



THESIS PROGRESSION

Phage genome characterization and effect of phage-antibiotic combination against *Burkholderia pseudomallei* in *ex vivo* and *in vivo*

Phage precipitation for genome extraction and recovery of phage from phage/CAZ combination

Date: March 6, 2024 Time: 11.30-12.00 PM

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 Co-advisor: Dr. Auttawit Sirichoat



CONTENTS

INTRODUCTION

- Literature review
- Hypothesis
- Objective
- Conceptual framework

SUMMARY

- Conclusion
- Further work

METHODS & RESULTS

- Study design
- Phage DNA extraction : PEG precipitation
- Phage DNA extraction : extraction, gel electrophoresis and Nanodrop
- Recovery of phage from phage/CAZ combination

THESIS PLAN

- Thesis progress overview

INTRODUCTION

Burkholderia pseudomallei (BP)

Gram **negative** bacilli

Soil Water

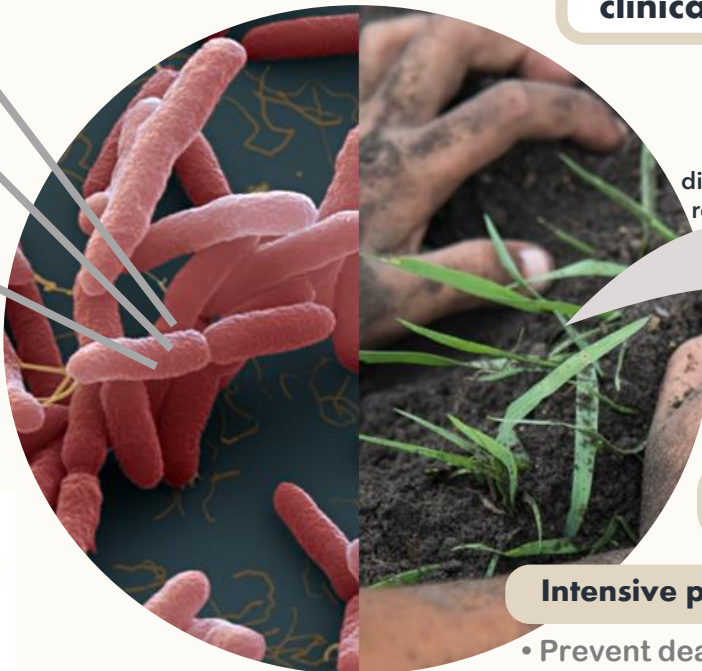
Causing agent of **melioidosis**

High mortality
20-50% worldwide
(Robert, M., et al, 2020)



Jing Hong Fong et al, 2021

Melioidosis is **endemic disease** in tropical and subtropical area



Melioidosis

clinical sign & symptoms

Wound, digestion, or respiratory tract



Skin ulcer / skin tissue abscess

Splenic, liver, pulmonary abscess

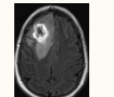
Brain abscess / encephalomyelitis

Bacteraemia / Mycotic aneurysm

Acute pyelonephritis



Currie B. J., 2003



Owen, W., et al, 2020



Tong, T. K., et al, 2021



Ling, T. T., et al, 2023

Treatment



Intensive phase

- Prevent death

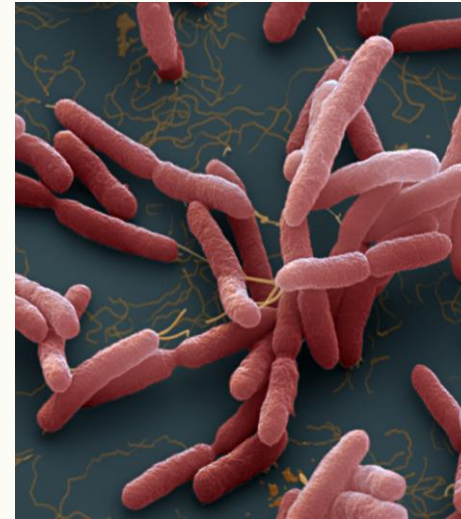
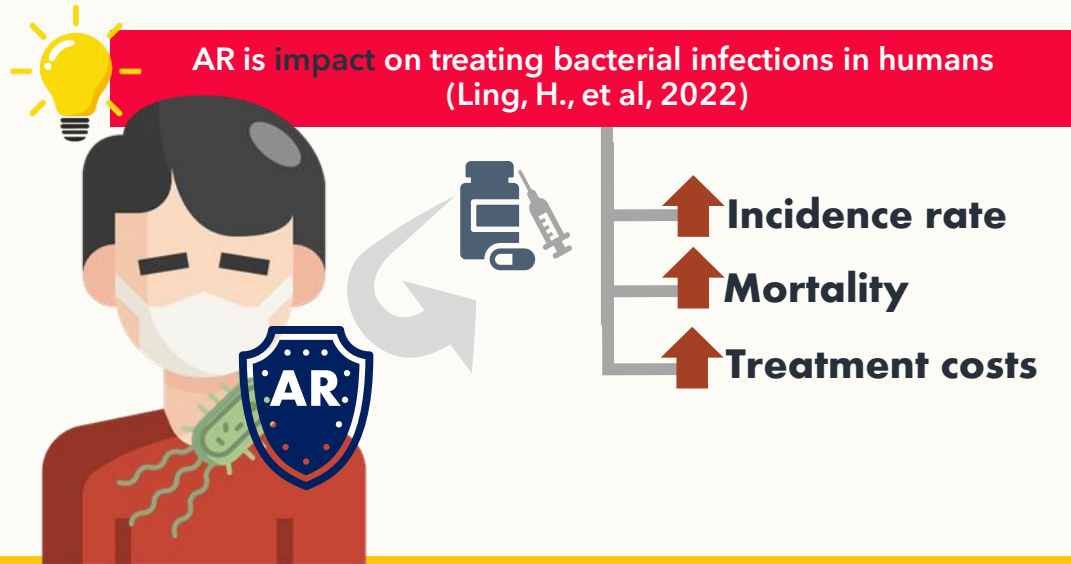
| Application | Drug | IV | Duration |
|---------------------|----------------|----|-----------|
| Primary therapy | Ceftazidime | | 2-8 weeks |
| Alternative therapy | Meropenem | | |
| Adjunct therapy | Co-trimoxazole | | |

