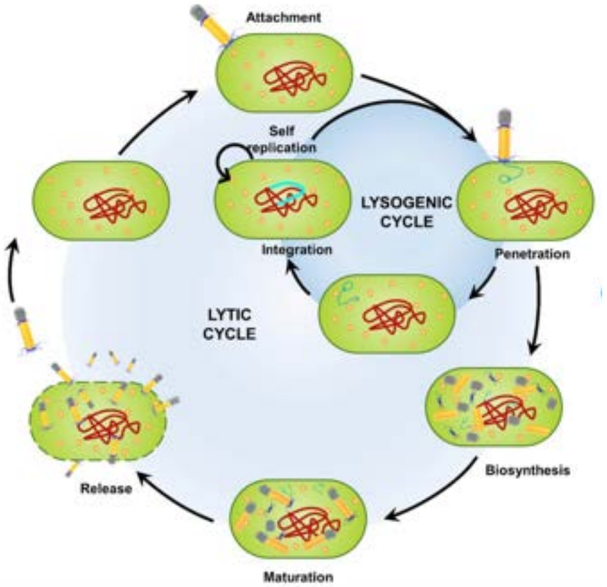
- Control of bacterial populations
- Potential medical applications: Researchers are exploring **“phage therapy”** to treat antibiotic-resistant bacterial infections.

Phage structure:



Sapkota.A, 2022

Life cycle of bacteriophage:



Monish B., Kusum K., Sakshi M., 2024

Introduction

Phage therapy:

To assess how effective phage therapy can be in treating bacterial infections, In preclinical infection models

Efficacy of phage therapy in preclinical models of bacterial infection: a systematic review and meta-analysis

Sergio Alejandro Gómez-Ochoa, Melissa Pitton, Luca G Valente, Cristian David Sosa Vesga, Jorge Largo, Andrea Carolina Quiroga-Centeno, Juliana Alexandra Hernández Vargas, Silvia Juliana Trujillo-Cáceres, Taulant Muka, David R Cameron, Yok-Ai Que**

	Country	Animal	Infection setting	Pathogen	Number of phages; phage name(s)	Phage administration route	Outcomes assessed	Overall results	Included in meta-analysis?	Reason for exclusion
Albac et al (2020)[16]	France	Mice	Skin or burn	*Staphylococcus aureus*	3; 1493, 1815, and 1957	Subcutaneous	Bacterial load	Phages reduce bacterial load	Yes	NA
Alemayehu et al (2012)[17]	Ireland	Mice	Respiratory	*Pseudomonas aeruginosa*	2; PHIMR299-2 and PHINH-4	Respiratory*	Bacterial load	Phages reduce bacterial load	No	Insufficient information
Cha et al (2018)[18]	South Korea	Mice	Respiratory	*Acinetobacter baumannii*	5; PBAB08, PBAB25, PBAB68, PBAB80, and PBAB93	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chadha et al (2016)[19]	India	Mice	Skin or burn	*Klebsiella pneumoniae*	5; Kpn1, Kpn2, Kpn3, Kpn4, and Kpn5	Topical or superficial	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies
Chadha et al (2017)[20]	India	Mice	Skin or burn	*Klebsiella pneumoniae*	5; KØ1, KØ, KØ3, KØ4, and KØ5	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chang et al (2018)[21]	Australia	Mice	Respiratory	*Pseudomonas aeruginosa*	1; PEV20	Respiratory	Bacterial load	Phages reduce bacterial load	Yes	NA
Chen et al (2021)[22]	China	Mice	Respiratory	*Pseudomonas aeruginosa*	2; MYY9 and HX1	Respiratory	Bacterial load	Phages reduce bacterial load	Yes	NA

The Lancet Microbe, 2022

- Applicable to multiple infection models and many MDR pathogens
- Very flexible administration route
- Almost all studies report positive outcomes

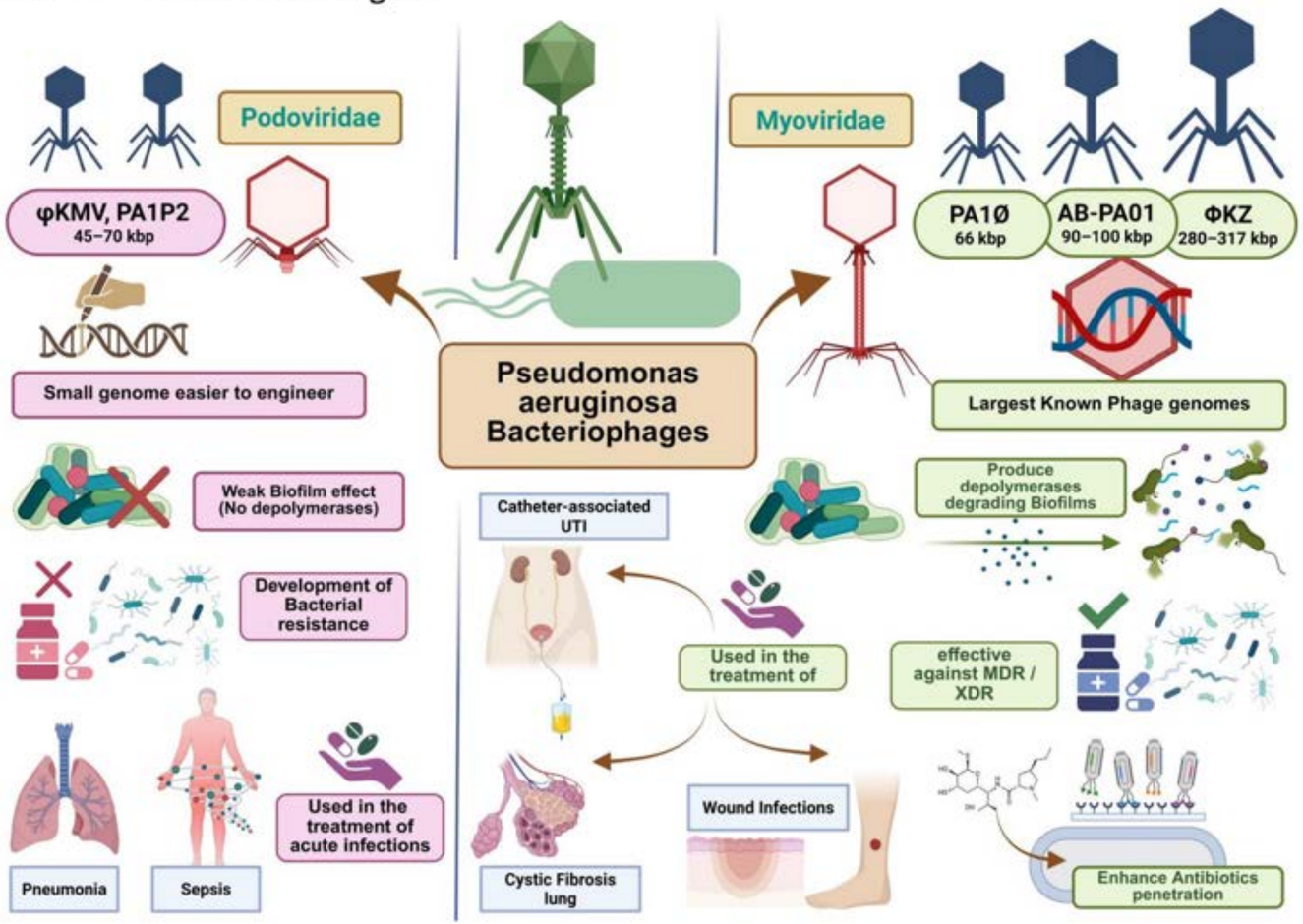
➤ **strong consistency of efficacy**

Preclinical studies demonstrated that phage therapy effectively reduced bacterial burden and mortality across multiple infection models, pathogens, and administration routes

Introduction

Bacteriophage therapy in clinical practice: case studies of *Pseudomonas aeruginosa* infections

Ahmed Elfadadny, Rokaia F. Ragab, Osama Aldesoky Abd Alaziz, Habiba Y. Essa, Esraa M. Eltony, Mohamed M. Ammar & Wedad M. Nageeb



Elfadadny et al., 2025

Seminar papers

Paper 1:

Characterization of the novel broad-spectrum lytic phage Phage_Pae01 and its antibiofilm efficacy against *Pseudomonas aeruginosa*

Zhixin Shi [1 2], Xin Hong [1], Zexuan Li [1], Meijuan Zhang [1], Jun Zhou [1], Zhe Zhao [3], Shengfeng Qiu [1], Genyan Liu [1 2]

Impact factor: 5.2
Quartile: Q1

Paper 2:

Therapeutic effect and anti-biofilm ability assessment of a novel phage, phiPA1-3, against carbapenem-resistant *Pseudomonas aeruginosa*

Yu-Chuan Tsai [a,1], Yi-Pang Lee [b,1], Nien-Tsung Lin [c,1], Hsueh-Hui Yang [d], Soon-Hian Teh [e,**], Ling-Chun Lin [a,c,*]

Impact factor: 4.4
Quartile: Q2

Paper 1: Overview

Characterization of the novel broad-spectrum lytic phage Phage_Pae01 and its antibiofilm efficacy against *Pseudomonas aeruginosa*

Zhixin Shi [1,2], Xin Hong [1], Zexuan Li [1], Meijuan Zhang [1], Jun Zhou [1], Zhe Zhao [3], Shengfeng Qiu [1], Genyan Liu [1,2]

Rationale:

isolating a broad-spectrum and genomically safe lytic phage may provide an alternative strategy to control antibiotic-resistant *P. aeruginosa* infections

Objectives:

- To isolated and identified **novel lytic phage**, Phage_Pae01
- To characterized and genome sequence of Phage_Pae01
- To observe the ability of the phage alone or in combination with gentamicin to **eliminate biofilm *P. aeruginosa***

Overview:

isolation and characterization of a novel virulent bacteriophage

Phage_Pae01

isolated from hospital sewage

↓

Morphological & biological characterization

↓

Genome sequencing & functional annotation

↓

Stability assessment (temperature & pH)

↓

Evaluation of antibiofilm activity

↓

Phage–antibiotic combination analysis

Method & Result: Plaque & phage morphology of Phage_Pae01

Bacterial strains and phage isolation

A sample of **the sewage treatment** centre of Jiangsu Women and Children Health Hospital → The host bacterium (*P. aeruginosa* PaO21)

The untreated sewage → Phage

A complete and transparent plaque was randomly selected for further purification and amplification

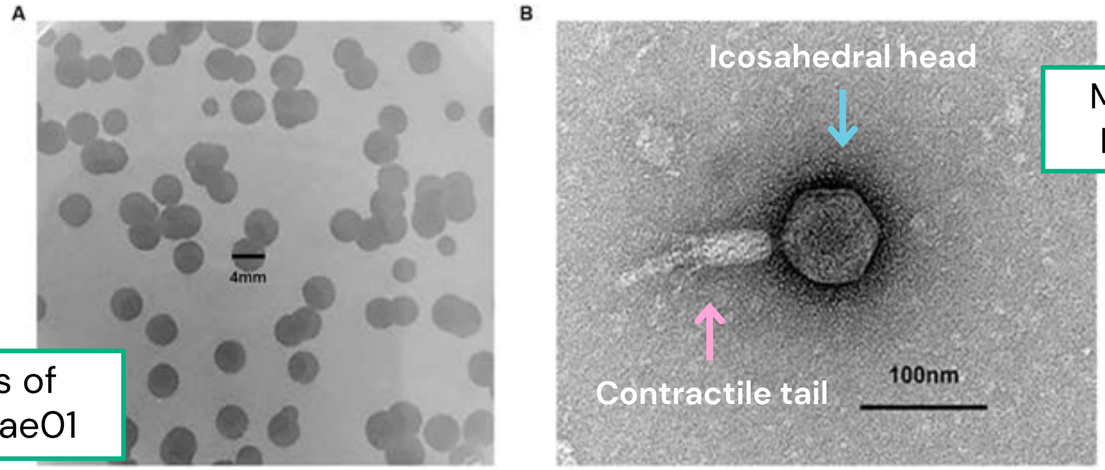
"Phage_Pae01"

Transmission electron microscopy (TEM) observation:

Plaque assay
clear plaques with a diameter of 3 to 4 mm on the plate

↓

Lytic activity



Plaques of Phage_Pae01

Morphology of Phage_Pae01

- Icosahedral head (~80 nm)
- Contractile tail (~110 nm)

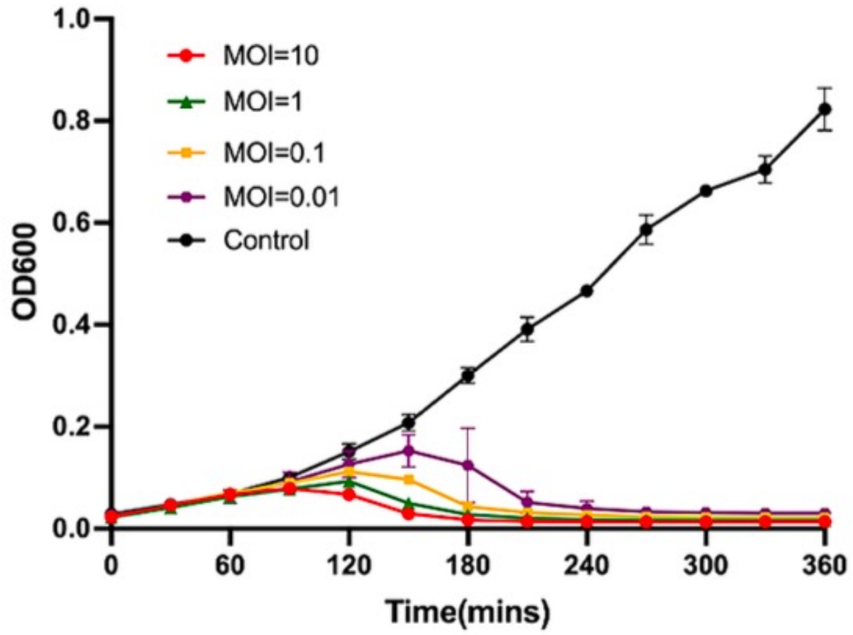
belongs to ***Myoviridae,*** family of the order ***Caudovirales***

Fig. 1: The plaque and phage morphology of Phage_Pae01.

