



# Mathematical Modeling of Bacteriophage Therapy

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# Outline

Bacteriophage Therapy란?

M&S of Phage Therapy

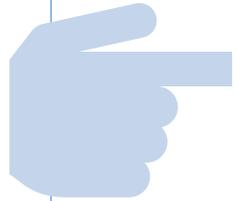
연구 사례 소개

토의 및 결론



# BACTERIOPHAGE THERAPY란?

# Phage therapy란?



100년 전부터 사용되어  
옴 (주로 동유럽권)



항생제가 나오면서  
서구권에서는 잊혀짐



항생제 내성의 증가에  
따라 다시 관심이 증가

# 박테리오파지

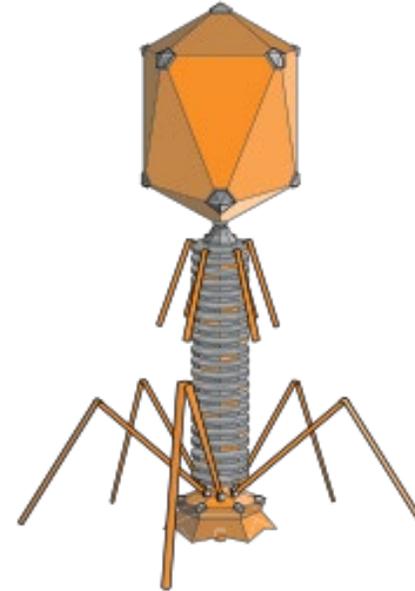
박테리아를 선택적으로 감염시키고 **용균**하는 바이러스

**항생제 다제내성균**에도 활성을 가짐

**매우 높은 숙주 특이성**

# 간략한 역사 (1)

- Phage는 세균이 존재하는 모든 곳에 존재 (지구상에  $>10^{31}$  추정)
- Frederick Twort (1915) 와 Felix d'Herelle (1917) 에 의해 발견. d'Herelle이 phage therapy 개념 제안. 1919년 처음으로 임상 적용 시도



# 간략한 역사 (2)

- **George Eliava (1892-1937)** 는 **d'Herelle** 과의 공동 연구를 통해 **phage therapy** 를 정착시키는데 기여
- **1923년 Tbilisi, Georgia** 에 연구소를 설립 후 **phage therapy** 를 지속적으로 도모
- 해당 연구소는 **1988년**에 **George Eliava Institute**로 개명



# Eliava Institute

## Bacteriophage 연구소

Eliava Phage Therapy Center 에서  
personalized phage therapy 를 환자들에  
게 제공

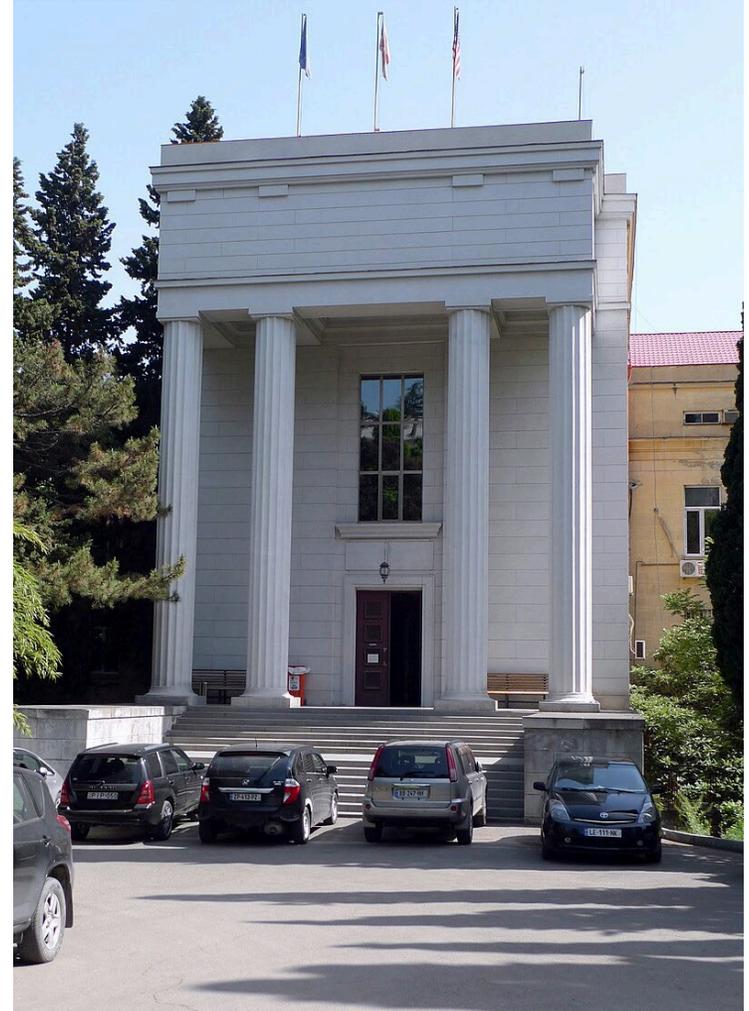
### Treatment Options

#### Local Treatment Course

The main course the EPTC offers is the local treatment course. It involves coming to our clinic in Tbilisi, Georgia, and receiving treatment locally, including outpatient surgical and non-surgical consultations, procedures, examinations, investigations, etc.

#### Distance Treatment Course

Distance treatment is for patients who could potentially benefit from phage therapy but are unable to travel due to various reasons. The clinic can send packages of phages to multiple countries all over the globe and provide necessary recommendations suitable for the patient's treatment.