Eradication phase

- Prevent relapse

| Application | Drug | Oral | Duration |
|---------------------|----------------|------|------------|
| Primary therapy | Co-trimoxazole | | 3-6 months |
| Alternative therapy | Co-amoxiclav | | |

Antibiotic resistance



BP resist to...

- Penicillin
- Macrolides
- Colistin
- Cephalosporins

BP suscept to...

- Co-amoxiclav
- Ceftazidime
- Carbapenems
- Co-trimoxazole

β -lactamase and CAZ-resistant BP infection have occurred. (Perumal Samy, R., et al, 2006) & (Holden, M. T., et al, 2004)

Mechanisms of antibiotic resistance *B. pseudomallei*

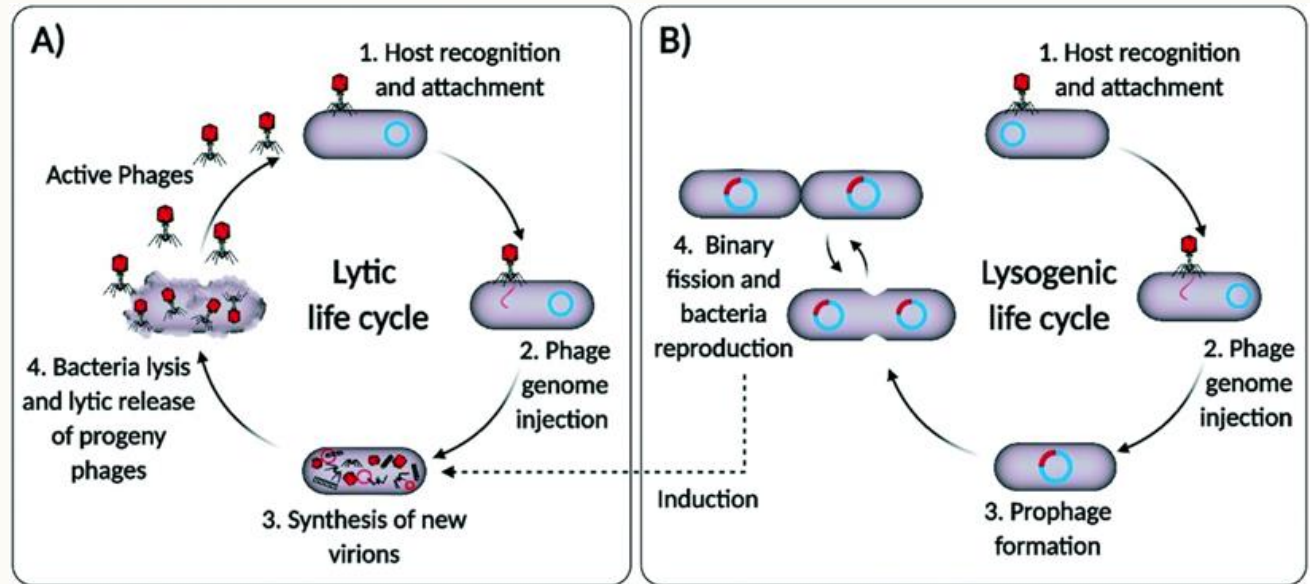


Other mechanisms

- Produce transient
- Biofilm formation
- Colony adaptation
- Metabolic dormancy

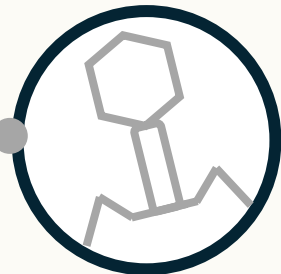
Bacteriophage

Phage replication



Phage lifecycle. (A) Lytic lifecycle, (B) lysogenic lifecycle

Applications



Phage therapy

Food preservation

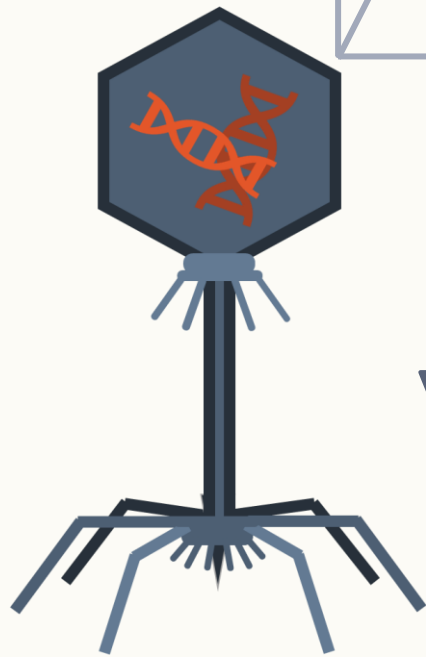
Agriculture /Aquaculture

Phage display

Phage therapy

Beneficial of phage therapy

- Host Specificity
- Biofilm Degradation
- Kill AR bacteria



Bacteriophage

VS



Antibiotic

Phage therapy studies in *B. pseudomallei*



RESEARCH ARTICLE

Experimental Phage Therapy for *Burkholderia pseudomallei* Infection

Ong Guang-Han¹, Choh Leang-Chung¹, Kumutha Malar Vellasamy¹, Vanitha Mariappan¹, Chang Li-Yen^{1,2}, Jamuna Vadivelu^{1*}