Result: MOI, one-step growth curve and lysis kinetics of Phage_Pae01

Objective: To identify the most efficient infection condition for phage propagation and to evaluate the replication cycle of Phage_Pae01 inside the bacterial host

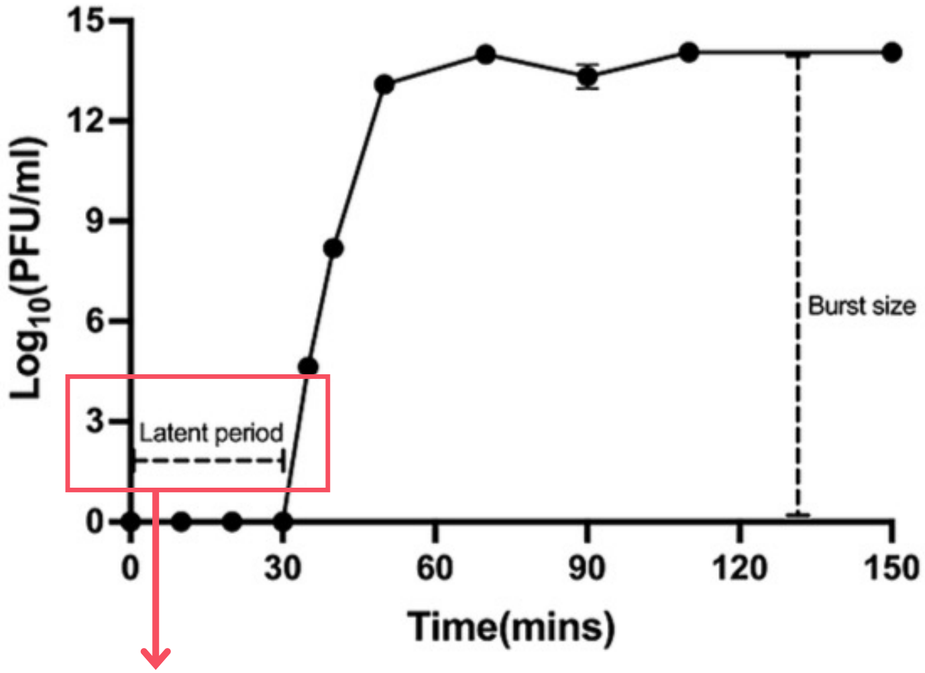
Killing curves of *P. aeruginosa* strain PaO21 by Phage_Pae01 at various MOIs



Optimal MOI for Phage_Pae01: **0.01**

The antibacterial effect of phage can last up to 9 hours

One-step growth curve of Phage_Pae01 on *P. aeruginosa* strain PaO21



Latent period ~30 min

burst size 10^6 PFU/ml

Result: Host range of Phage_Pae01

Objective: To assess the lytic efficiency based on plaque morphology

The lytic ability of Phage_Pae01

- Spot assay
- Plaque assay

found that Phage_Pae01 could effectively lyse 87 out of the 104 strains of *P. aeruginosa*

(83.6 %)

broad host spectrum

P. aeruginosa subtype	Host range
Sensitive	54/65(83.1%)
CRPA	28/31(90.3%)
MDRPA	33/39 (84.6%)
Total	87/104 (83.6%)

TABLE 1 Host range of Phage_Pae01 against *P. aeruginosa*.

The results showed that phage **Phage_Pae01** showed strong cleavage ability to 82 of the 87 sensitive strains, and the remaining 5 strains showed turbid plaque

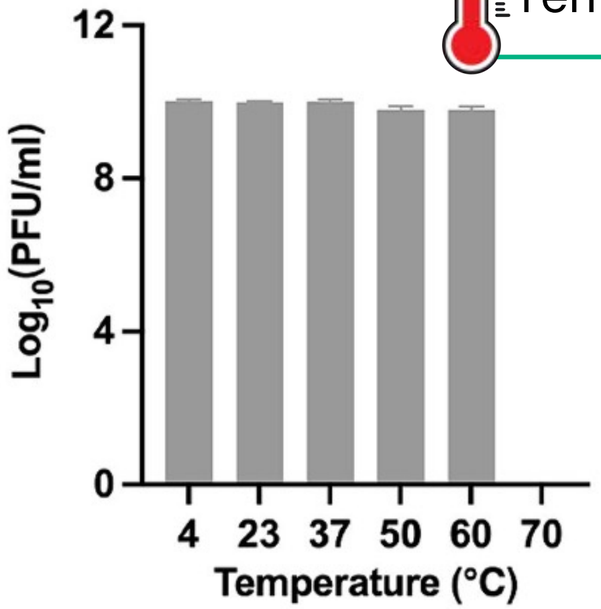
Result: Phage_PaeO1 temperature and pH stability

Objective: To assess the ability to survive to the environment

:

Temperature Stability

Temp: 4-60°C

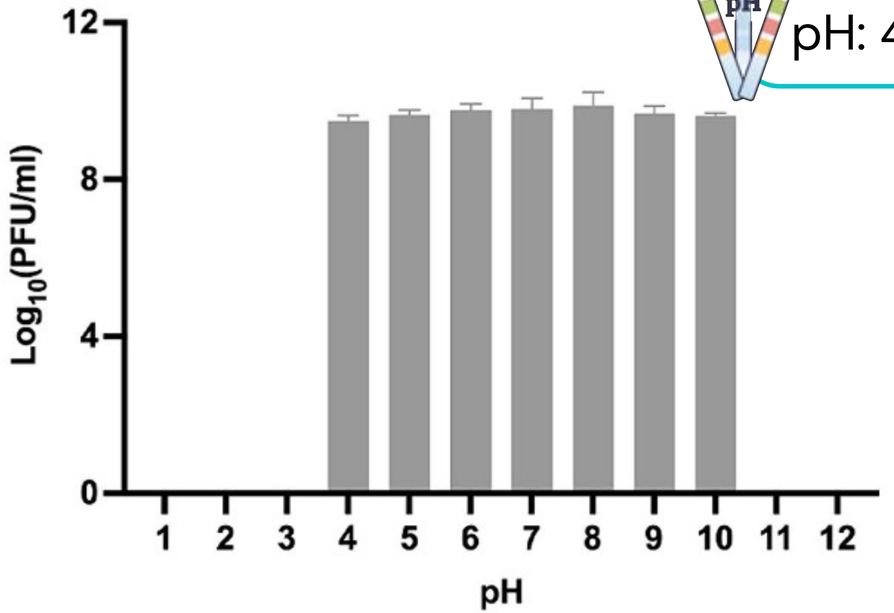


Phage_PaeO1:

- Remained stable at 4–60 °C
- Highest viability observed at 25–37 °C

pH Stability

pH: 4-10



Phage_PaeO1:

- Showed high stability between **pH 5–9**
- Optimal lytic activity near neutral pH (pH 7)

stable under wide environmental conditions

Result: Genome analysis of Phage_PaeO1

Objective: To confirm the lytic nature of the phage by identifying genes related to DNA replication, structural proteins, and lysis systems

Phage_PaeO1

double-stranded DNA (dsDNA)

- Total genome size of **93,182 bp**
- GC content of **49.4%**

typical modular architecture of lytic phages

No antibiotic resistance genes
→ CARD database
Virulence genes were not detected
→ VFDB database comparison

Phage_PaeO1 is safe

The genome of Phage_PaeO1 contains **176** ORFs by NCBI BLASTp

- **119 ORFs** → hypothetical proteins
- **57 ORFs** → functional coding sequences (CDSs)

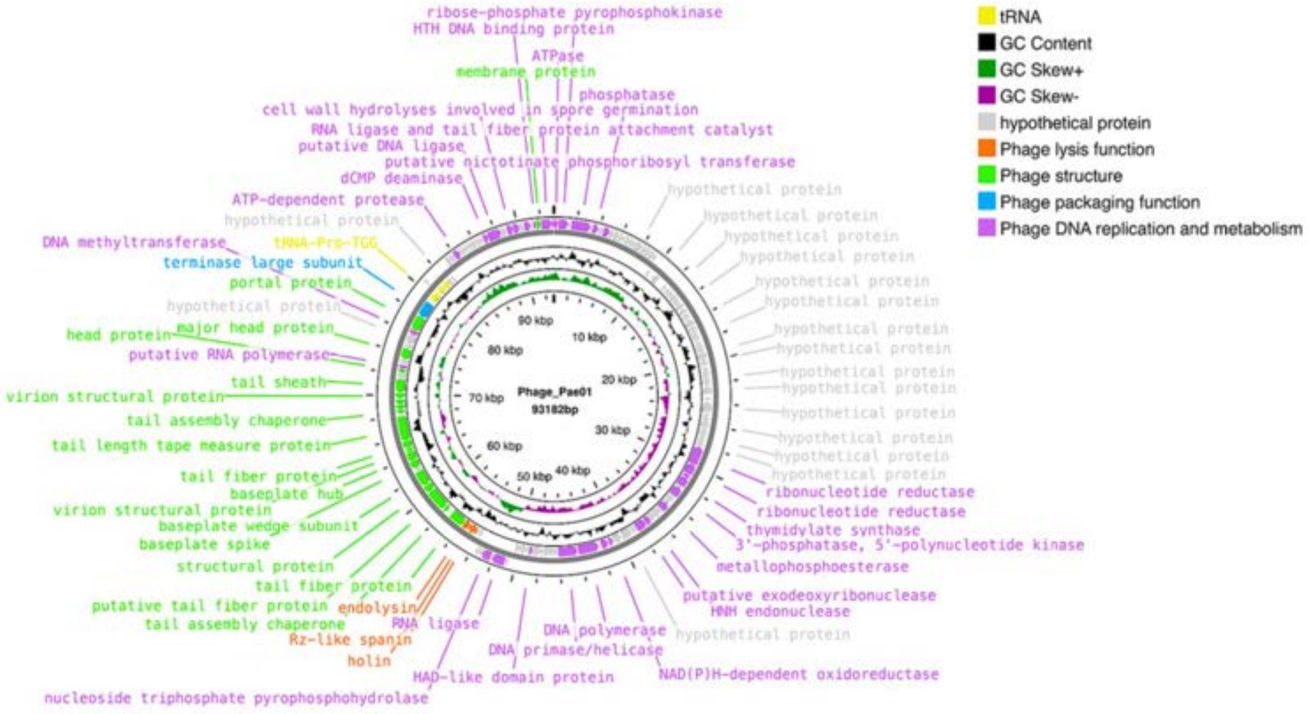


Fig. 4: Whole-genome map of Phage_PaeO1.

Result: Genome analysis of Phage_Pae01 (Cont.)