# Phage therapy의 장점

다제내성균  
효능

높은 숙주  
특이성

자가 증식능

정상 균총에  
미미한 영향

부작용 거의  
없음

Biofilm  
침투 가능

# 단점

표적균을 사전에  
알아야만 함

Lytic phage만  
사용 가능

최적 용량/용법  
모름

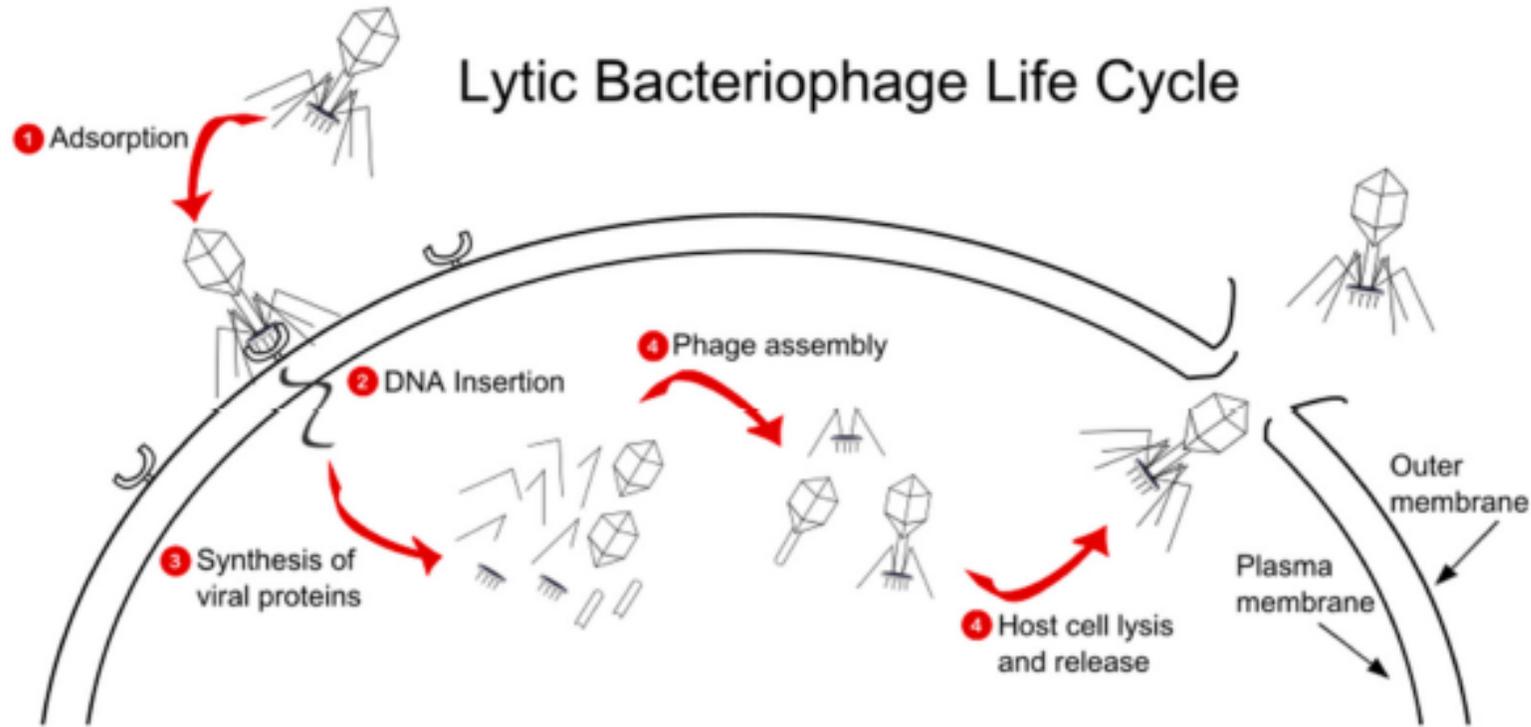
DNA를 host  
사이에서 전달할  
위험

FDA/EMA 승인  
사례 무



# M&S OF PHAGE THERAPY

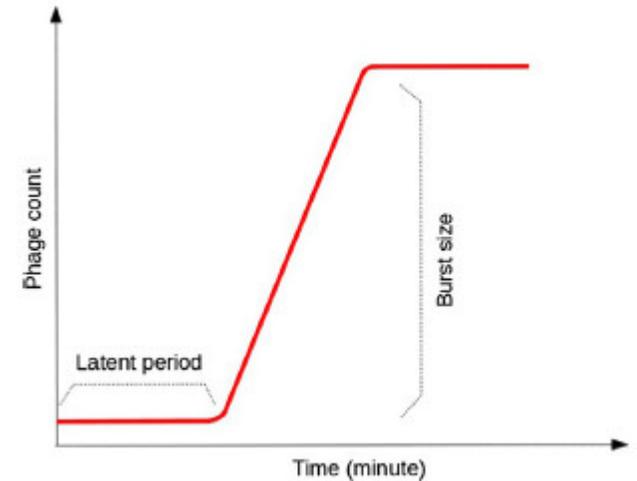
# Lytic phage의 생활사



Phage therapy에서는 **strictly lytic** phages (lysogenic이 아닌)만 사용 가능

# 핵심 phage 효능 파라미터

- **Phage adsorption rate (mL/min)**
  - Number of phage binding sites per bacteria
  - Diffusion rate constant of the phage
  - Binding affinity
- **Latent period (min), burst size (PFU)**
  - Latent period: 파지 감염 이후 방출까지 걸리는 시간
  - Burst size: 용균과 함께 유리되는 파지의 승수
  - 통상적으로 latent period와 burst size는 양의 상관 관계를 보이며 latent period 는 숙주균의 성장 속도에 반비례, burst size 는 비례



# 주요 역치

- Proliferation threshold

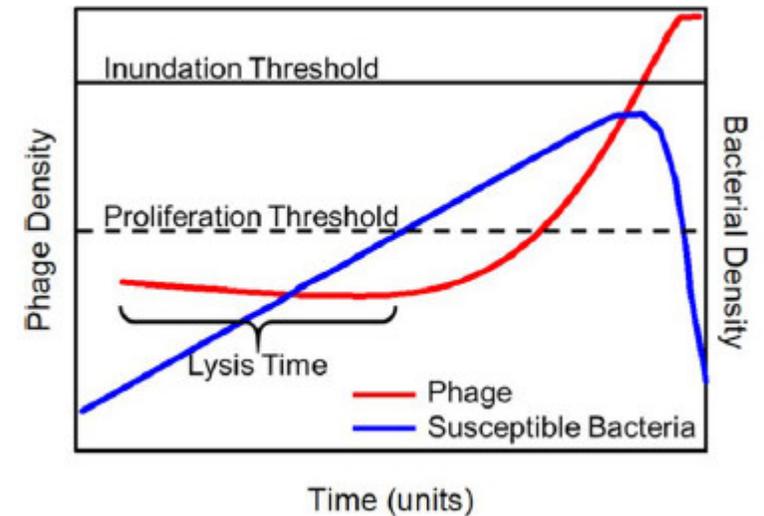
Phage가 증식하기 위한 균의 최소 농도

- Inundation threshold

균 농도의 net 감소를 일으키기 위한 phage의 최소 농도

- Multiplicity of infection (MOI)

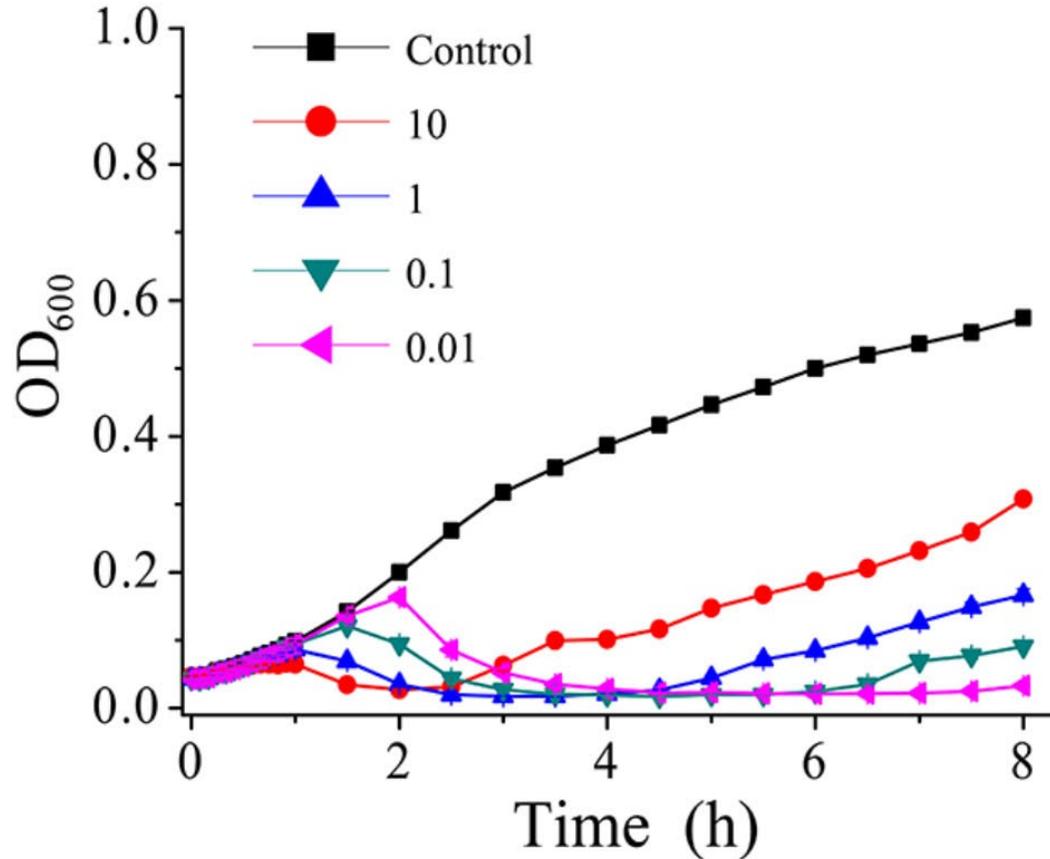
Phage:Bacteria의 비율. 높을 수록 균의 성공적인 감염 확률 증가.



# 파지 치료 전략의 2가지 형태

- 수동 치료 (Passive therapy)
  - 파지를 inundation threshold (IT) 이상의 고용량으로 투여하는 전략
  - 파지 증식 없이도 균 감소가 일어남 (이미 파지 농도가 IT 이상이므로)
- 능동 치료 (Active therapy)
  - 파지를 IT 이하의 저용량으로 투여하되 파지의 증식에 의해 파지 농도가 IT에 도달하는 것을 겨냥한 전략
  - 해당 전략이 유효하기 위해서는 균의 농도가 proliferation threshold (PT) 이상으로 존재해야만 함