¹ Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, ² Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, Kuala Lumpur, Malaysia

2016

Application of phage successfully rescued 33.3% of mice infected

RESEARCH ARTICLE

Open Access

A novel lytic phage potentially effective for phage therapy against *Burkholderia pseudomallei* in the tropics

Yanshuang Wang^{1,2†}, Xuemiao Li^{1†}, David A. B. Dance^{3,4,5}, Han Xia^{6,7}, Chen Chen¹, Nini Luo¹, Anyang Li¹, Yanmei Li¹, Qiao Zhu¹, Qinghui Sun¹, Xingyong Wu¹, Yingfei Zeng¹, Lin Chen², Shen Tian^{1*} and Qianfeng Xia^{1*}



2022

A significantly reduced mortality (10%) and a decreased pathogen load

Effect of phage antibiotic combination for treatment

Phage-antibiotic combination better than phage or antibiotic alone

- Manohar, P., et al, 2022 are using **phages with a low antibiotic concentration** is an effective therapeutic strategy
- Phage antibiotic combination studies in numerous of pathogen

BRIEF REPORT



Piperacillin and ceftazidime produce the strongest synergistic phage-antibiotic effect in *Pseudomonas aeruginosa*

Jumpei Uchiyama¹ · Ryu Shigehisa² · Tadahiro Nasukawa¹ · Keijiro Mizukami¹ · Iyo Takemura-Uchiyama¹ · Takako Ujihara² · Hironobu Murakami¹ · Ichiro Imanishi³ · Koji Nishifuji³ · Masahiro Sakaguchi¹ · Shigenobu Matsuzaki²

ACS Infectious Diseases

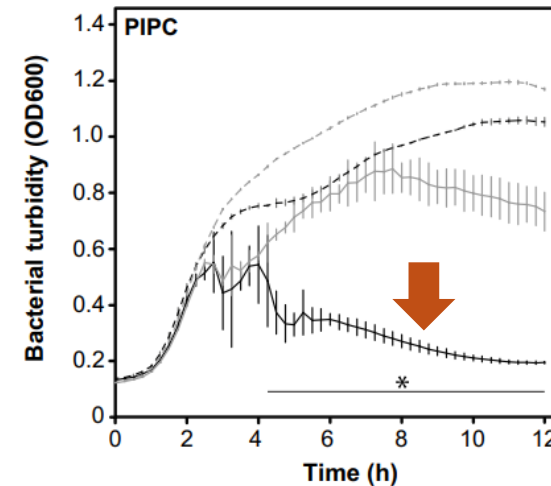
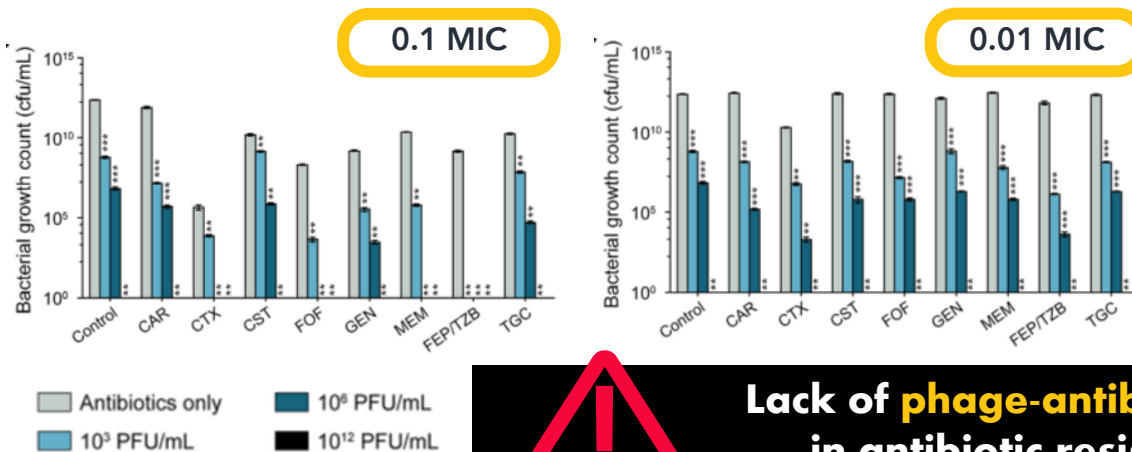
pubs.acs.org/journal/aidtbc



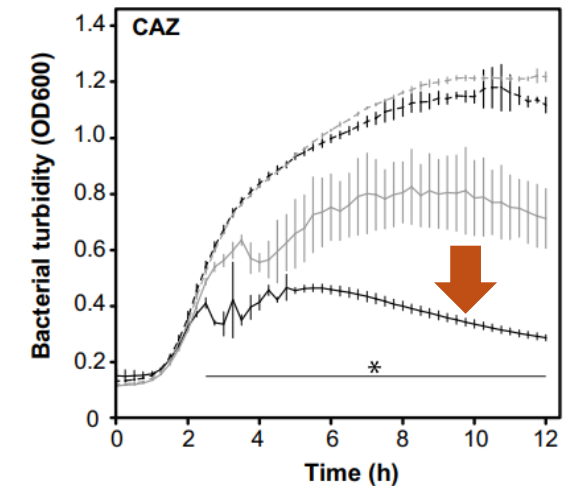
Letter

Synergistic Effects of Phage-Antibiotic Combinations against *Citrobacter amalonoticus*