Objective: To confirm the lytic nature of the phage by identifying genes related to DNA replication, structural proteins, and lysis systems

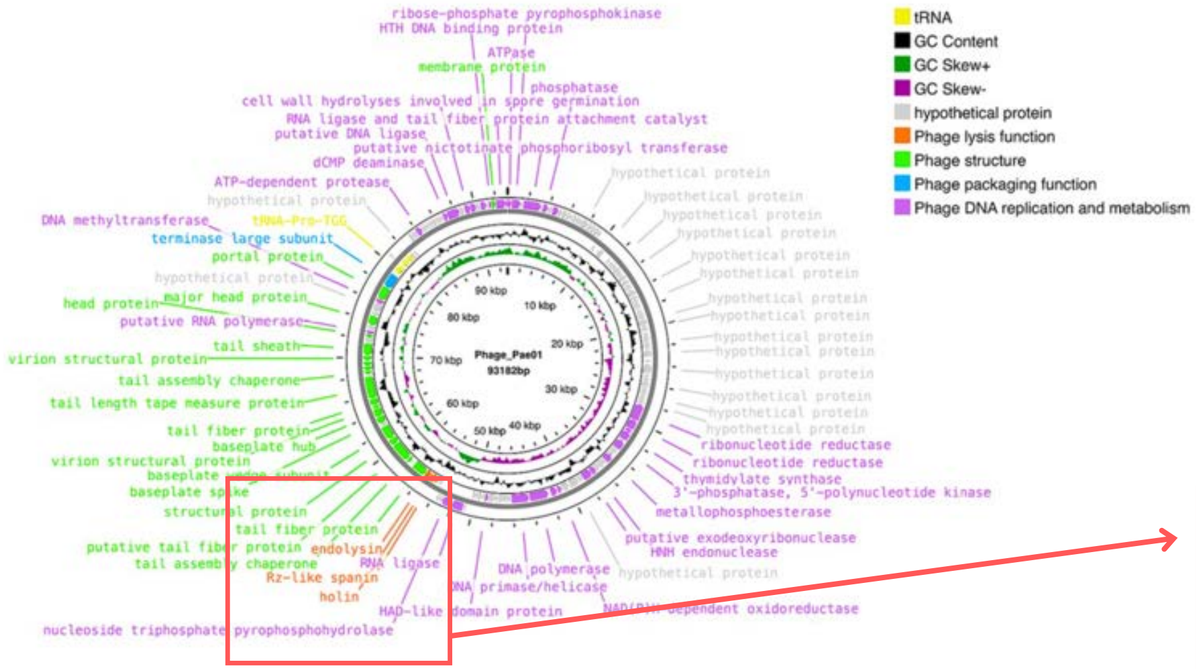


Fig. 4: Whole-genome map of Phage_Pae01.

The ORFs found are proteins involved in phage DNA replication and metabolism, such as:

1.DNA Replication

- **ORF166** — DNA ligase

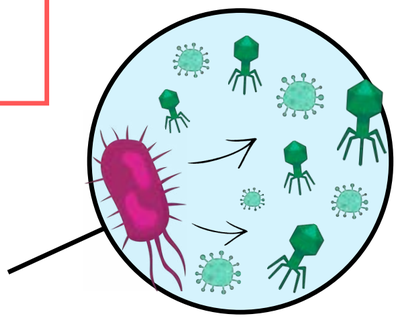
2.Adsorption Module

- **ORF122** and **132** — Tail fiber proteins
  - To infection and the adsorption of host

3.Lysis System of Phage_Pae01

- **ORF120** — Holin
- **ORF121** — Endolysin
- **ORF118** and **ORF119** — Spanin

RELEASE OF PROGENY PHAGES



Result: Genome analysis of Phage_Pae01 (Cont.)

Objective: To compare the genome organization of Phage_Pae01 with closely related Pseudomonas bacteriophages.
Potential as a therapeutic agent against drug-resistant *Pseudomonas aeruginosa*

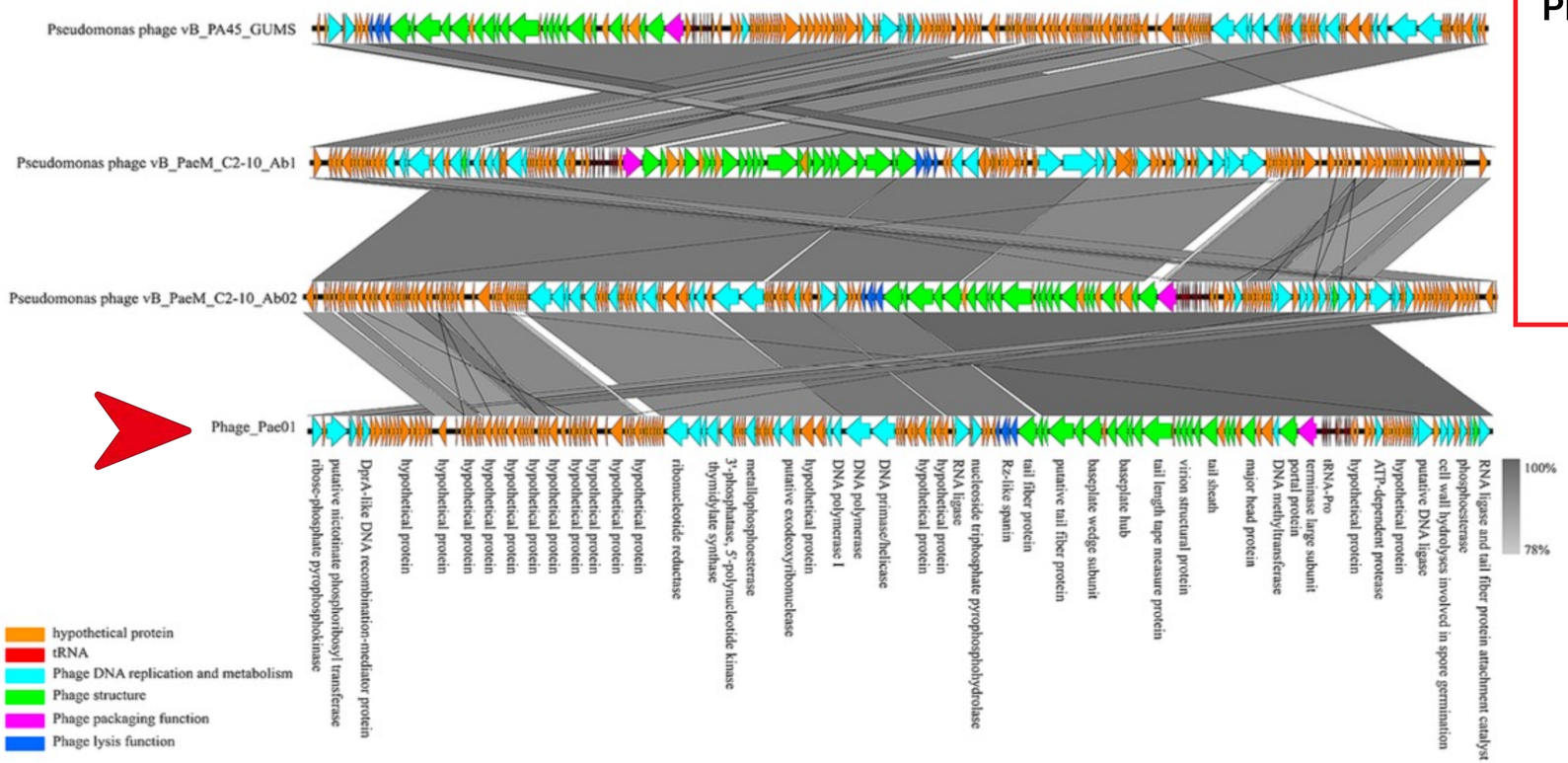


Fig. 5: Comparative genome analysis of the *P. aeruginosa* phages Phage_Pae01

Phage_Pae01 was relatively similar to

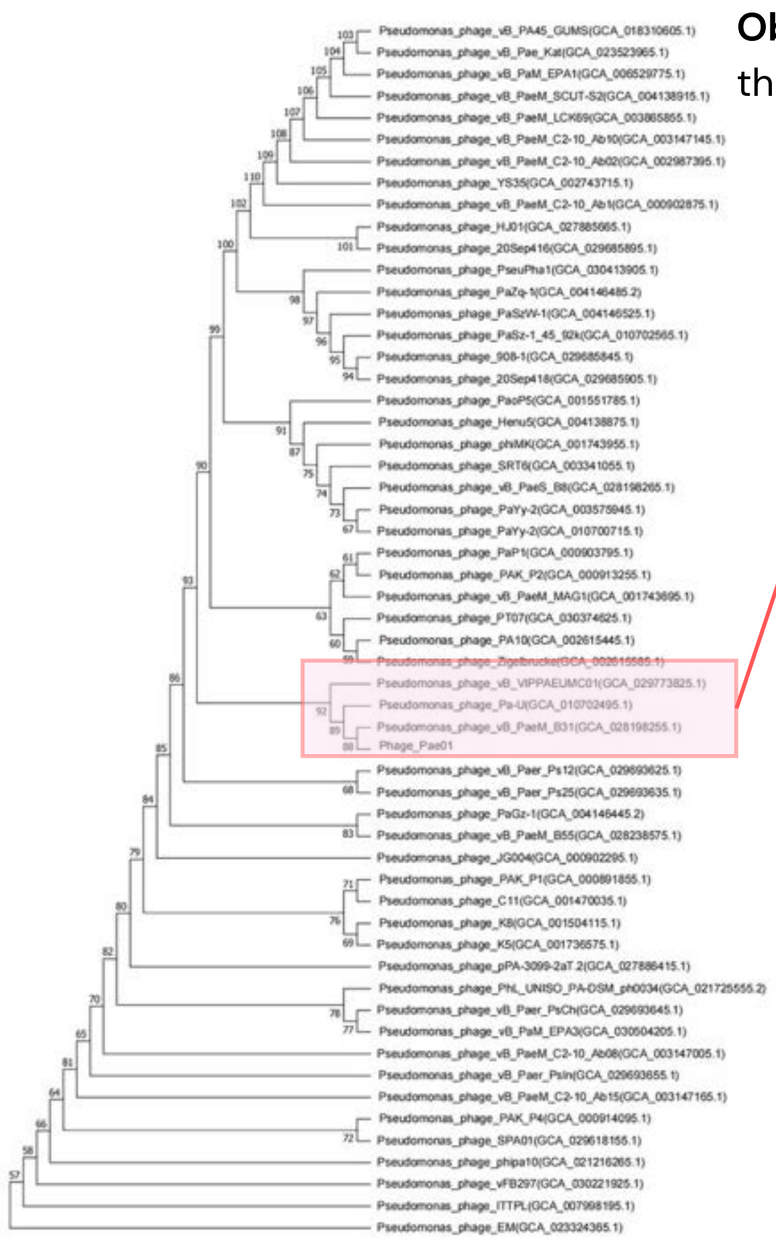
- **vB_PaeM_C2-10_Ab02**
  (96% coverage, 97.47% identity)
- **vB_PaeM_C2-10_Ab1**
  (95% coverage, 97.19% identity)
- **vB_PA45_GUMS**
  (94% coverage, 97.2% identity)

- High Synteny & Homology
- Modular Genome
- Confirmation of Safety

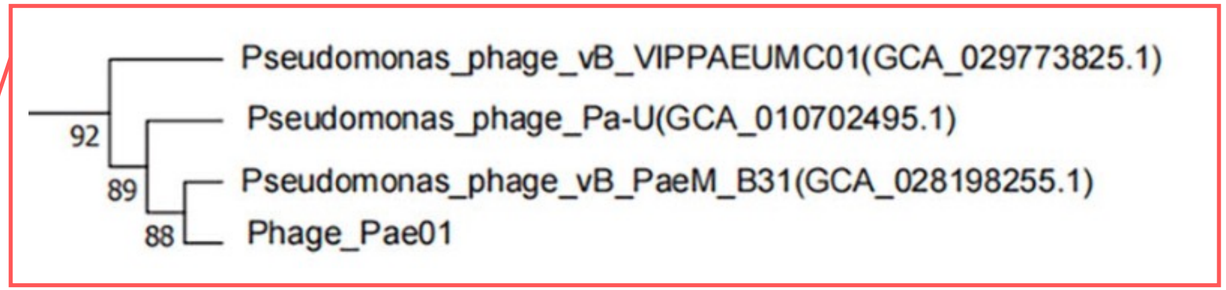
Phage_Pae01 genome against three other closely related phage strains

Phage_Pae01 possesses a conserved lytic phage genome architecture with high similarity to known Pseudomonas phages, supporting its potential application as a therapeutic phage

Result: Genome analysis of Phage_Pae01 (Cont.)