# 용량 증가의 역설

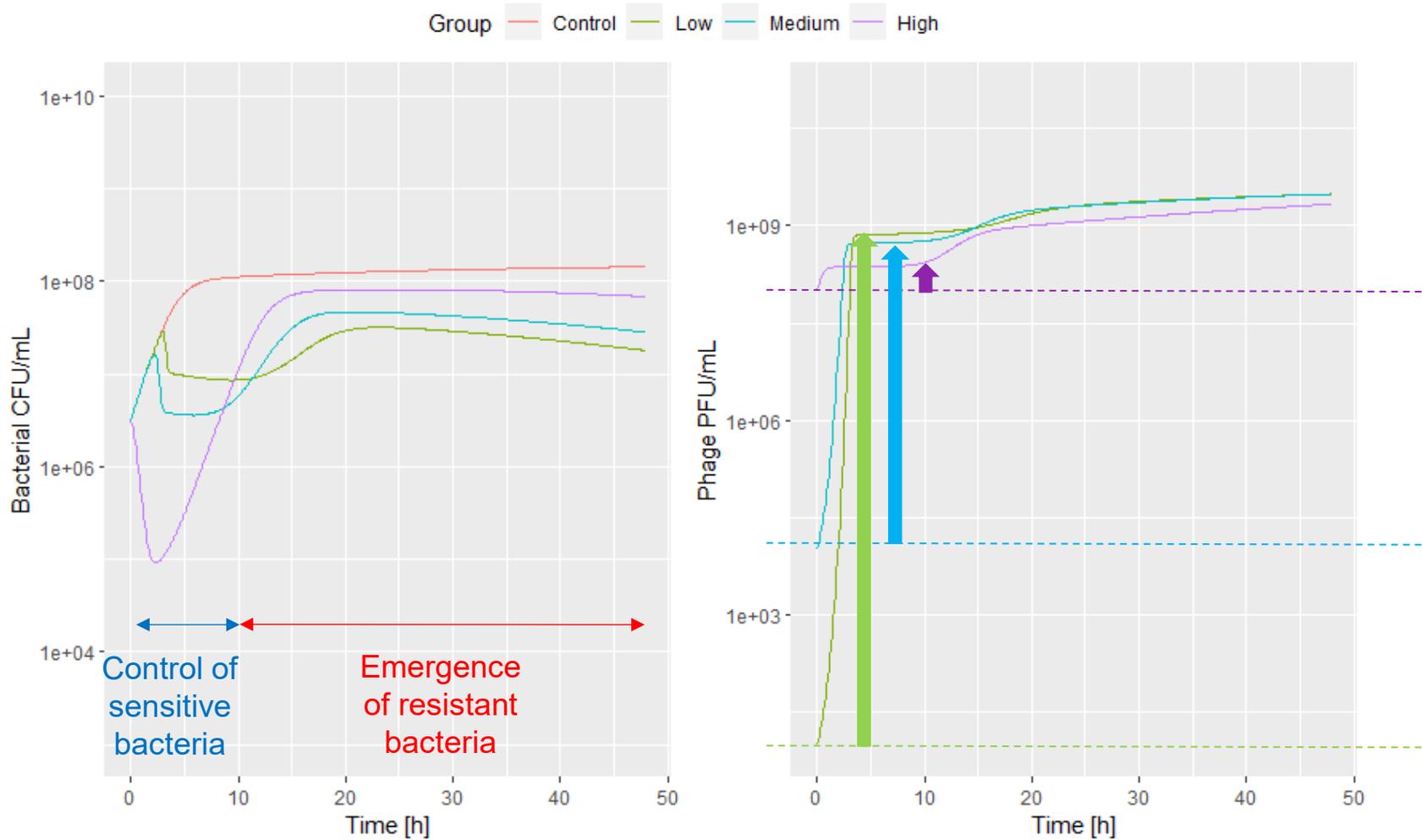


Chen et al., Front. Microbiol., 2018

- 용량(=MOI)가 증가할 수록 초기 균감 소능은 증진
- 내성균이 등장하는 시점은 빨라짐

# Typical *in vitro* time kill profile

파지 투여 직후  
균 감소가  
나타나지만 이내  
파지 내성균이  
성장하게 됨



파지 용량이  
증가할 수록  
초기 농도는  
높아지지만 파지  
증식은 억제되며  
이는 뒤이어  
나타나는  
내성균의 성장을  
역설적으로  
촉진함



# 초창기 발전

- 수학자들은 오래 전부터 phage-host 상호작용을 모델링하여 옴
- 초창기에는 주로 이론적인 측면에서 연구가 이루어짐

Campbell (1961)

$$\begin{aligned}\dot{S}(t) &= \alpha S(t) \left[ 1 - \left( \frac{S(t)}{C} \right) \right] - kS(t)P(t) - aS(t) \\ \dot{P}(t) &= bkS(t-T)P(t-T) - kS(t)P(t) - \mu_p P(t) - aP(t)\end{aligned}$$

Bremermann (1983)

$$\begin{aligned}\dot{S}(t) &= \alpha S(t) \left[ 1 - \left( \frac{S(t)}{C} \right) \right] - kS(t)P(t) \\ \dot{I}(t) &= kS(t)P(t) - \mu_i I(t) \\ \dot{P}(t) &= b\mu_i I(t) - \mu_p P(t),\end{aligned}$$

Beretta and Kuang (1998)

$$\begin{aligned}\dot{S} &= \alpha S \left[ 1 - \left( \frac{N}{C} \right) \right] - kSP \\ \dot{I} &= kSP - \mu_i I \\ \dot{P} &= -kSP - \mu_p P + b\mu_i I,\end{aligned}$$

Payne and Jansen (2000)

$$\begin{aligned}\dot{S} &= \alpha S - kSP - H(t)S \\ \dot{I} &= \alpha I + kSP - \mu_i I - H(t)I \\ \dot{P} &= b\mu_i I - kSP - \mu_p P - h(t)P.\end{aligned}$$

# 실험실 자료 기반 모델링 연구

OPEN ACCESS Freely available online

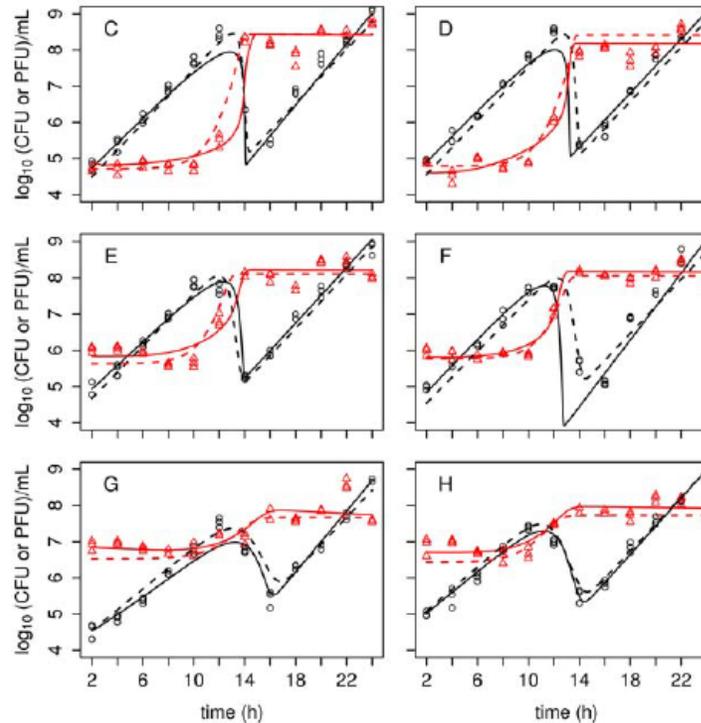
PLoS PATHOGENS

## Quantitative Models of *In Vitro* Bacteriophage–Host Dynamics and Their Application to Phage Therapy

Benjamin J. Cairns<sup>1</sup>, Andrew R. Timms<sup>2</sup>, Vincent A. A. Jansen<sup>3</sup>, Ian F. Connerton<sup>2</sup>, Robert J. H. Payne<sup>1\*</sup>

<sup>1</sup> School of Biological Sciences, University of Bristol, Bristol, United Kingdom, <sup>2</sup> Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom, <sup>3</sup> School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, United Kingdom

$$\begin{aligned}\frac{dS}{dt} &= aS - fS - bSV, \\ \frac{dR}{dt} &= aR + fS, \\ \frac{dI}{dt} &= bSV - bS(t-K)V(t-K), \\ \frac{dV}{dt} &= hbS(t-K)V(t-K) - bSV - mV.\end{aligned}$$