Prasanth Manohar,[†] Madhav Madurantakam Royam,[†] Belinda Loh, Bulent Bozdogan, Ramesh Nachimuthu,* and Sebastian Leptihn*



--- No KPP22; no PIPIC
 --- No KPP22; 1.25 µg/ml of PIPIC
 — KPP22; no CAZ
 — KPP22; 0.31 µg/ml of CAZ



--- No KPP22; no CAZ
 --- No KPP22; 0.31 µg/ml of CAZ
 — KPP22; no CAZ
 — KPP22; 0.31 µg/ml of CAZ

Lack of phage-antibiotic combination studies in antibiotic resistance *B. pseudomallei*

Previous studies

Autchasai Siriprayong's thesis (Unpublish)



S4+1/2CAZ &
CT+1/2CAZ



A549 cell

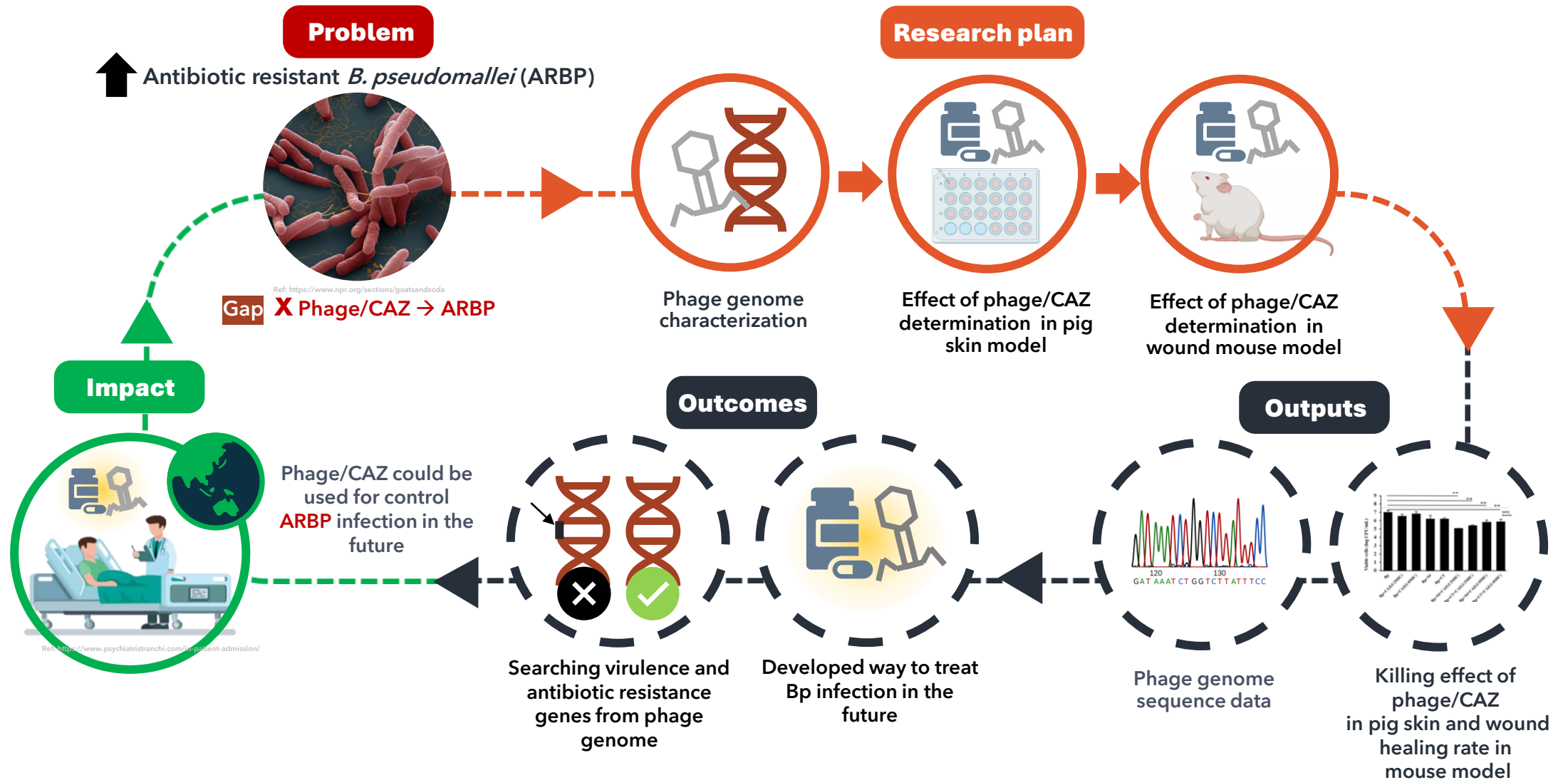
Hypothesis

Phages which specifically lyse *B. pseudomallei* do not contain virulence and antibiotic-resistant genes in genome. Phages and ceftazidime combination has high efficacy in treating *B. pseudomallei* on pig skin and mouse wound models. Phages and ceftazidime combination has potentially effective and can be safely used in clinical trial.