Objective: To evaluate the evolutionary relationship, an analysis is performed by comparing the amino acid sequences of the phage terminase large subunit

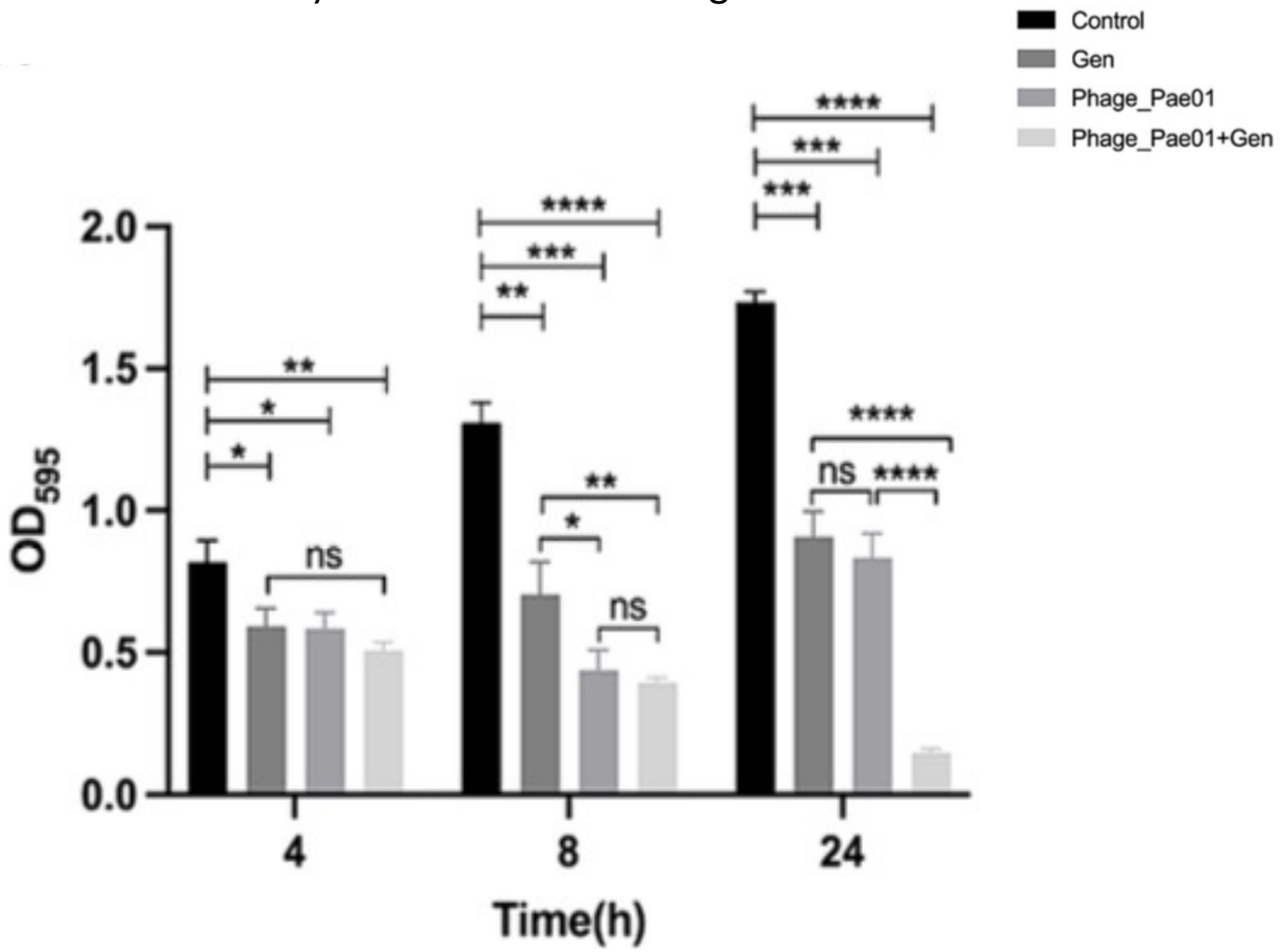


- **Sequence alignment** → ClustalW
- **The phylogenetic tree**
  → The neighbor-joining method with 1,000 bootstrap replications

"These genomic characteristics, together with the observed morphology consisting of an icosahedral head and a contractile long tail, support the classification of **Phage_Pae01** within the family ***Myoviridae*** of the order ***Caudovirales***."

Result: Effect of Phage_PaeO1 and/or antibiotics on the biofilm formed

Objective: To evaluate the antibiofilm activity of Phage_PaeO1, antibiotics, and their combined treatment against biofilm formed by Pseudomonas aeruginosa



Tested on mature biofilms

Time 4 h: **Gentamicin**
Phage_PaeO1
Phage + Gen → Best reduction

Time 8 h:
Phages break down biofilm structures more effectively over time

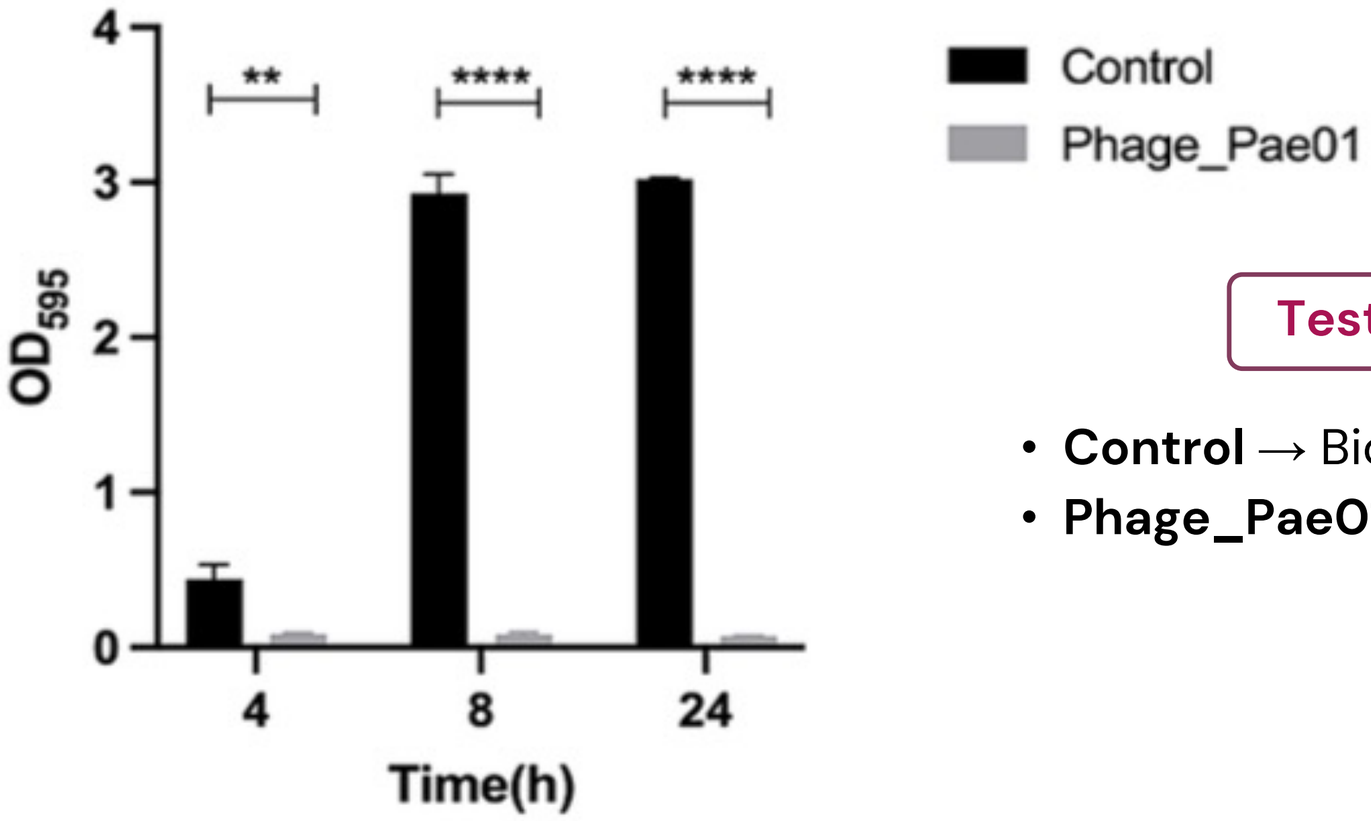
Time 24 h:
Phage + Gen → almost completely reduced biofilm

STRONG SYNERGISTIC ANTIBIOFILM ACTIVITY

Phage_PaeO1 can **destroy existing biofilm**

Result: Effect of Phage_PaeO1 and/or antibiotics on the biofilm formed

Objective: To evaluate the antibiofilm activity of Phage_PaeO1, antibiotics, and their combined treatment against biofilm formed by Pseudomonas aeruginosa



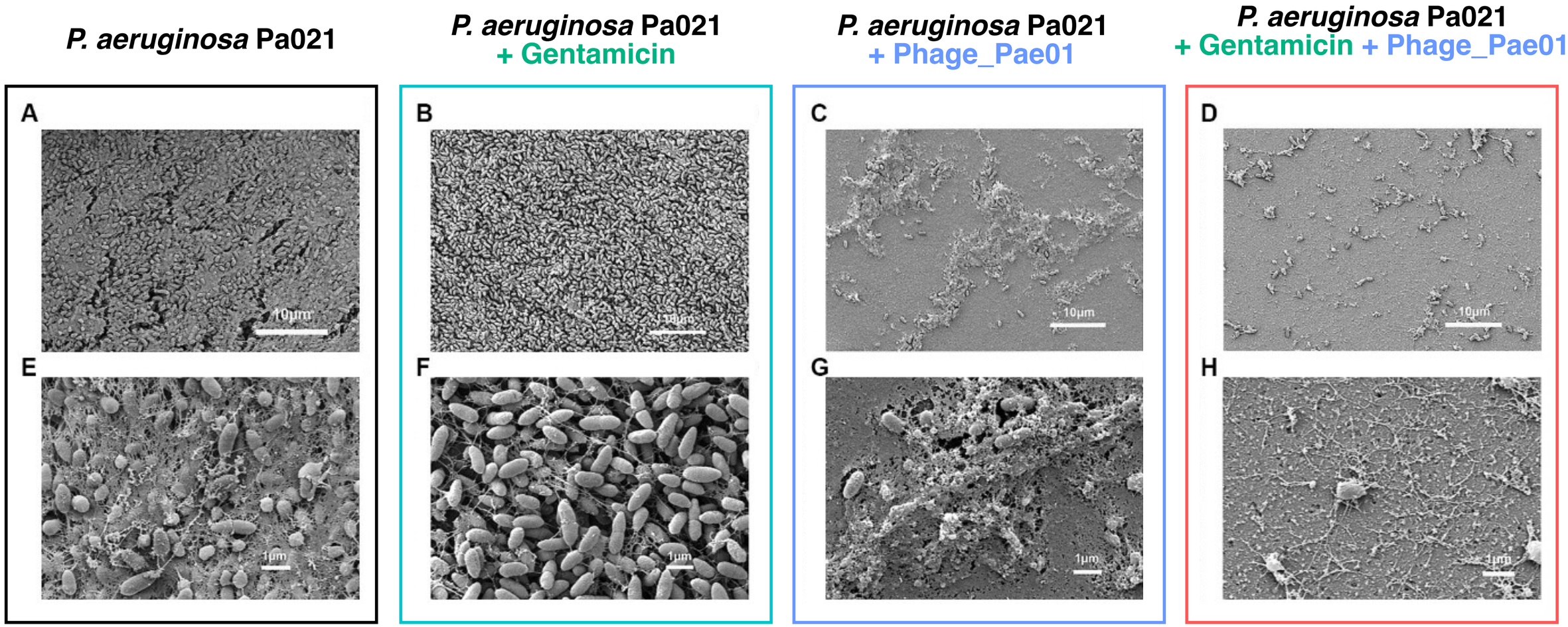
Tested on biofilms formation

- **Control** → Biofilm continuously increasing (4 → 24 h)
- **Phage_PaeO1** → OD value very low at all times