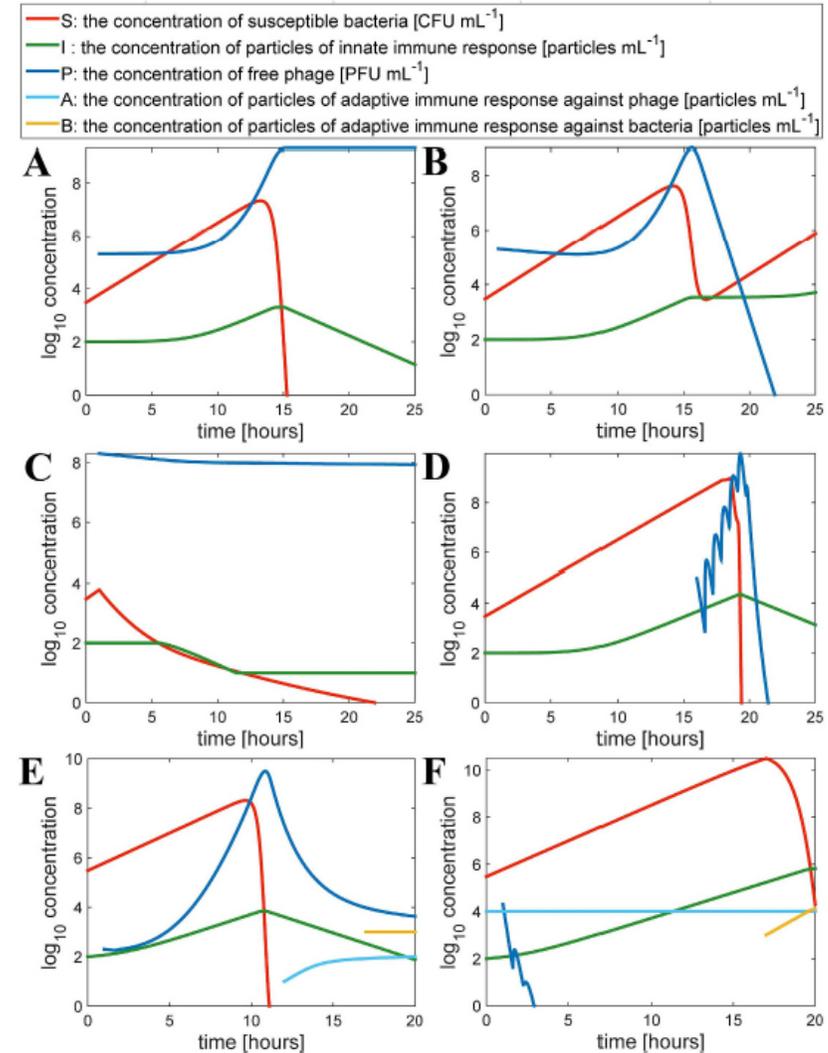
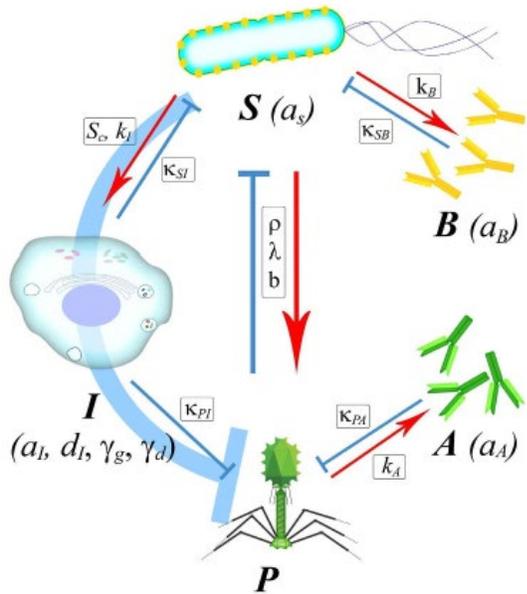
# In vivo phage PKPD에 대한 연구

SCIENTIFIC REPORTS

OPEN **Mammalian Host-Versus-Phage immune response determines phage fate *in vivo***

Received: 27 May 2015  
Accepted: 09 September 2015  
Published: 06 October 2015

Katarzyna Hodyra-Stefaniak<sup>1</sup>, Paulina Miernikiewicz<sup>2</sup>, Jarosław Drapała<sup>2</sup>, Marek Drab<sup>3</sup>, Ewa Jorńczyk-Matysiak<sup>4</sup>, Dorota Lecion<sup>2</sup>, Zuzanna Kaźmierczak<sup>4</sup>, Weronika Beta<sup>4</sup>, Joanna Majewska<sup>2</sup>, Marek Harhala<sup>2</sup>, Barbara Bubak<sup>2</sup>, Anna Kłopot<sup>2</sup>, Andrzej Górski<sup>2</sup> & Krystyna Dąbrowska<sup>4</sup>



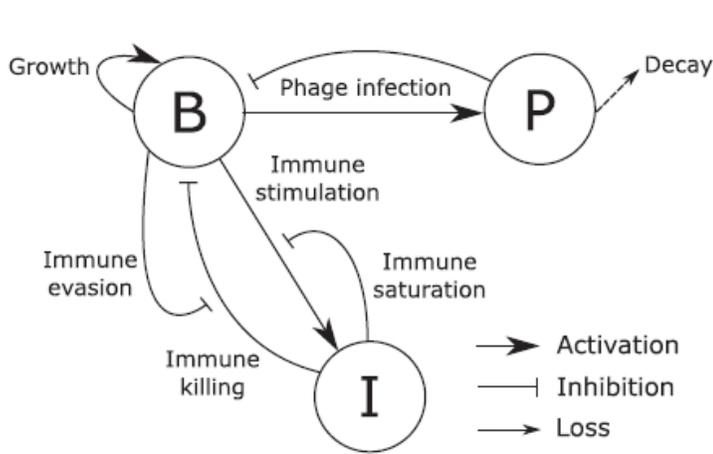
# Immune-phage synergy 고찰



## Modeling the synergistic elimination of bacteria by phage and the innate immune system

Chung Yin (Joey) Leung<sup>a,b,\*</sup>, Joshua S. Weitz<sup>a,b,\*</sup>

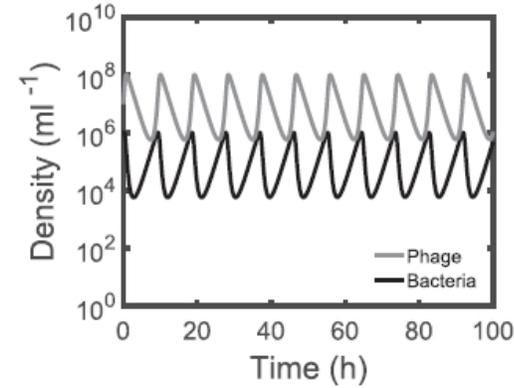
<sup>a</sup>School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332, USA  
<sup>b</sup>School of Physics, Georgia Institute of Technology, Atlanta, Georgia 30332, USA



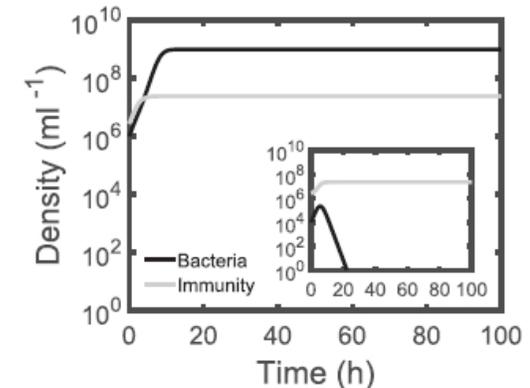
$$\dot{B} = \underbrace{rB\left(1 - \frac{B}{K_C}\right)}_{\text{Growth}} - \underbrace{\phi BP}_{\text{lysis}} - \underbrace{\epsilon IB / \left(1 + \frac{B}{K_D}\right)}_{\substack{\text{Innate immune killing} \\ \text{Immune evasion}}}$$

$$\dot{P} = \underbrace{\beta \phi BP}_{\text{Replication}} - \underbrace{\omega P}_{\text{Decay}}$$

$$\dot{I} = \underbrace{\alpha I \left(1 - \frac{I}{K_I}\right)}_{\text{Innate immune stimulation}} \frac{B}{B + K_N}$$



(a)

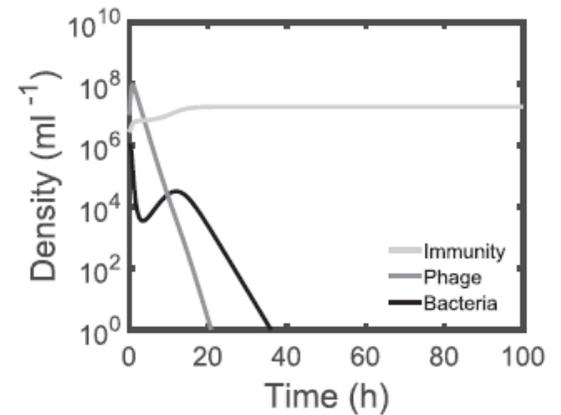


(b)

(1)

(2)

(3)



(c)



# Immune-phage synergy 실험 + 모델링

Cell Host & Microbe  
Article

## Synergy between the Host Immune System and Bacteriophage Is Essential for Successful Phage Therapy against an Acute Respiratory Pathogen

Dwayne R. Roach,<sup>1,6</sup> Chung Yin Leung,<sup>2,3,6</sup> Marine Henry,<sup>1</sup> Eric Morello,<sup>1</sup> Devika Singh,<sup>2</sup> James P. Di Santo,<sup>4,5</sup> Joshua S. Weitz,<sup>2,3,\*</sup> and Laurent Debarbieux<sup>1,7,\*</sup>