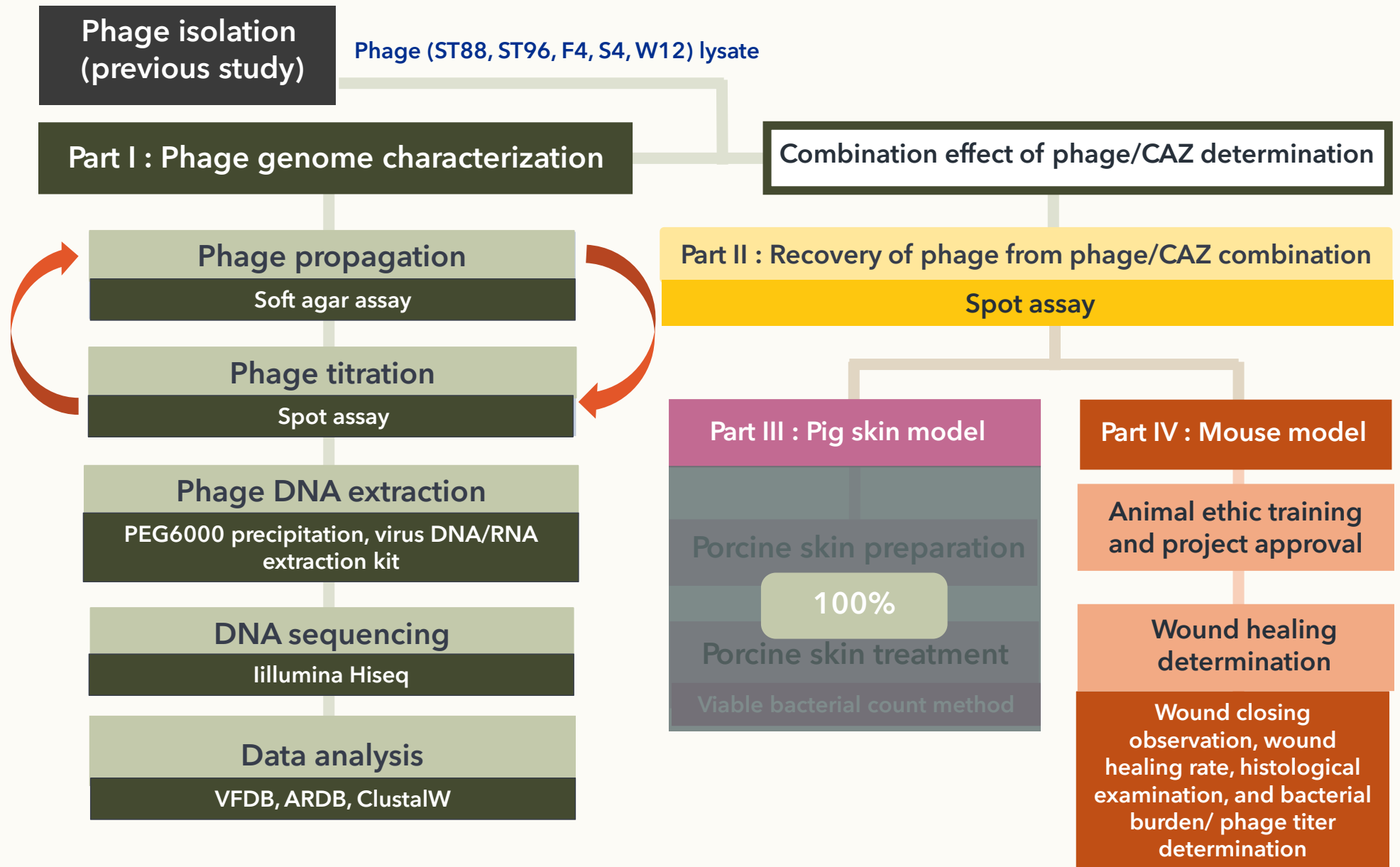
Objectives

1. To characterize and analyze phage genomes for searching and detecting virulence and antibiotic-resistant genes
2. To determine effect of phages and ceftazidime combination against *B. pseudomallei* in a pig skin model
3. To determine effect of phages and ceftazidime combination against *B. pseudomallei* through topical administration onto wound in a mouse model