Phage_PaeO1 can **prevent biofilm formation** from the early stages

Result: Effect of Phage_PaeO1 and/or antibiotics on the biofilm formed

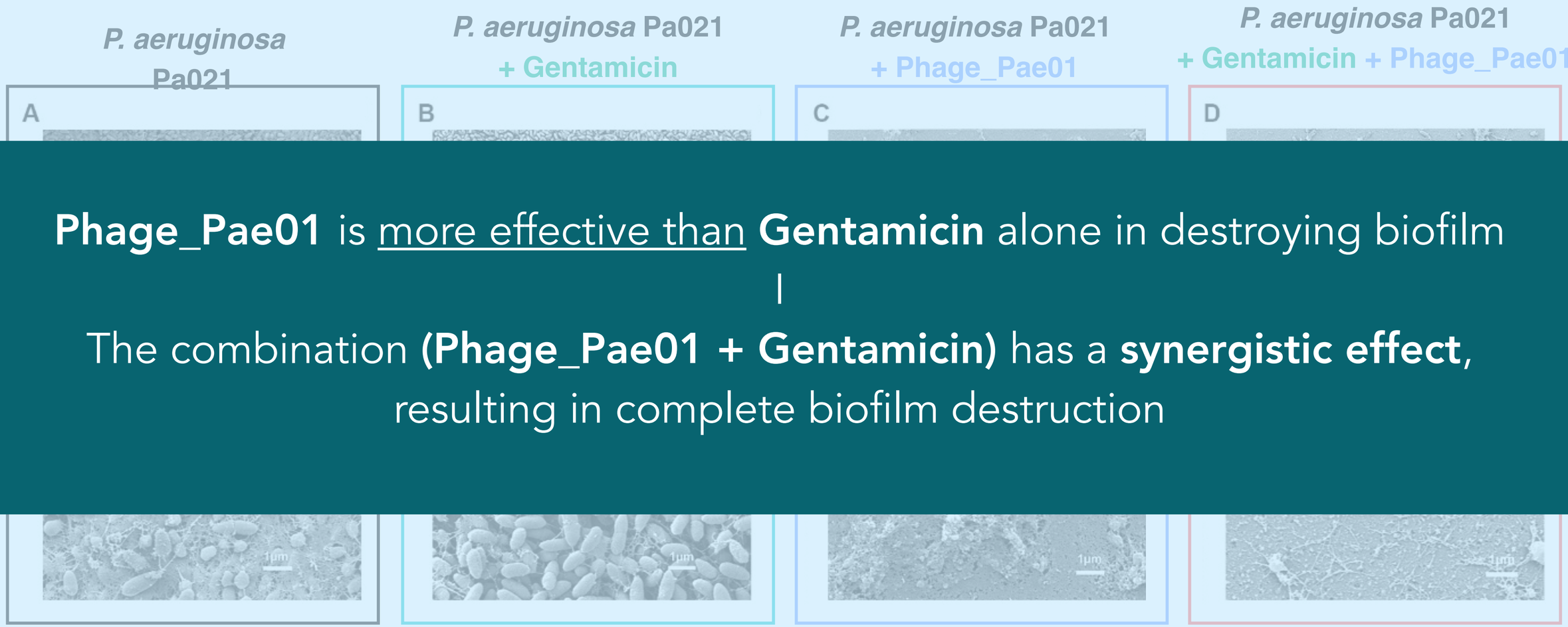
Fig. 8: Scanning electron microscopy images of *P. aeruginosa* PaO21 treated with Phage_PaeO1 and/or antibiotic



***P. aeruginosa* PaO21 + Gentamicin + Phage_PaeO1**: Biofilm completely destroyed, bacteria greatly reduced

Result: Effect of Phage_PaeO1 and/or antibiotics on the biofilm formed

Fig. 8: Scanning electron microscopy images of P. aeruginosa PaO21 treated with Phage_PaeO1 and/or antibiotic



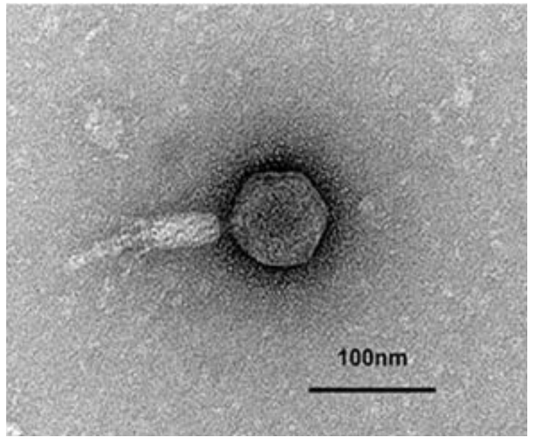
Phage_Pae01 is <u>more effective than</u> **Gentamicin** alone in destroying biofilm

|

The combination **(Phage_Pae01 + Gentamicin)** has a **synergistic effect**, resulting in complete biofilm destruction

P. aeruginosa **PaO21** **+ Gentamicin + Phage_PaeO1**: Biofilm completely destroyed, bacteria greatly reduced

Conclusions: Paper 1



Phage_PaeO1

is a **novel broad-spectrum lytic phage** with strong antibacterial activity

- prevent biofilm formation
- eradicate mature biofilm

Genome & Morphology

confirm **efficient infection** and **bacterial lysis mechanisms**

Phage_PaeO1 alone
& in combination with antibiotics → +

effectively controls *P. aeruginosa* biofilms, highlighting its therapeutic potential and safety for clinical use

Paper 2: Overview

Therapeutic effect and anti-biofilm ability assessment of a novel phage, phiPA1-3, against carbapenem-resistant *Pseudomonas aeruginosa*

Yu-Chuan Tsai [a,1], Yi-Pang Lee [b,1], Nien-Tsung Lin [c,1], Hsueh-Hui Yang [d], Soon-Hian Teh [e,*], Ling-Chun Lin [a,c,*]

Rationale:

Identifying a safe and effective anti-biofilm bacteriophage is crucial for developing alternative therapies against drug-resistant *P. aeruginosa*

Objectives:

- Characterize **biological** and **genomic properties** of phage phiPA1-3
- Evaluate **antibacterial activity** against CRPA
- Assess **biofilm prevention** and **eradication ability**
- Determine **therapeutic** effectiveness using an ***in vivo* infection model**

Overview:

Phage Isolation

Biological Characterization

Genomic Analysis
- Whole-genome sequencing ~73 kb dsDNA

Confirmed:
- Lytic lifestyle
- No virulence genes
- No antibiotic resistance genes

→ **Suitable for phage therapy**

Effective antibiofilm activity

***In vivo* therapeutic efficacy**

- Reduced bacterial burden
- Improved survival after phage treatment

Method & Result: Plaque and Phage Morphology of phiPA1-3

Objective: To find phages that kill CRPA

Bacterial strains

***P. aeruginosa* strain PA001**

↓ Isolated from

Pateint's sputum

Phage host → Clinical isolates

45 *P. aeruginosa* → **71%** were CRPA

- High antibiotic resistance
- High genetic diversity **(RAPD-PCR)**
- No correlation between genotype and phage susceptibility

3 *Klebsiella pneumoniae*

3 *Enterobacter cloacae*

2 *Escherichia coli*

Phage Isolation via spot assay

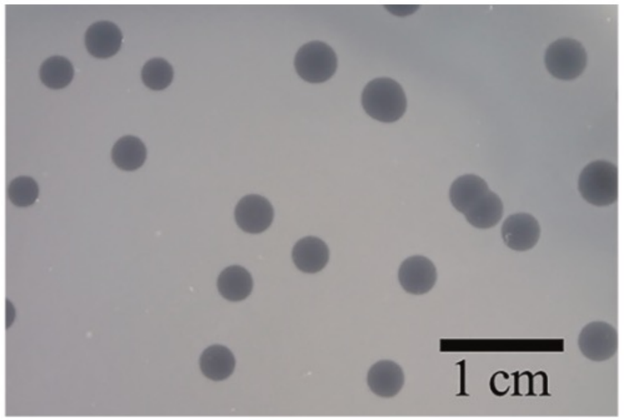
collected wastewater sample taken from a sewer at Tzu Chi Hospital, Hualien, Taiwan

"phiPA1-3"

Result: Plaque and Phage Morphology of phiPA1-3

Objective: To find phages that kill CRPA

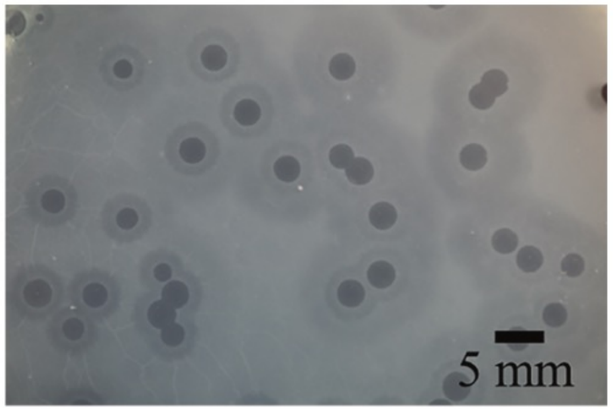
Plaque morphology



Phage phiPA1-3 is infected with *P. aeruginosa* PAO01, it is found that:

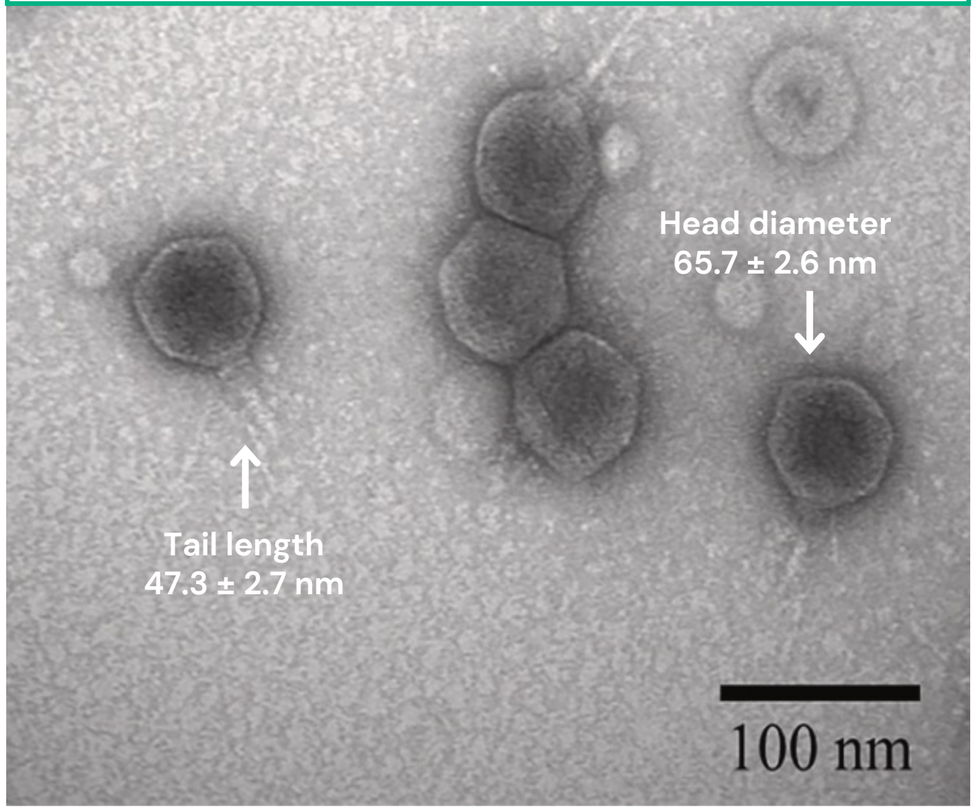
- Clear plaques form
  → **lytic phage**

Halo expansion over time



The halo surrounding the plaque enlarges over time
→ **phage-encoded depolymerase enzyme**

Transmission Electron Microscopy; TEM



Result: Host spectrum determination and EOP analysis

Objective: To determine the Host Range and evaluate Lytic Efficiency via Efficiency of Plating

Host Range

phiPA1-3
+
***P. aeruginosa* clinical isolates 45 strains**

→ **Moderate host range**

9 strains are susceptible to infection,

including the primary host PAO01,

representing **20% of the host range (9/45)**

Efficiency of Plating; EOP

Some strains have **higher** EOP than PAO01

↓

Phages can kill certain clinical strains of bacteria
more effectively than the primary host

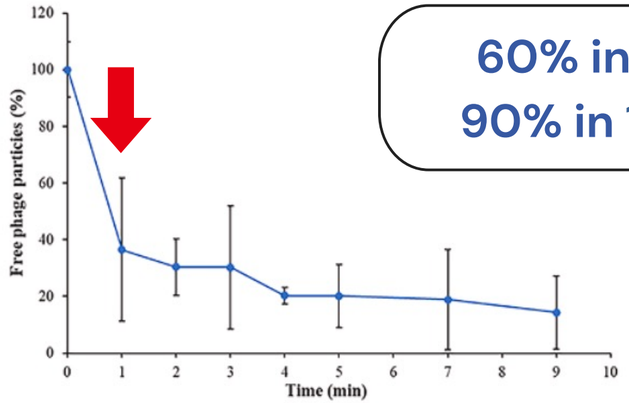
Table 1
Host range of phiPA1-3 against *P. aeruginosa* and other species.