<sup>1</sup>Department of Microbiology, Institut Pasteur, Paris 75015, France

<sup>2</sup>School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

<sup>3</sup>School of Physics, Georgia Institute of Technology, Atlanta, GA 30332, USA

<sup>4</sup>Innate Immunity Unit, Department of Immunology, Institut Pasteur, Paris 75015, France

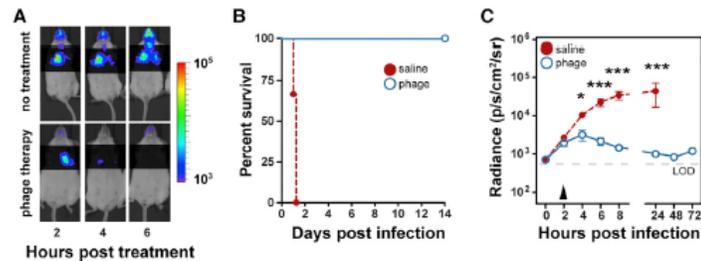
<sup>5</sup>Inserm U1223, Paris 75015, France

<sup>6</sup>These authors contributed equally

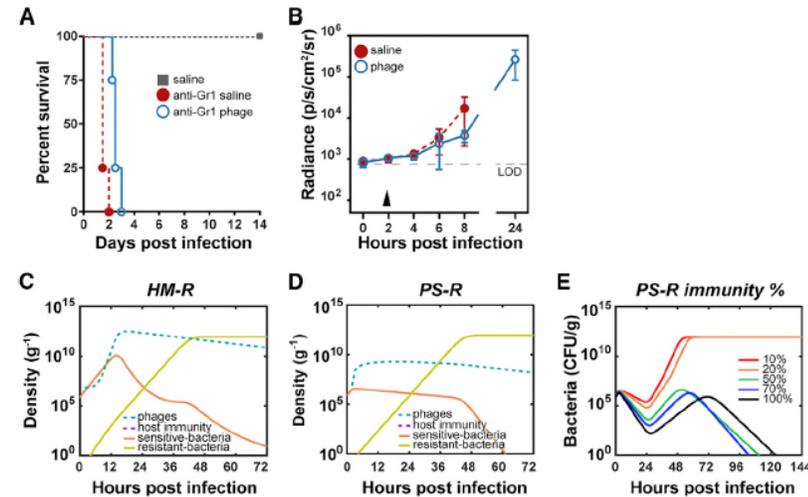
<sup>7</sup>Lead Contact

\*Correspondence: jsweitz@gatech.edu (J.S.W.), laurent.debarbieux@pasteur.fr (L.D.)

<http://dx.doi.org/10.1016/j.chom.2017.06.018>



as mean radiance over time indicating phage antibacterial activity by a significant reduction in bacterial burden beyond 2 hr post-treatment. Arrow marks treatment point; *in vivo* radiance limit of detection (LOD); error bars indicate SEM ( $^*p < 0.05$ ;  $^{***}p < 0.001$ ).



**Figure 5. Phage Therapy Is Ineffective in the Neutropenic Host**

(A) Anti-granulocyte receptor-1 (Gr1) monoclonal antibody was used to deplete neutrophils in WT mice 24 hr before an intranasal inoculum of *Pseudomonas aeruginosa* ( $10^5$  CFU;  $n = 4$  per group). After a 2 hr delay, these neutropenic mice received a single-dose inhaled monophage therapy (MOI of 10), which was ineffective in preventing fatal pneumonia. Arrow marks treatment point; *in vivo* radiance limit of detection (LOD).

(B) Colonization pattern of the bioluminescent pathogen in the lungs of live mice plotted as mean radiance ( $\text{p/s}^2/\text{cm}^2/\text{s/r}$ ) over time showed no indication of phage antibacterial activity.

(C and D) Time series of bacteria, phage, and immune cell populations from (C) heterogeneous mixing (HM-R) and (D) phage saturation (PS-R) models, both predicting a decline of sensitive bacteria over time accompanied by dominance of phage-resistant outgrowth causing phage therapy failure.

(E) PS-R model prediction of the level of neutrophil activity relative to healthy hosts

required to eliminate phage-resistant bacterial outgrowth and restore phage therapy efficacy in neutropenic hosts. The curves show the total bacterial population densities. See Table S1 for *in silico* simulation parameters. *In silico* simulations of phage therapy in neutrophil-depleted hosts are modeled by setting host innate immune response to zero.

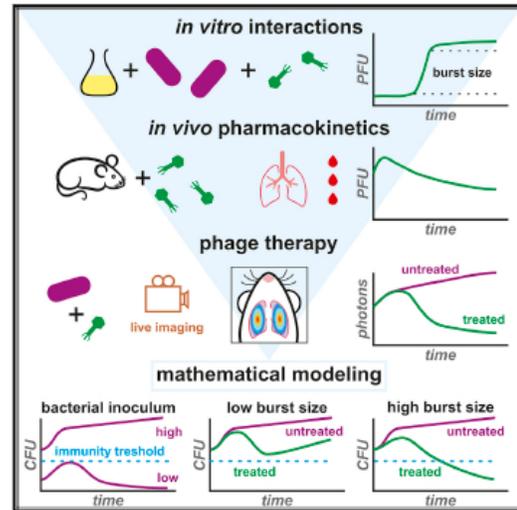


# 신약개발에의 응용

## Cell Reports

### Combination of *in vivo* phage therapy data with *in silico* model highlights key parameters for pneumonia treatment efficacy

#### Graphical abstract



#### Highlights

- Longitudinal records of pulmonary infections quantify the immunity threshold
- *In situ* amplification of phages is required for treatment efficacy
- A mathematical model predicts the outcome of phage-therapy treatment schemes

#### Article

#### Authors

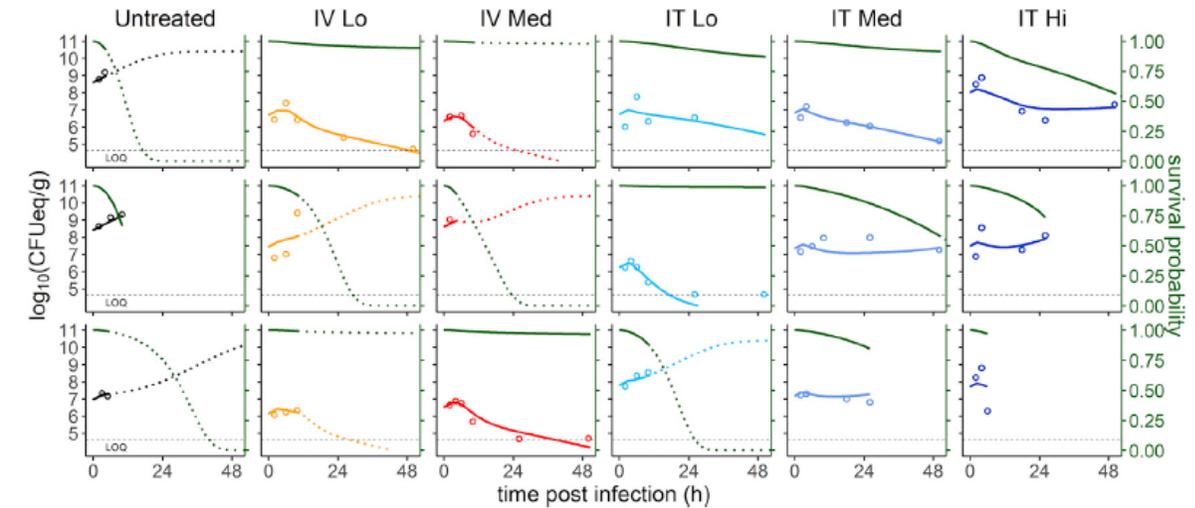
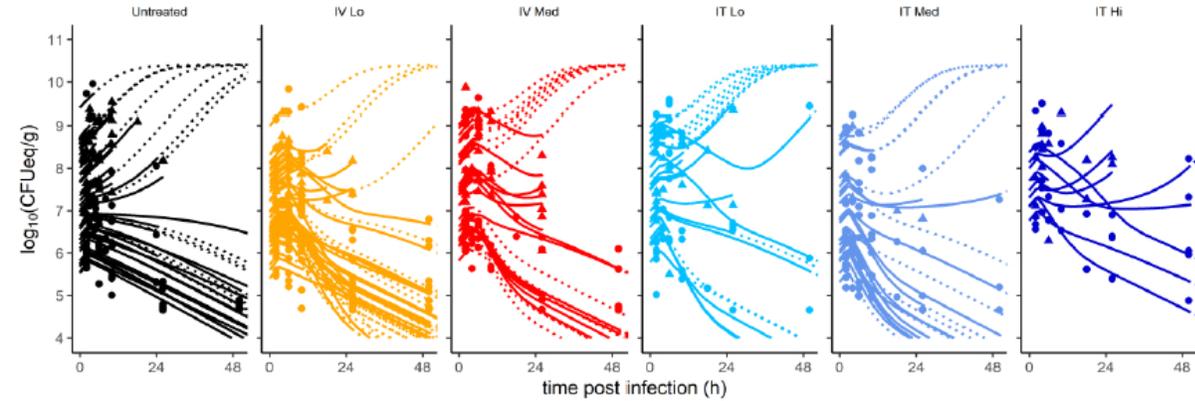
Raphaëlle Delattre, Jérémy Seurat, Feyrouz Haddad, ..., Jean-Damien Ricard, Jérémie Guedj, Laurent Debarbieux

#### Correspondence

jeremie.guedj@insem.fr (J.G.), laurent.debarbieux@pasteur.fr (L.D.)