CONCEPTUAL FRAMEWORK

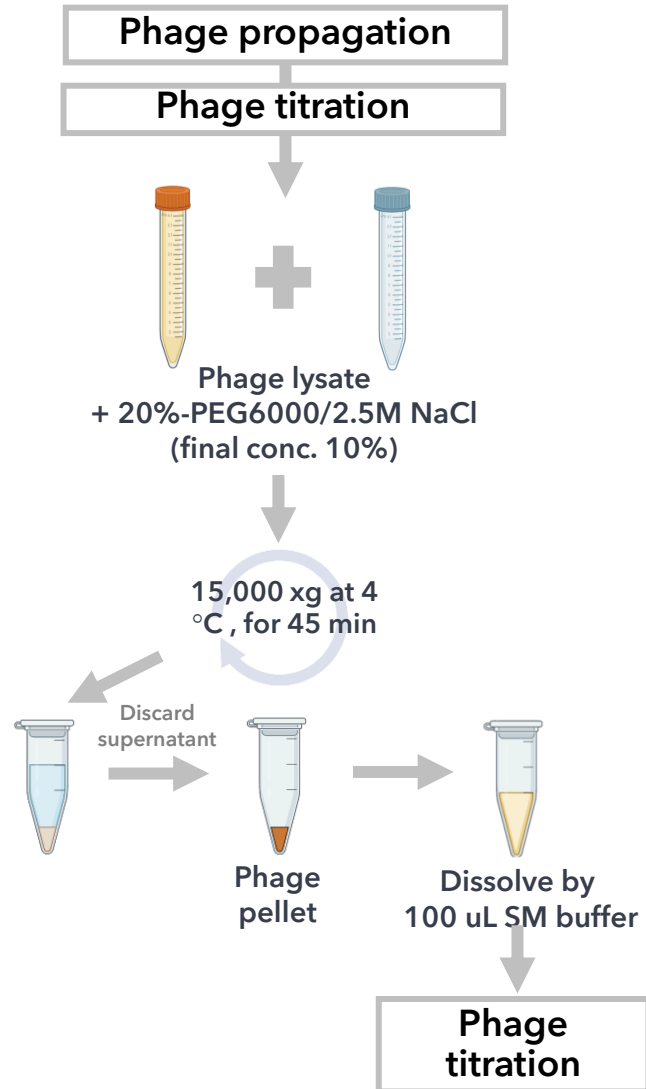


STUDY DESIGN



Methods

Phage **ST96, ST88, W12, S4, F4**

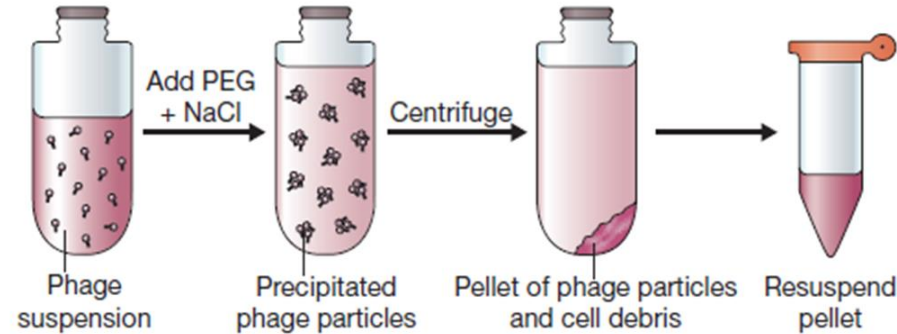


Phage genome extraction

Phage concentration by PEG precipitation

Objective : To increase concentration of phage lysate for phage DNA extraction.

PRINCIPLE



PEG : inert solvent sponge, reducing solvent availability
 High conc. of NaCl : Salting out

Results

Phages titer after precipitation

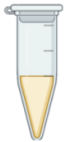
| Phages | Phage titer (PFU/mL) | |
|--------|----------------------|---------------------|
| | Before precipitation | After precipitation |
| ST96 | 7.25×10^6 | - |
| ST88 | 2×10^7 | - |
| W12 | 2×10^6 | - |
| S4 | 7.25×10^7 | - |
| F4 | 3×10^8 | 7.5×10^9 |

Methods



Treat w/ DNaseI 1U
at 37 °C for 45 min

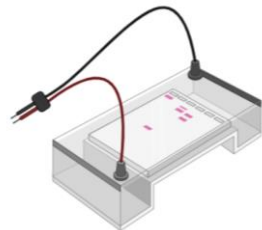
200 uL of phage lysate



Stop reaction
at 65 °C for 10 min



TIANamp Virus
DNA/RNA Kit, Tiangen,
Beijing, China



1% agarose gel electrophoresis in 1X
TAE buffer and run at 70V for 40 min



Phage DNA



NanoDrop: A260/280
and DNA conc.
measurement

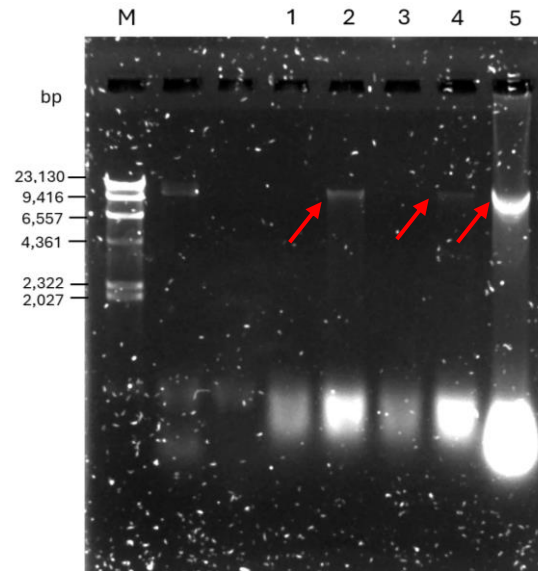
Phage genome extraction

Phage DNA extraction/
Length, amount & purity measurement

Objective : To extract and measure the amount and purity of DNA in a phage.