Bacterial Strains	Antibiotic Resistance Profile[a]	EOP (% ± SD)[b]
P. aeruginosa		
PA001	CRPA	100±2.16
LCL12	CRPA	–
LCL13	CRPA	–
LCL14	CRPA	–
PS-1	CRPA	–
PS-2	CRPA	–
PS-3	CRPA	–
PS-4	CRPA	–
PS-5	CRPA	–
PS-6	CRPA	–
PS-7	CRPA	–
PA10	CRPA	–
PA13	CRPA	–
PA18	CRPA	–
PA20	CRPA	0.67±6.84
PA22	CRPA	–
PA25	CRPA	–
PA75	CRPA	0.001±0.47
PA76	CRPA	–
PA77	CRPA	–
PA78	CRPA	–
PA79	CRPA	–
PA80	CSPA	–
PA81	CRPA	–
PA82	CSPA	–
PA83	CSPA	–
PA84	CRPA	–
PA85	CRPA	–
PA86	CRPA	–
PA87	CSPA	–
PA88	CRPA	–
PA89	CSPA	–
PA90	CSPA	–
PA91	CRPA	–
PA92	CSPA	–
PA002	CSPA	35.41±1.88
PA005	CRPA	–
PA006	CSPA	–
PA009	CSPA	16.56±5.35
PA010	CRPA	–
PA011	CRPA	–
PA022	CSPA	0.01±2.16
PA023	CSPA	0.008±3.29
PA024	CRPA	0.0008±5.43
PA025	CSPA	178.12±9.20
K. pneumoniae		
98,035	ND	–
95,509	ND	–
96,111	ND	–
E. coli		
78,030	MDR	–
70,751	MDR	–
E. cloacae		
57,546	CRE	–
48,742	CRE	–
55,269	CRE	–

Result: Analysis of the biological properties and stability of phiPA1-3

Objective: To assess reproductive capabilities

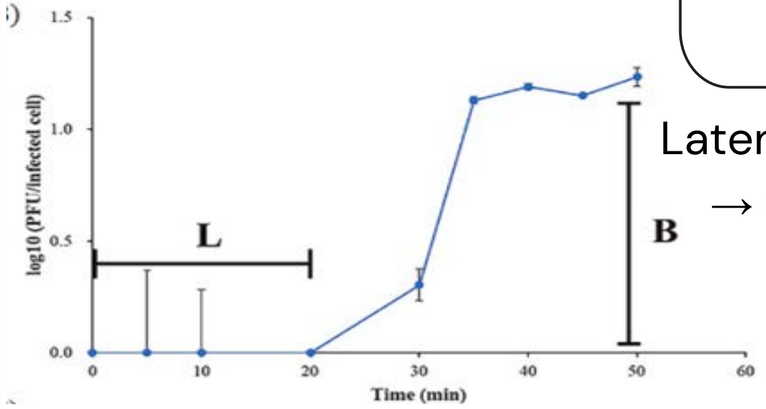
Adsorption assays of phiPA1-3 with host PAO01



60% in 1 min
90% in 10 min

phiPA1-3 has rapid adsorption efficiency

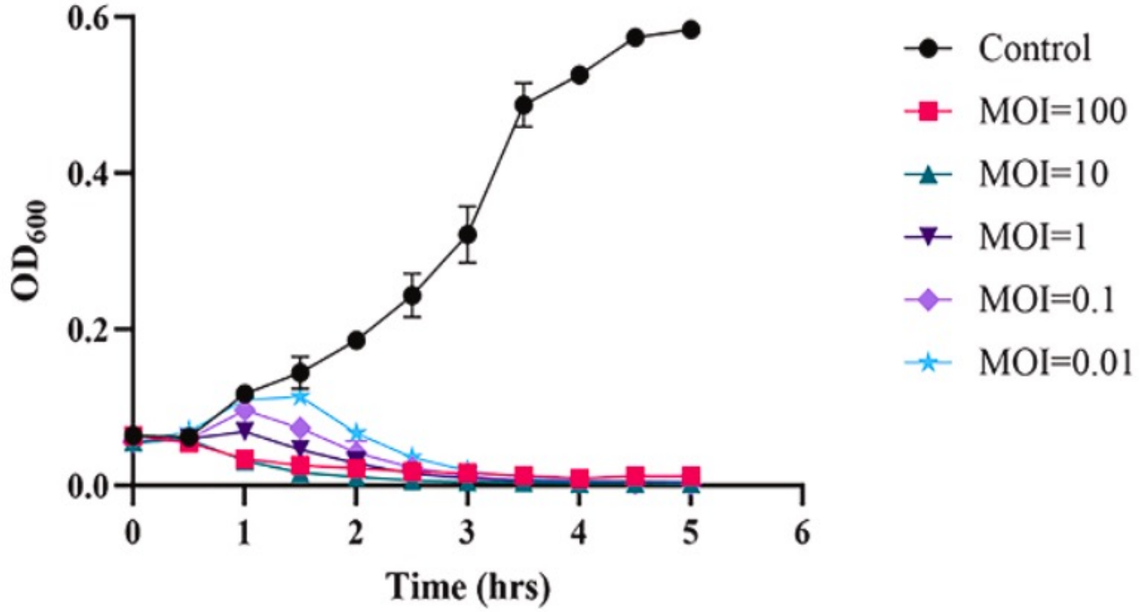
One-step growth curve



burst size
619 PFU/ml

Latent period ≈ **20 min**
→ strong lytic phage

Bacterial killing assay (MOI test)



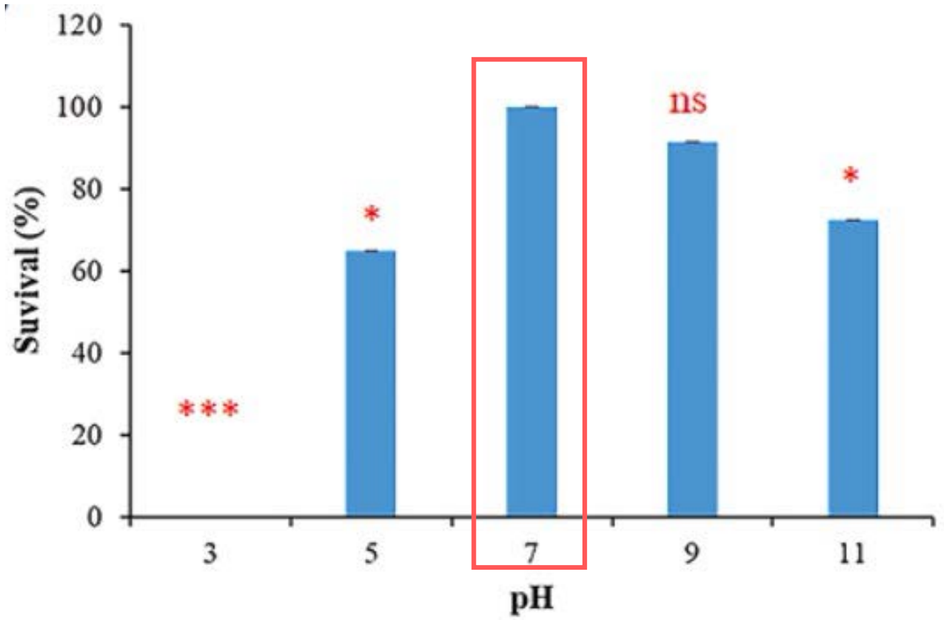
phiPA1-3 exhibits **strong** and **rapid lytic activity** even at low doses of phage

Optimal MOI of phiPA1-3 is **0.01**

Result: Analysis of the biological properties and stability of phiPA1-3

Objective: To determine phage stability

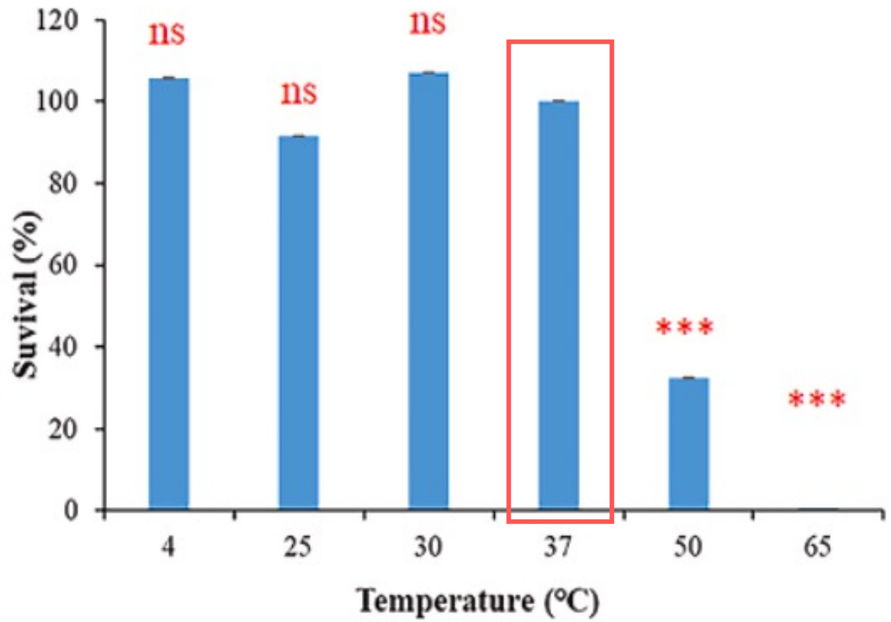
pH stability



High stability at pH **7-9**

Significant viability loss in acidic environment (< pH 5)

Temperature stability



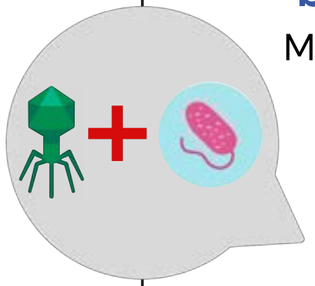
Optimal activity at physiological temperature (**37°C**)

Stable for 1h at elevated temp.

Result: Anti-biofilm activity of phiPA1-3

Objective: To evaluate whether phage phiPA1-3 can prevent biofilm formation and eliminate already mature biofilms

Co-culturing of phage and bacteria for biofilm-formation inhibition assay

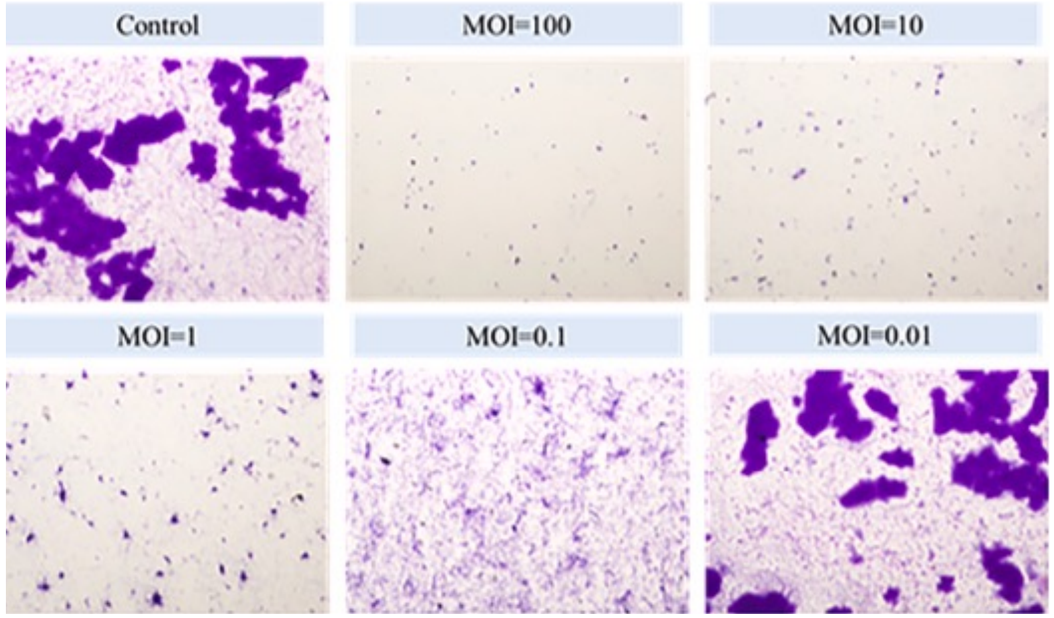


Mature biofilm of *P. aeruginosa* PA001 formed

after 6 h incubation

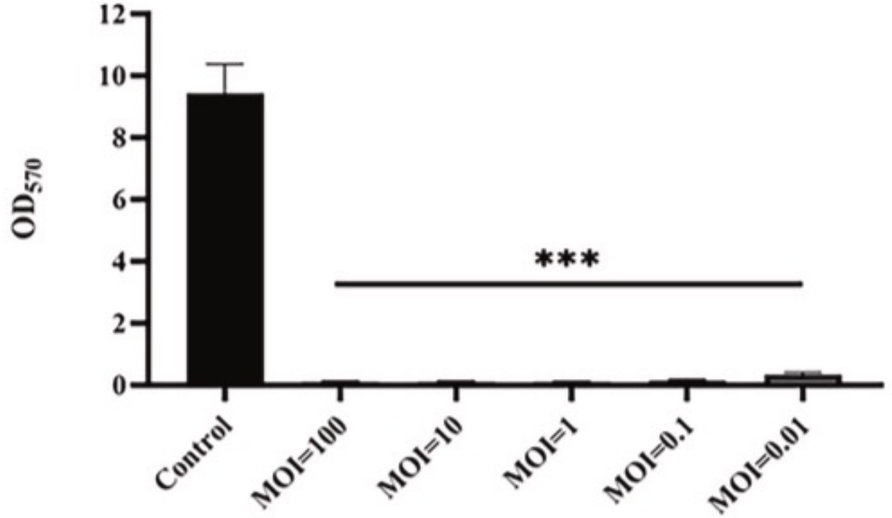
Biofilm Prevention Assay (Co-culture)