#### In brief

The clinical development of phage therapy to treat antibiotic-resistant bacterial infections requires understanding the dose-effect relationship. Delattre et al. use a murine pneumonia model to longitudinally record phage-bacteria interactions. Mathematical modeling characterizes the synergy between phages and the host immune response. Predictions of treatment outcome could guide clinicians.





# 연구사례 소개

# 마이크로바이오틱스 (주)

- 설립자/대표이사: 용동은 (연세대학교 의과대학 진단검사의학교실)
- 설립일: **2016년 11월 16일**
- 임직원: **39명 (24년 11월 기준)**
- 연구개발분야: 박테리오파지 치료제 개발

# 경영진

## 01 기업 개요

### 경영진 소개



#### 용동은 대표이사/CEO, CTO

마이크로바이오틱스 대표이사  
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(前) 연세의료원 진단검사의학과 과장  
대한민국 의학한림원 정회원

#### 주요 연구 분야 및 대표 성과

- 항균제 내성학, 임상 미생물학, 진단검사의학 관련 논문 약 430편 출판
- **H-index=61** (Google scholar, 2024.1.25)
- NDM-1 in AAC (**Google scholar citation 3,238회**)  
\*NDM: New Delhi Metallo-beta-lactamase-1. 카바페넴계열 내성유발 유전자

#### 수상 경력

- 2023.05.13 Golden Citations Award
- 2022.07.08 최우수논문상
- 2021.09.27 대한진단검사의학회 최우수 논문상
- 2021.04.25 KMA 2021년 제 28회 의당학술상



#### 채동우 상무이사

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■ 연세대학교 의과대학 학사/석사/박사



#### 박현희 상무이사

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**온코빅스 임상기획 이사, 한독 임상팀, SK바이오사이언스 임상팀**  
■ 고려대학교 생명과학대학 학사/석사, 통계학 박사



#### 신충렬 이사

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**한미약품 바이오제조개발 그룹장**, 대응제약, 이수애플리스  
■ 고려대학교 생명공학 생물법제학 석사/박사

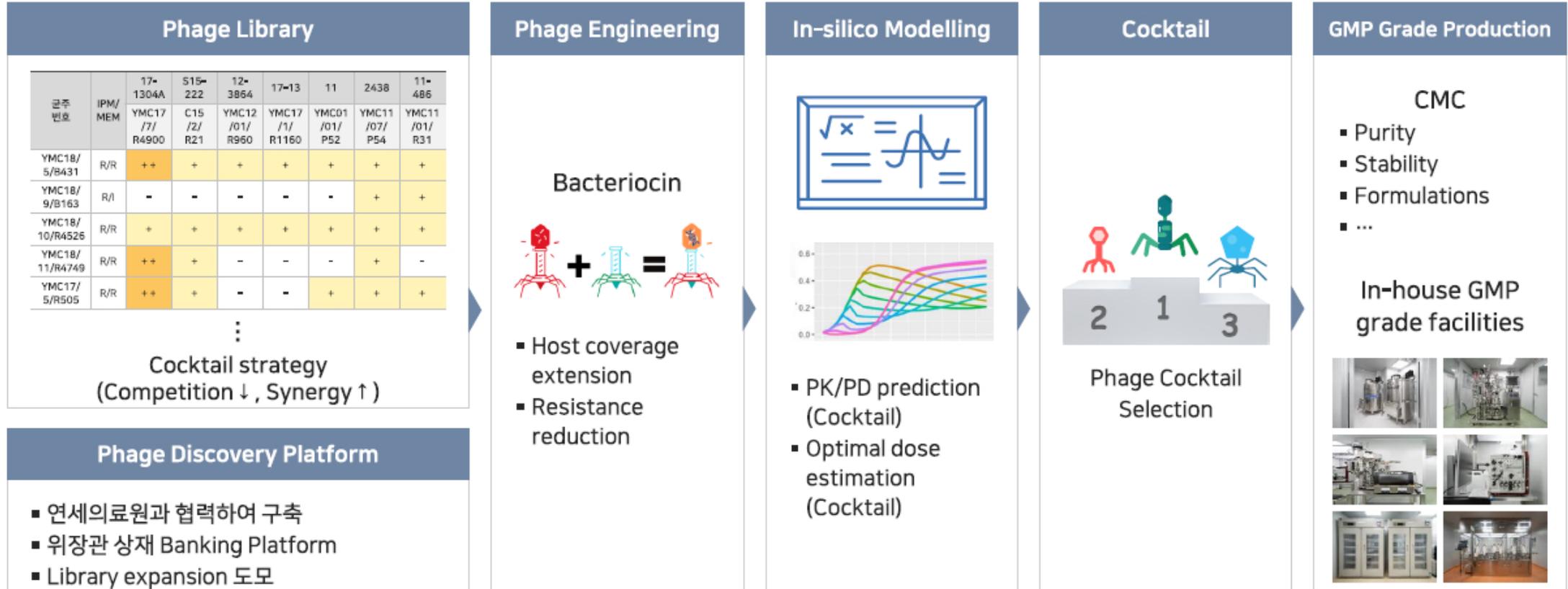


#### 이치영 상무이사/CFO

마이크로바이오틱스 상무이사  
**한국투자증권 IPO (기업금융부), KDB인베스트먼트 이사**  
PwC삼일회계법인 Deal본부, EY한영회계법인 감사본부  
■ 연세대학교 경영학과 학사, **한국공인회계사**, CFA Charterholder