Results

DNA size of phages in 1% agarose gel

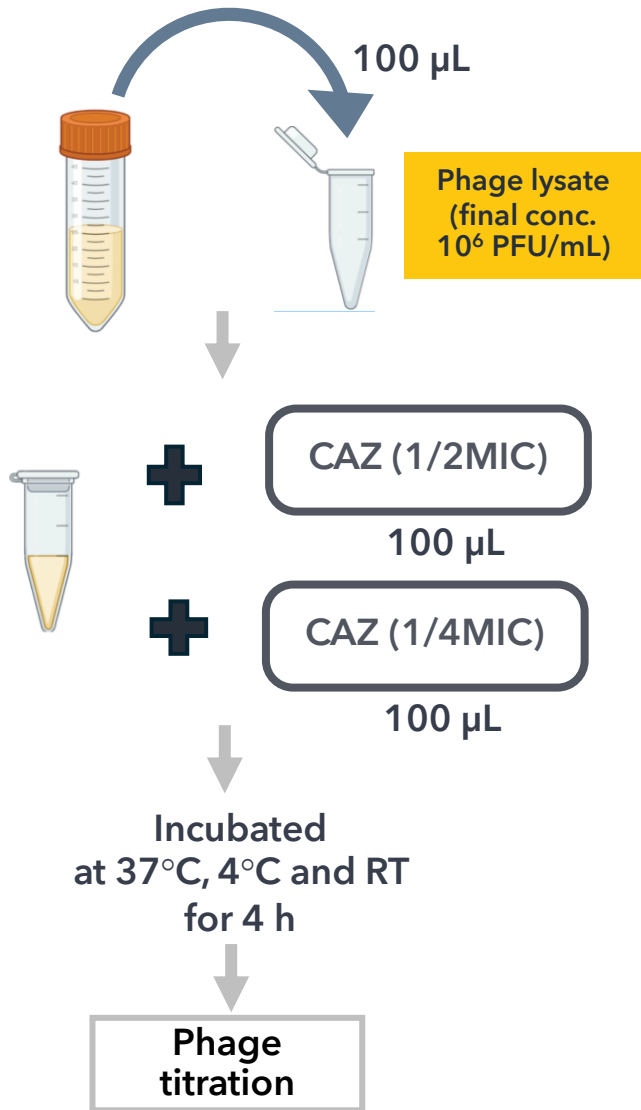


- M = Marker
- Lane 1 = ST96 phage DNA
- Lane 2 = ST88 phage DNA
- Lane 3 = W12 phage DNA
- Lane 4 = S4 phage DNA
- Lane 5 = F4 phage DNA

Nanodrop measurement
of DNA extracted from phages

| Phages | Phage titer (PFU/mL) | A260 | A280 | A230 | A260/280 | A260/230 | Concentration (ng/μL) |
|--------|-------------------------|-------|-------|-------|----------|----------|--------------------------|
| ST96 | 3×10^8 | 1.913 | 0.667 | 0.810 | 2.87 | 2.36 | 95.7 |
| ST88 | 1.1×10^9 | 2.473 | 0.935 | 0.985 | 2.65 | 2.51 | 123.7 |
| W12 | 2×10^8 | 1.917 | 0.667 | 0.949 | 2.87 | 2.02 | 95.8 |
| S4 | 1.2×10^9 | 2.606 | 0.920 | 0.797 | 2.83 | 3.27 | 130.3 |
| F4 | 7.5×10^9 | 8.529 | 3.748 | 3.824 | 2.28 | 2.23 | 426.4 |

Methods

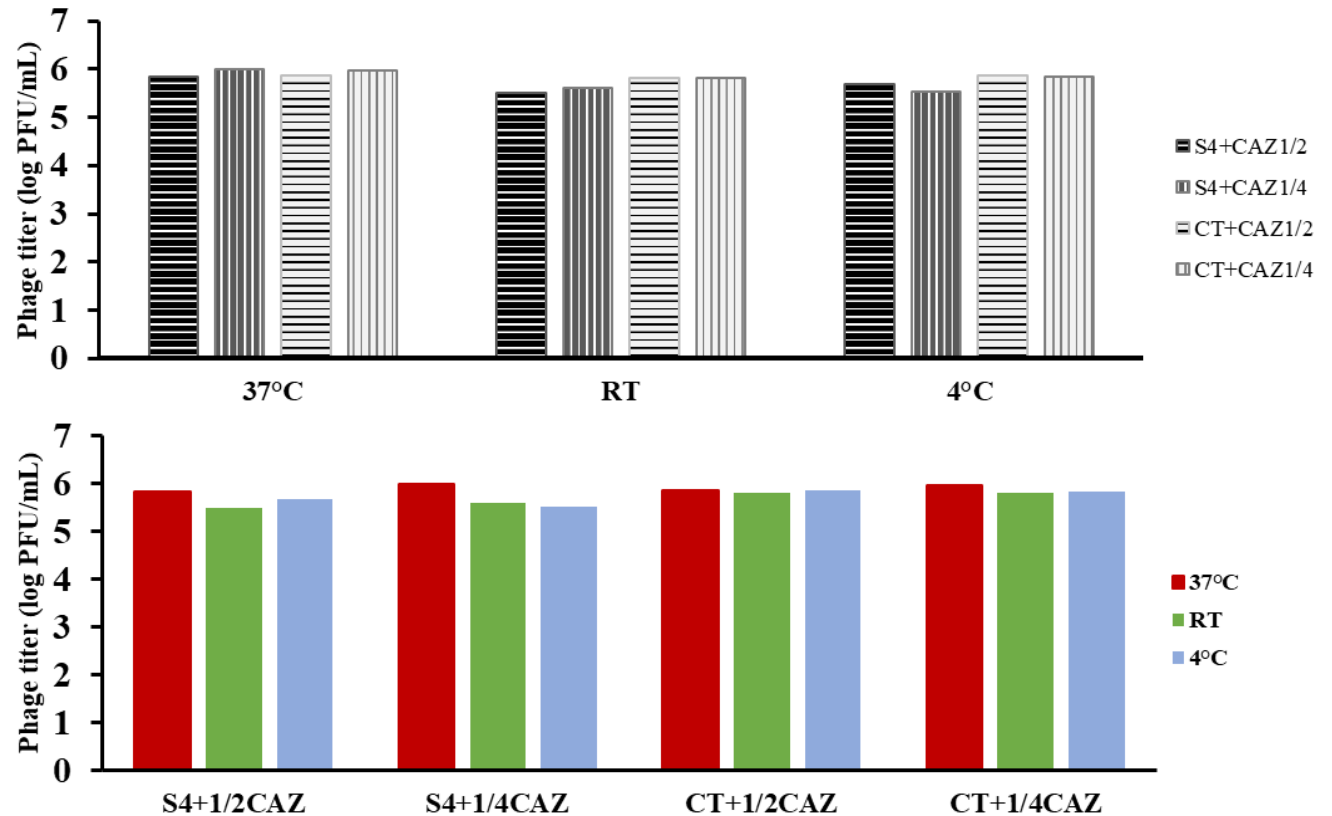


Recovery of phage from phage/CAZ combination

Objective : To determine stability of phage in phage and ceftazidime combination.