Phage + bacteria at different MOIs:



phiPA1-3 dose-dependent inhibition

Quanitification of the results for Co-culturing of phage and bacteria for biofilm-formation inhibition assay



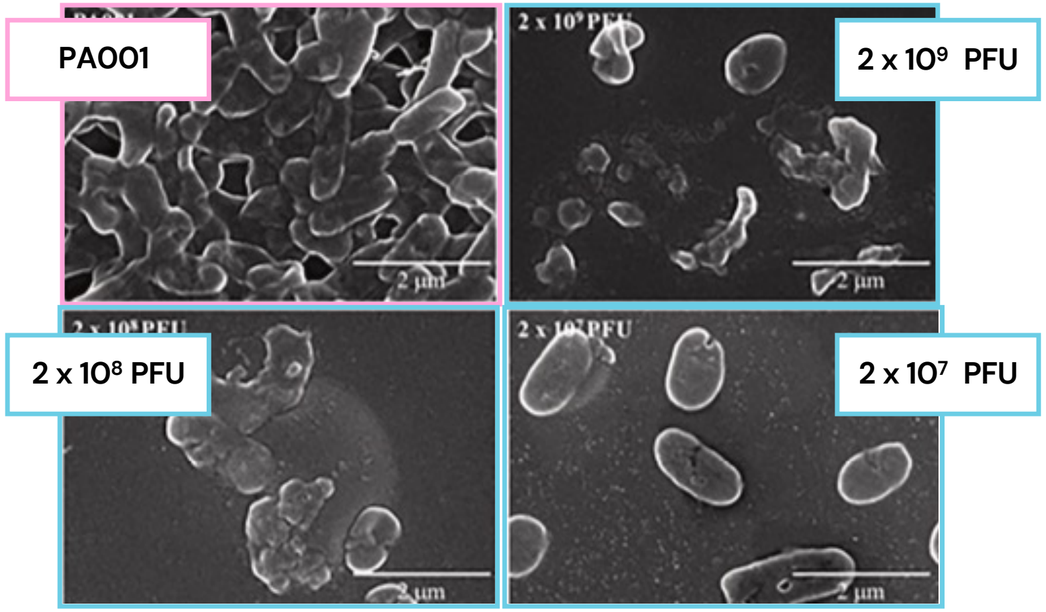
All MOI values significantly inhibited biofilm formation compared to the control group

Phages can prevent biofilm formation

Result: Anti-biofilm activity of phiPA1-3

Objective: To evaluate whether phage phiPA1-3 can prevent biofilm formation and eliminate already mature biofilms

SEM — Biofilm eradication



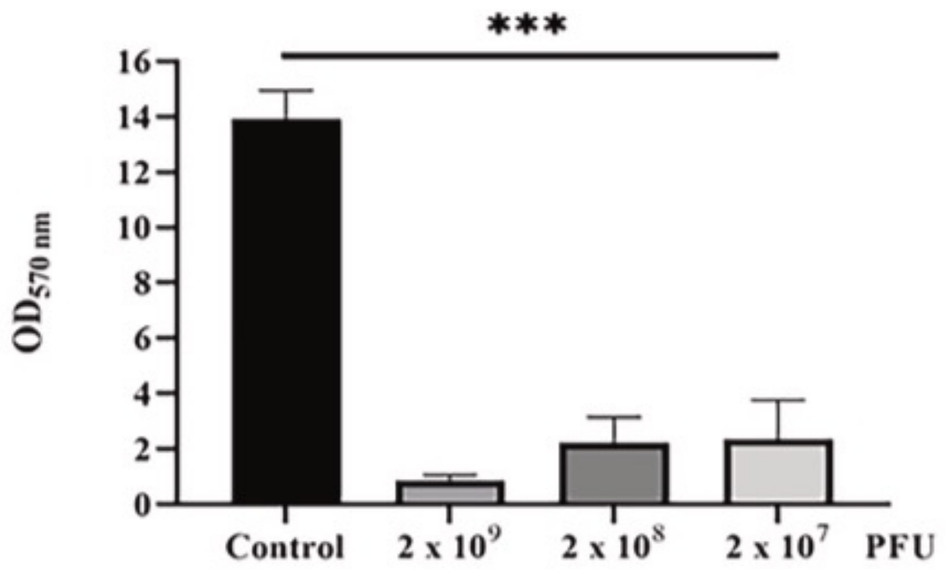
***Phage-treated

After added phage:

- Cell lysis
- Surface structure damaged
- Biofilm network destroyed

phiPA1-3 destroys mature biofilm structure

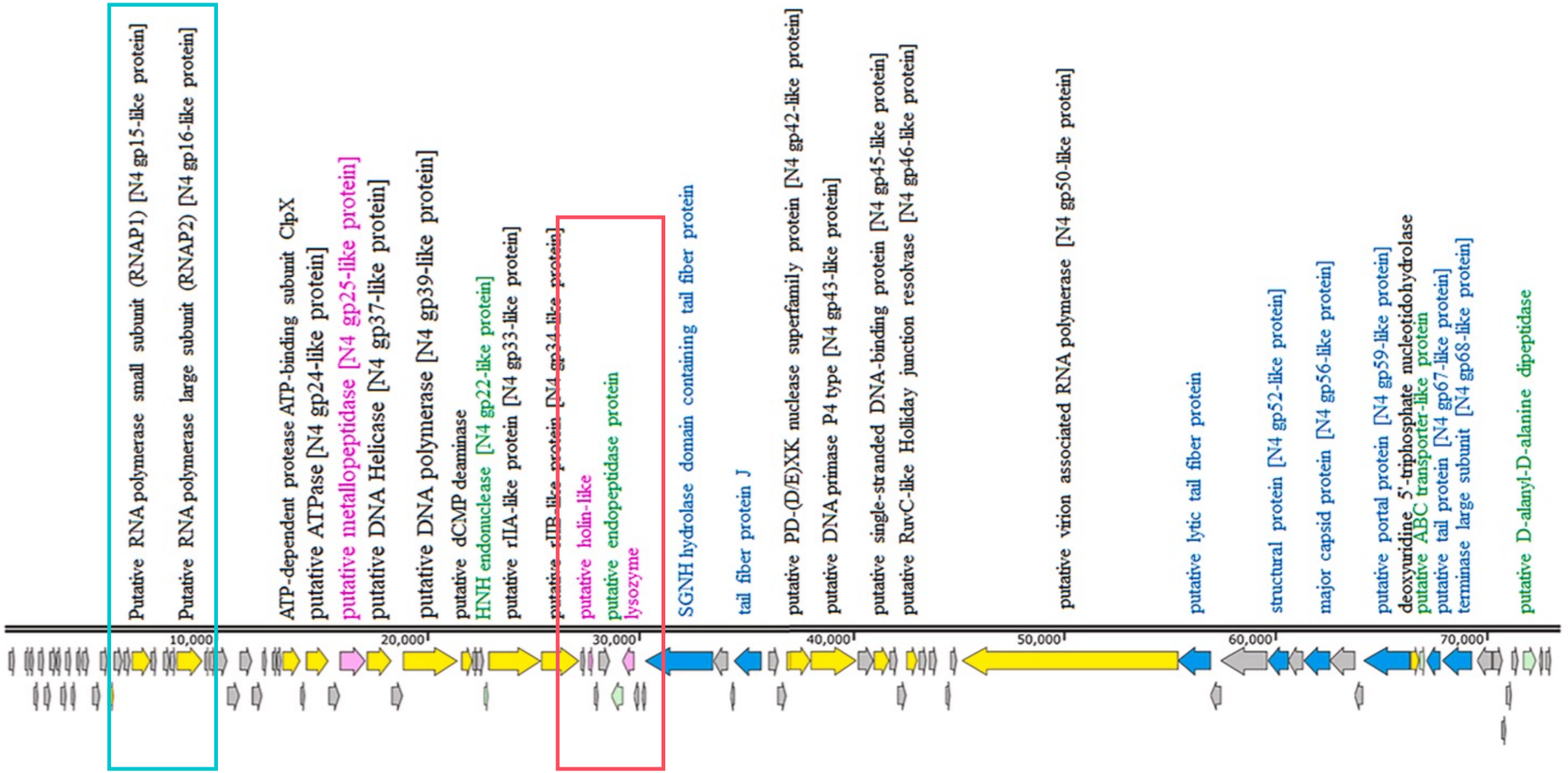
Quanitification of biofilm-eradication



phiPA1-3:

- Inhibit biofilm formation
- Eradicate mature biofilm

Result: Genome map & functional annotation of phage phiPA1-3

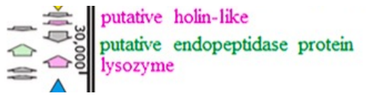


Objective: To analyze the genomic organization and predict functional genes of phage phiPA1-3 in order to determine its biological functions and therapeutic safety

Transcription machinery (N4 hallmark)

- RNA polymerase small & large subunits
- Virion-associated RNA polymerase

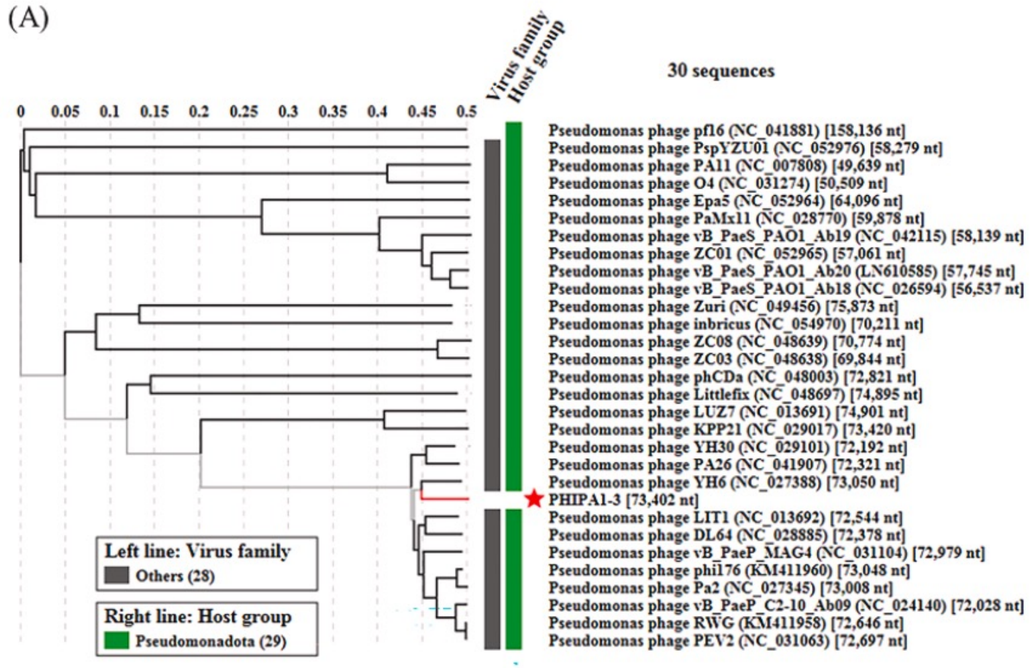
Host Lysis

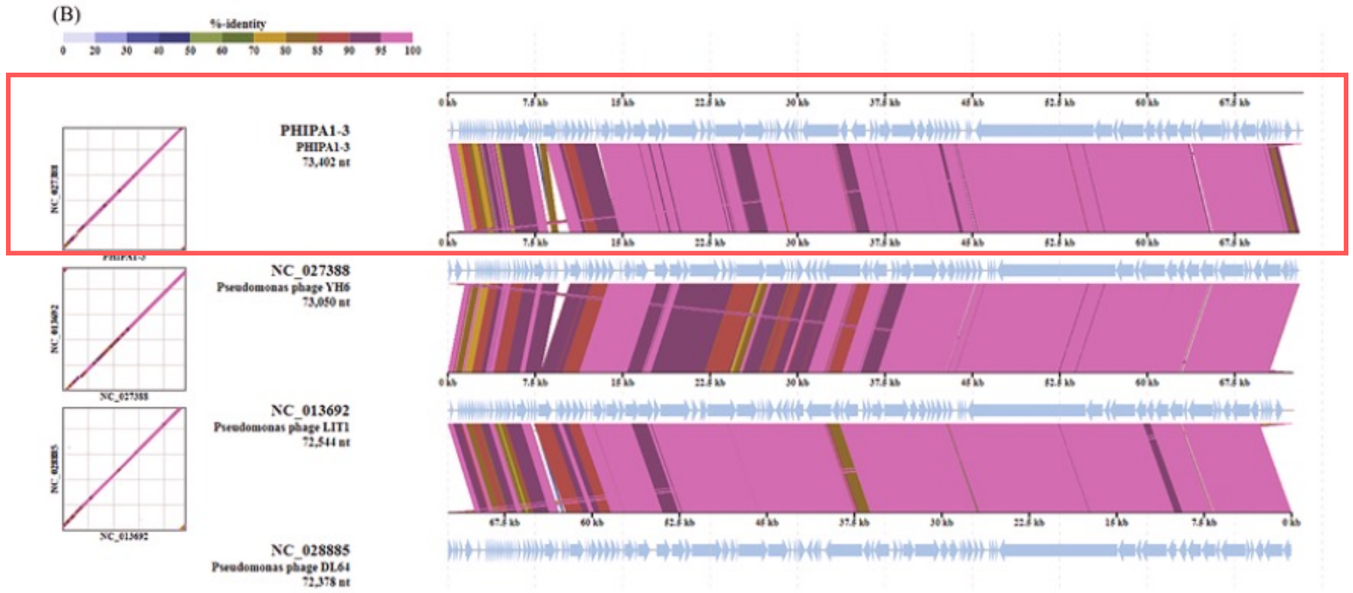


confirms the lytic mechanism used to lyse bacterial cells and release progeny phages