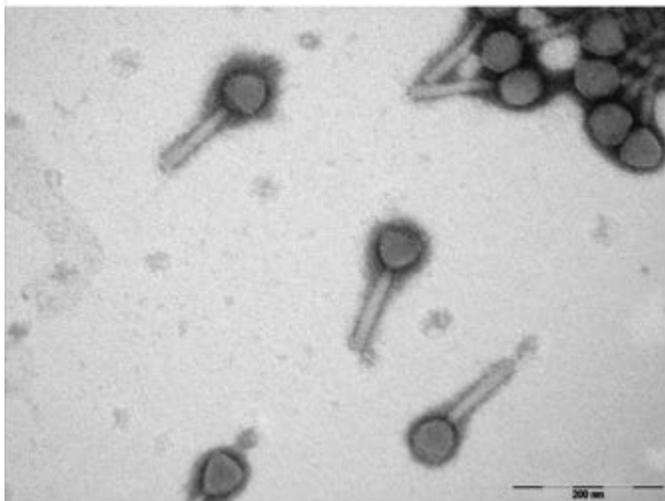
# 회사의 핵심 기술



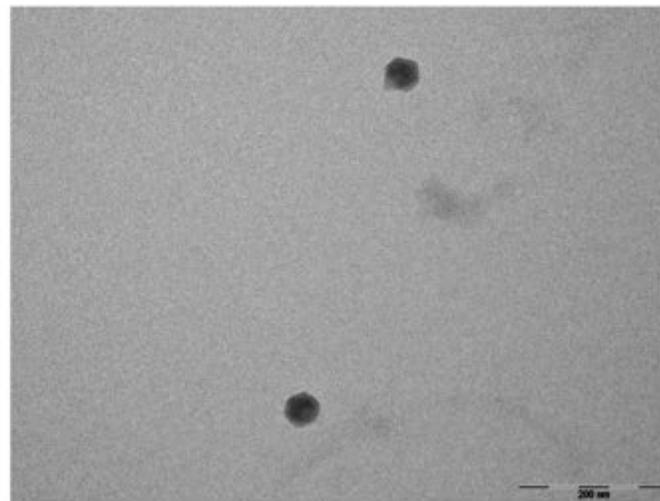
# 초기 개발 후보

- **Electron Microscopy**

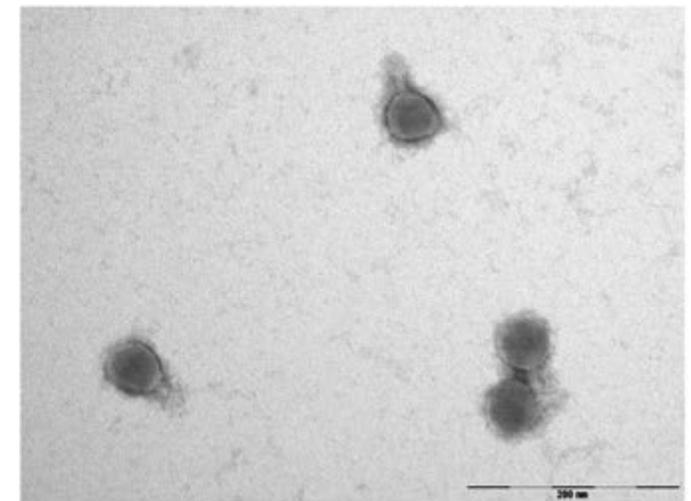
MXP1001



MXP1002

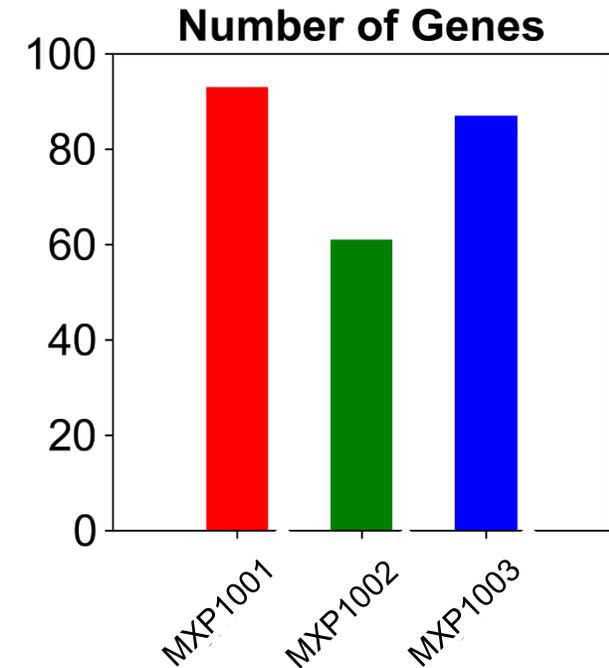
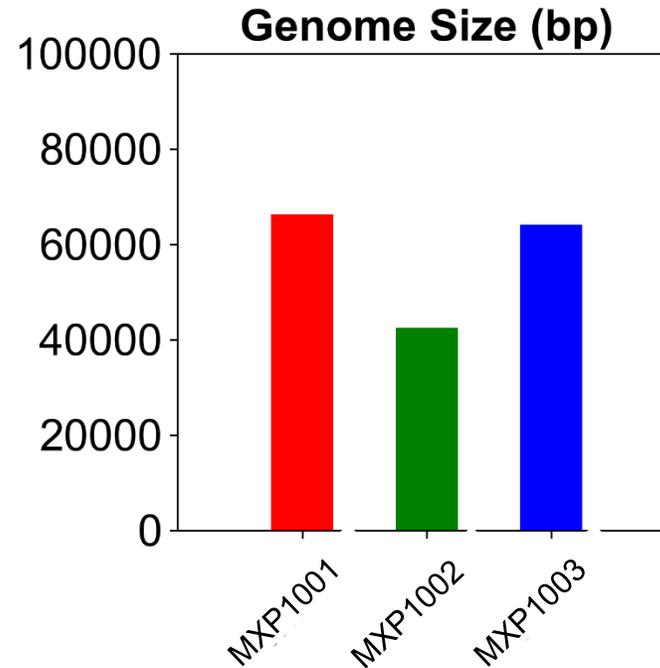
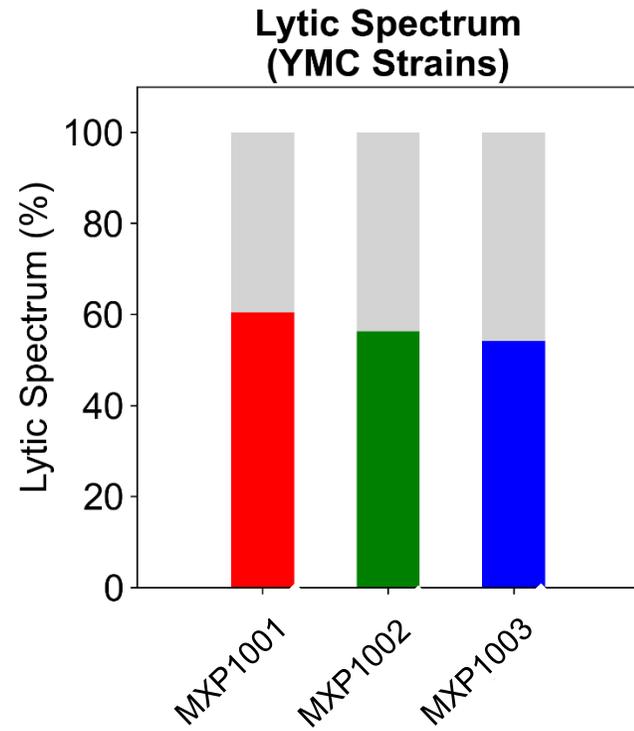