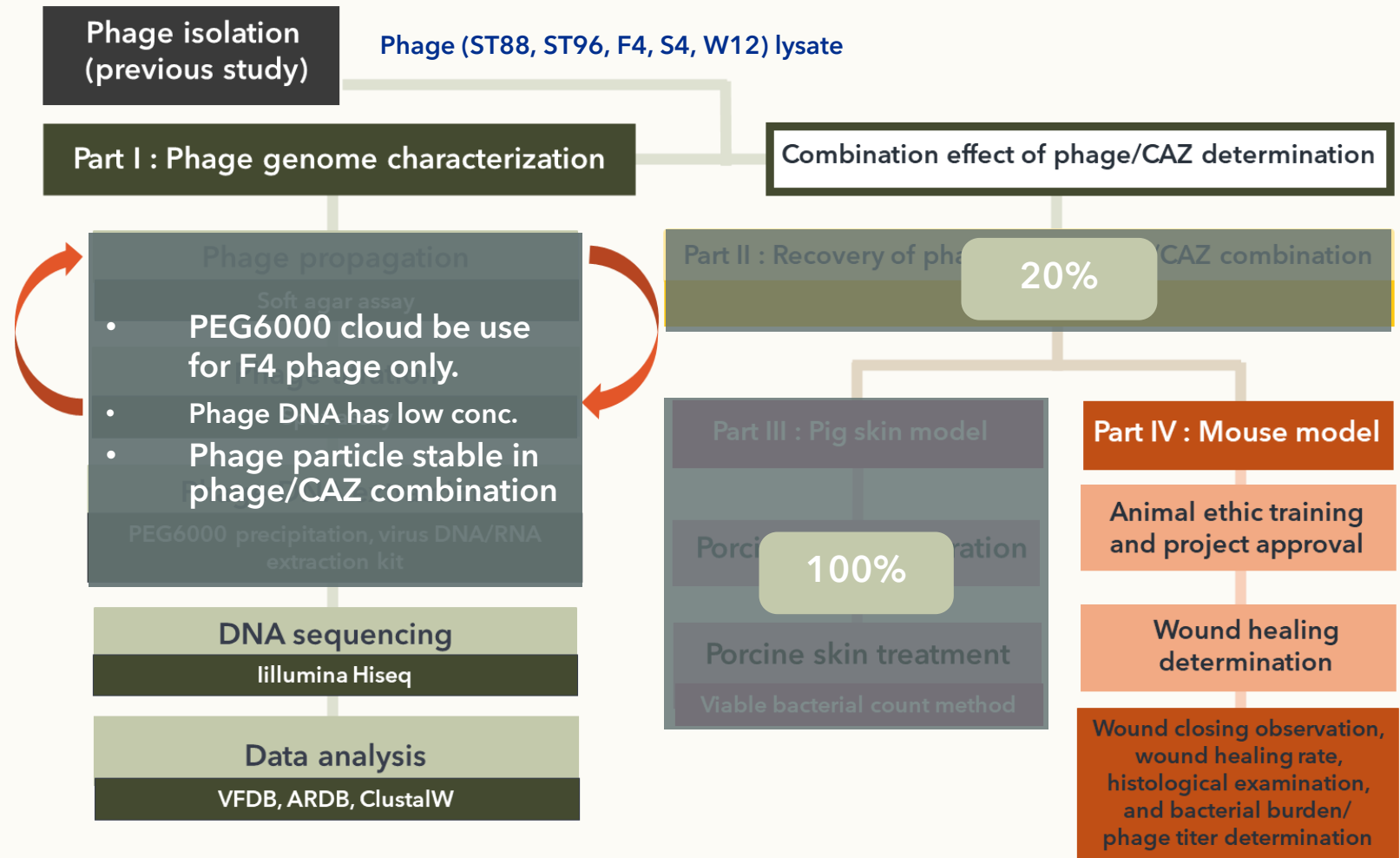
Results

S4 and CT phage titer from phage combine with CAZ in different temperatures



Note: One replicate (need 2 more replicates)

SUMMARY



Further work

- Phage will be propagated via liquid lysate
- Phage DNA will be extracted by phenol-chloroform method to compare extraction kit
- Phage DNA sequencing
- Recovery of phage from phage/CAZ combination (2 replicates)
- Request project to Northeast Laboratory Animal center for approval

THESIS PLAN

Finished
 Pending
 Future work

| Topics | Year 2023 | Year 2024 | | | | | | | | | | | | |
|--|-----------|-----------|---|---|---|---|---|---|---|---|----|----|----|--|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| Literature review and proposal writing | | | | | | | | | | | | | | |
| Proposal examination | | | | | | | | | | | | | | |
| Phage genome extraction | | | | | | | | | | | | | | |
| Phage genome sequencing | | | | | | | | | | | | | | |
| Phage genome analysis | | | | | | | | | | | | | | |
| Recovery of phage/CAZ combination | | | | | | | | | | | | | | |
| Pig skin preparation and treatment | | | | | | | | | | | | | | |
| Animal ethic training and approval | | | | | | | | | | | | | | |
| Combination effect of phage/CAZ for wound healing determination in mouse model observation | | | | | | | | | | | | | | |
| Histological examination and bacterial burden/phage titer determination | | | | | | | | | | | | | | |
| Results analysis, discussion and thesis report writing | | | | | | | | | | | | | | |
| Article writing for publication | | | | | | | | | | | | | | |
| Thesis examination | | | | | | | | | | | | | | |
| Thesis editing and thesis sending to graduate school | | | | | | | | | | | | | | |

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Thank you
for your kind attention