Result: Comparative genomics and phylogenetic analysis

Objective: To determine the evolutionary relationship, taxonomic classification, and genomic relatedness of phage phiPA1-3 with previously reported *Pseudomonas* bacteriophages





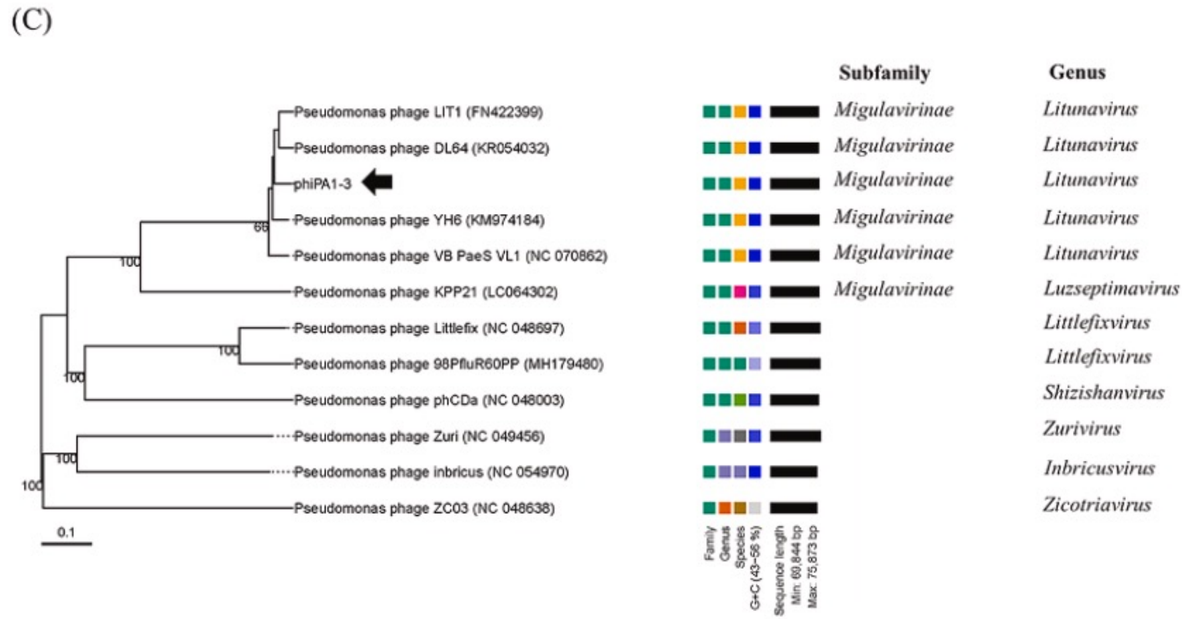
phiPA1-3 showed highest similarity to ***Pseudomonas* phage YH6**

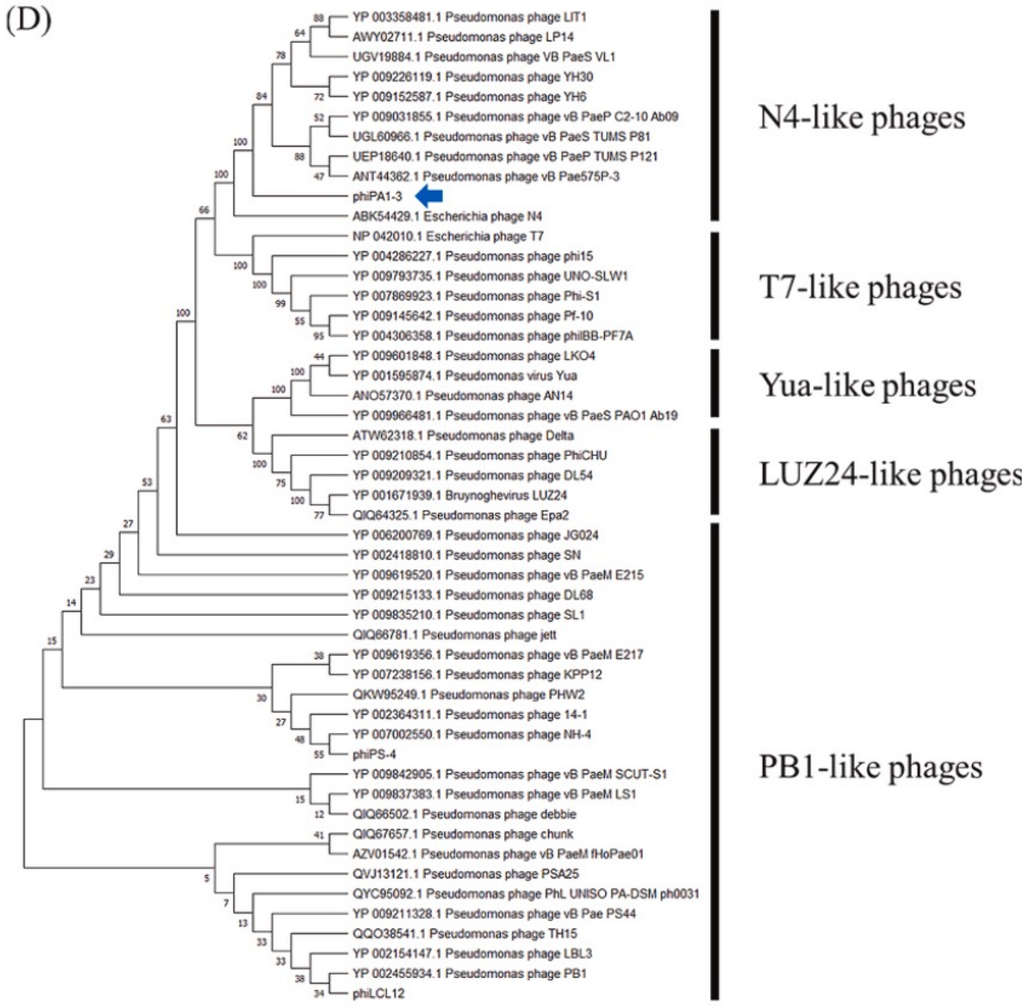
- 96.3% genome identity
- 98% genome coverage

→ **close relationship but still genetically distinct**

Result: Comparative genomics and phylogenetic analysis

Objective: To determine the evolutionary relationship, taxonomic classification, and genomic relatedness of phage phiPA1-3 with previously reported *Pseudomonas* bacteriophages





The phylogenetic tree was generated using VICTOR, and sequence comparison was performed using MEGA11

phiPA1-3 grouped within N4-like phages

Family: *Schitoviridae*

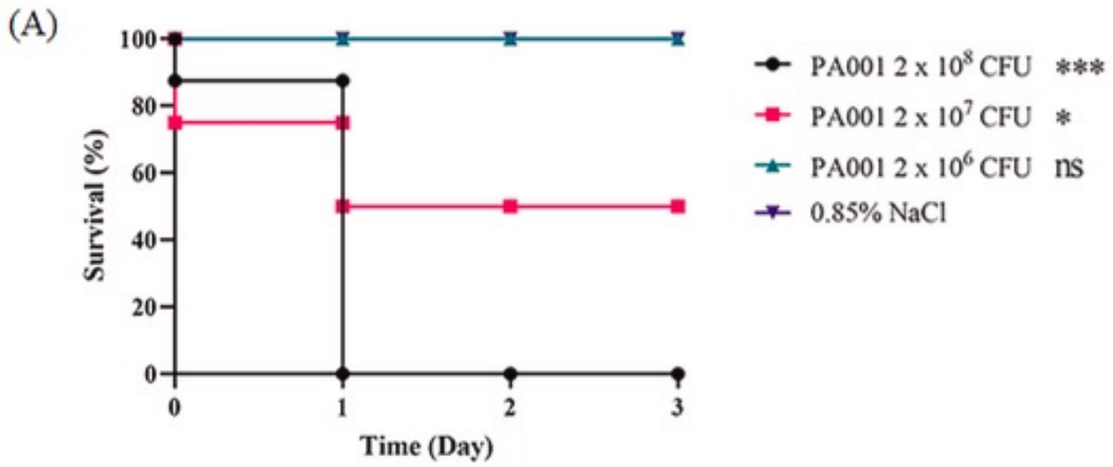
Subfamily: *Migulavirinae*

Genus: *Litunavirus*

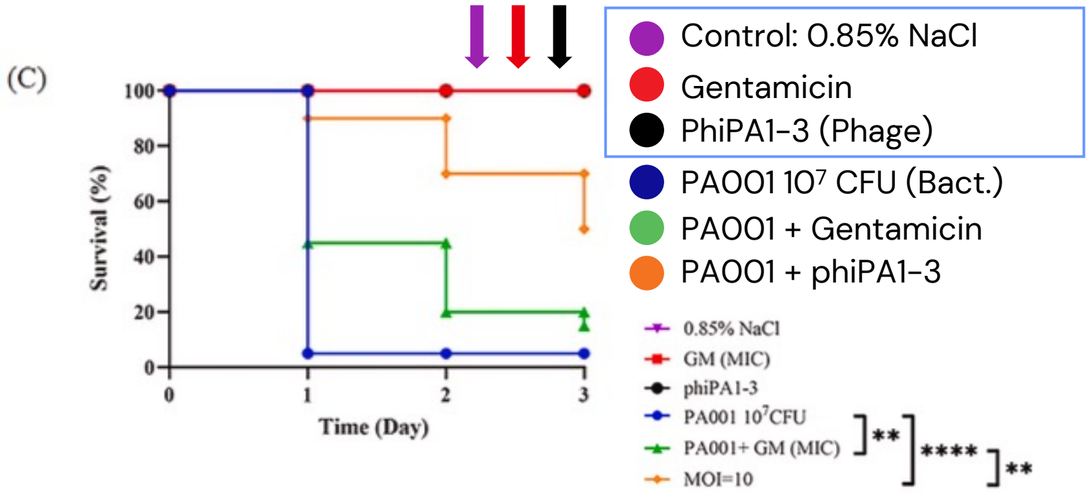
Result: *In vivo* Therapeutic Assessment

Objective: To evaluate the therapeutic efficacy and safety of phage phiPA1-3 in treating *P. aeruginosa* infection using an *in vivo* zebrafish model

Determination of lethal infection dose



Therapeutic rescue experiment



Demonstrates pathological symptoms observed in infected zebrafish

***P. aeruginosa* PA001 infection**

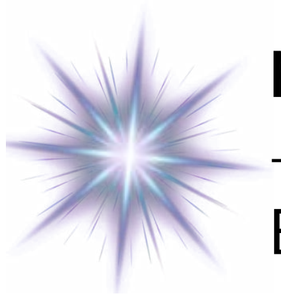
- sepsis-like symptoms

Infection + phiPA1-3 (MOI=10)

- phage reduces pathological damage

MOI: multiplicity of infections

Conclusions: Paper 2



phiPA1-3:

→ lytic phage

Burst Size: **619** PFU/cell

Specificity

target CRPA

moderate host range (20%)

biofilm eradication
&
prevent biofilm formation

Non-Toxic *In Vivo*

Phage treatment was more effective than gentamicin

(Zebrafish model)

Criticisms

	STRONG POINTS	WEAK POINTS
1st Paper	• **Broad host range** lytic phage • Genomically safe therapeutic candidate • Strong antibiofilm efficacy • **Synergistic activity** with antibiotics	The experiments were conducted at the ***in vitro*** level, The evaluation of phage efficacy and biofilm removal was carried out under laboratory conditions only
2nd Paper	• Targets carbapenem-resistant strains (CRPA) • Genomic safety confirmation • *In vivo* therapeutic efficacy	• Moderate host range • Single-phage therapy tested without combination with antibiotics • Resistance development not deeply investigated

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MDKKU

Thank you for your kind attention

Q&A or Suggestion