MXP1003



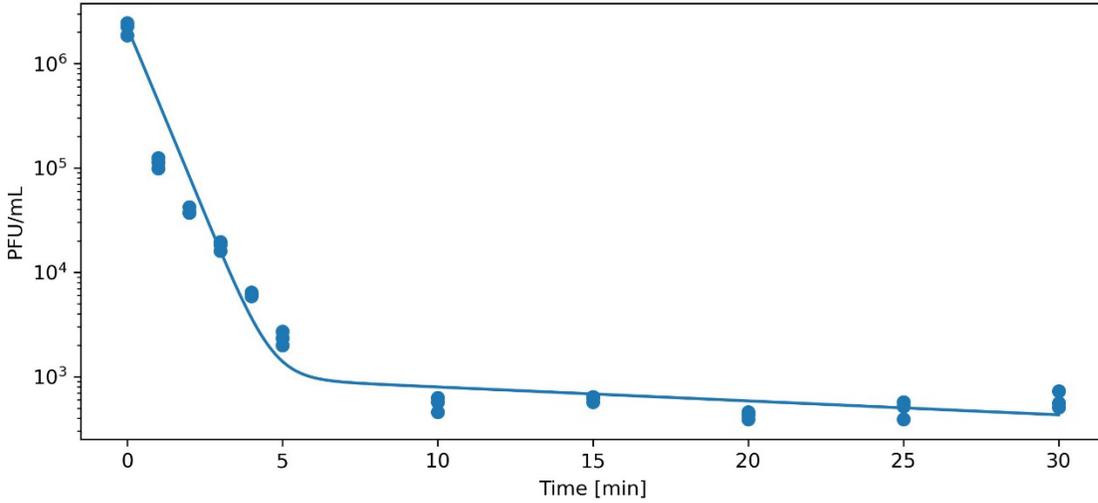
# Bacteriophage Characterization

- Lytic Spectrum

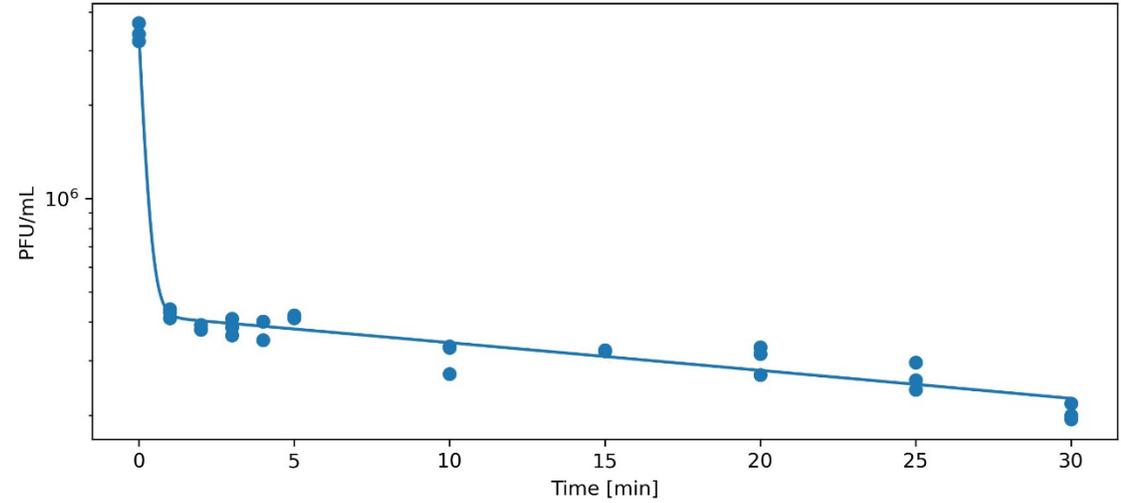


# Adsorption test

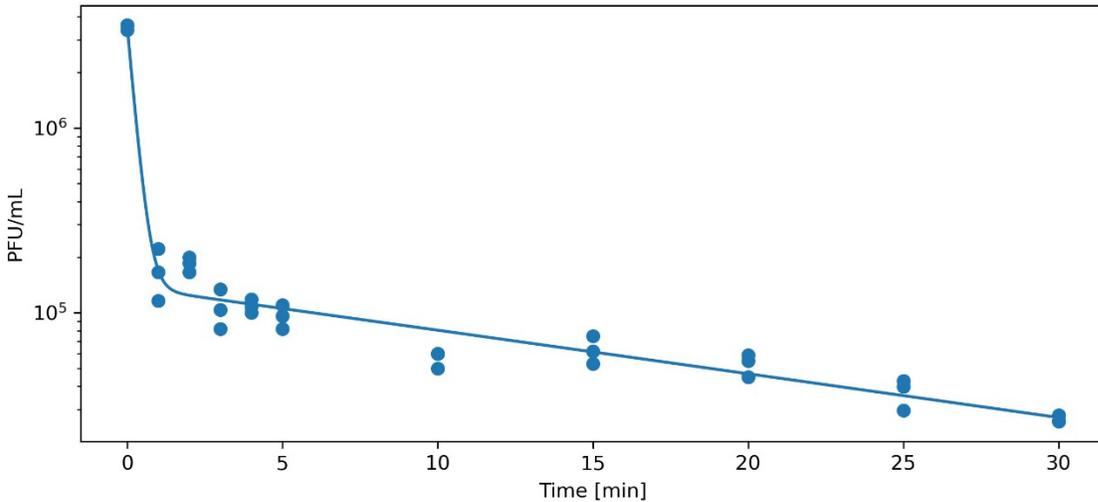
MXP1001



MXP1002



MXP1003



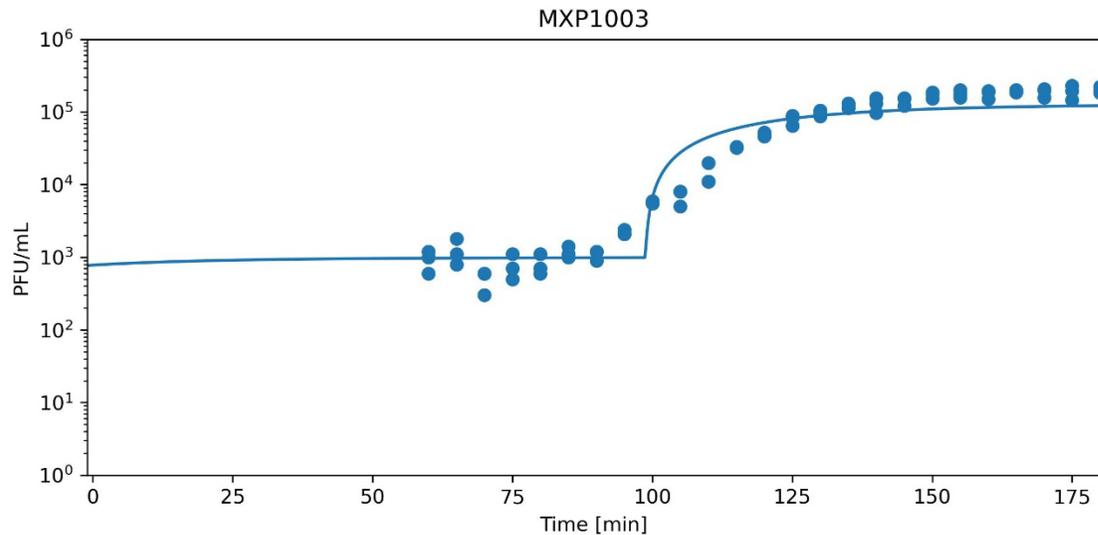
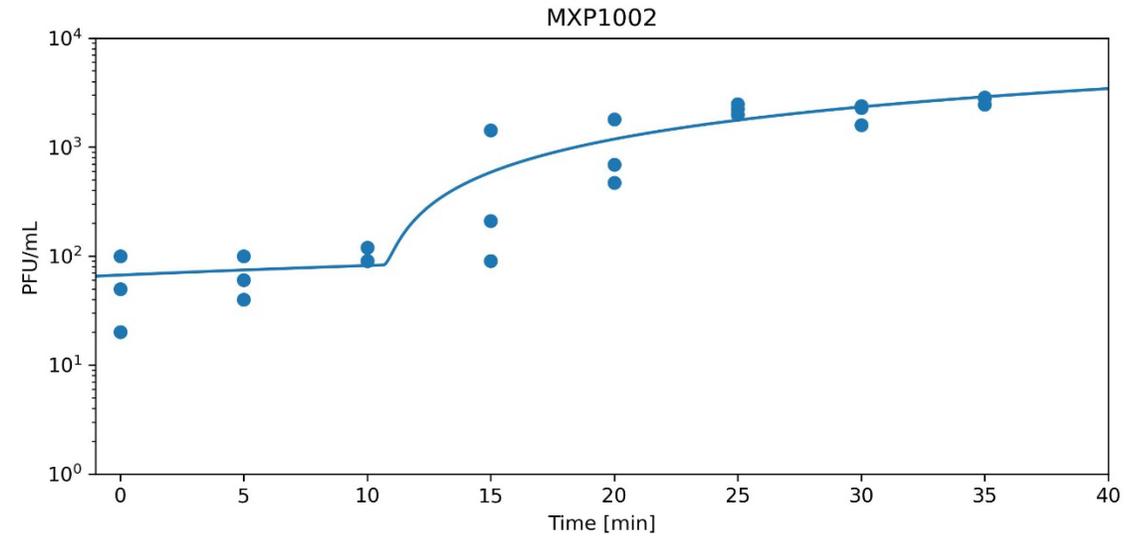
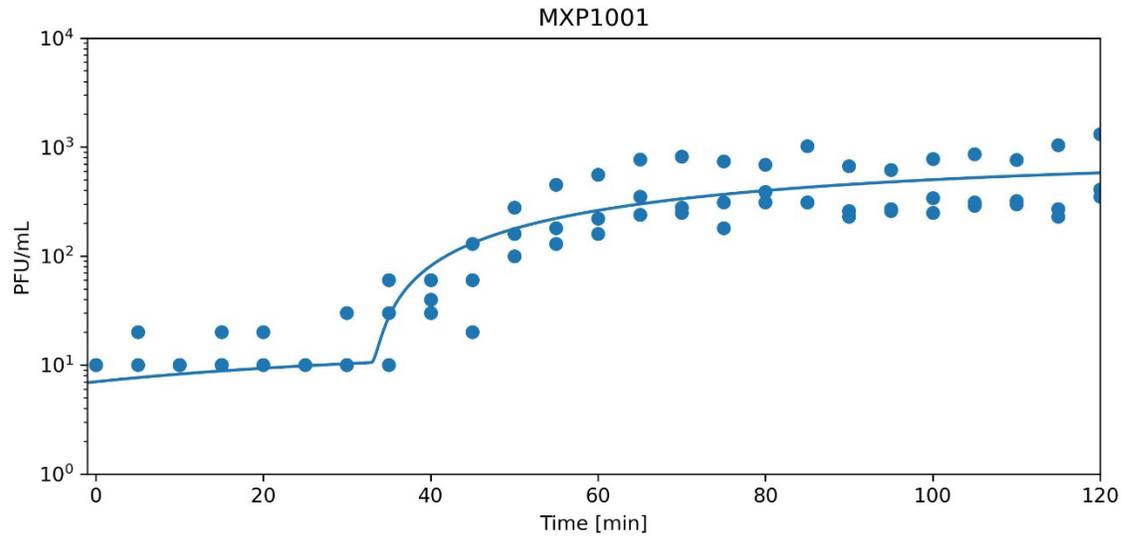
Biphasic decline 패턴

Standard two stage model 로 적합

Free phage + Bacteria ↔ Bound phage  
Bound phage → Infected bacteria



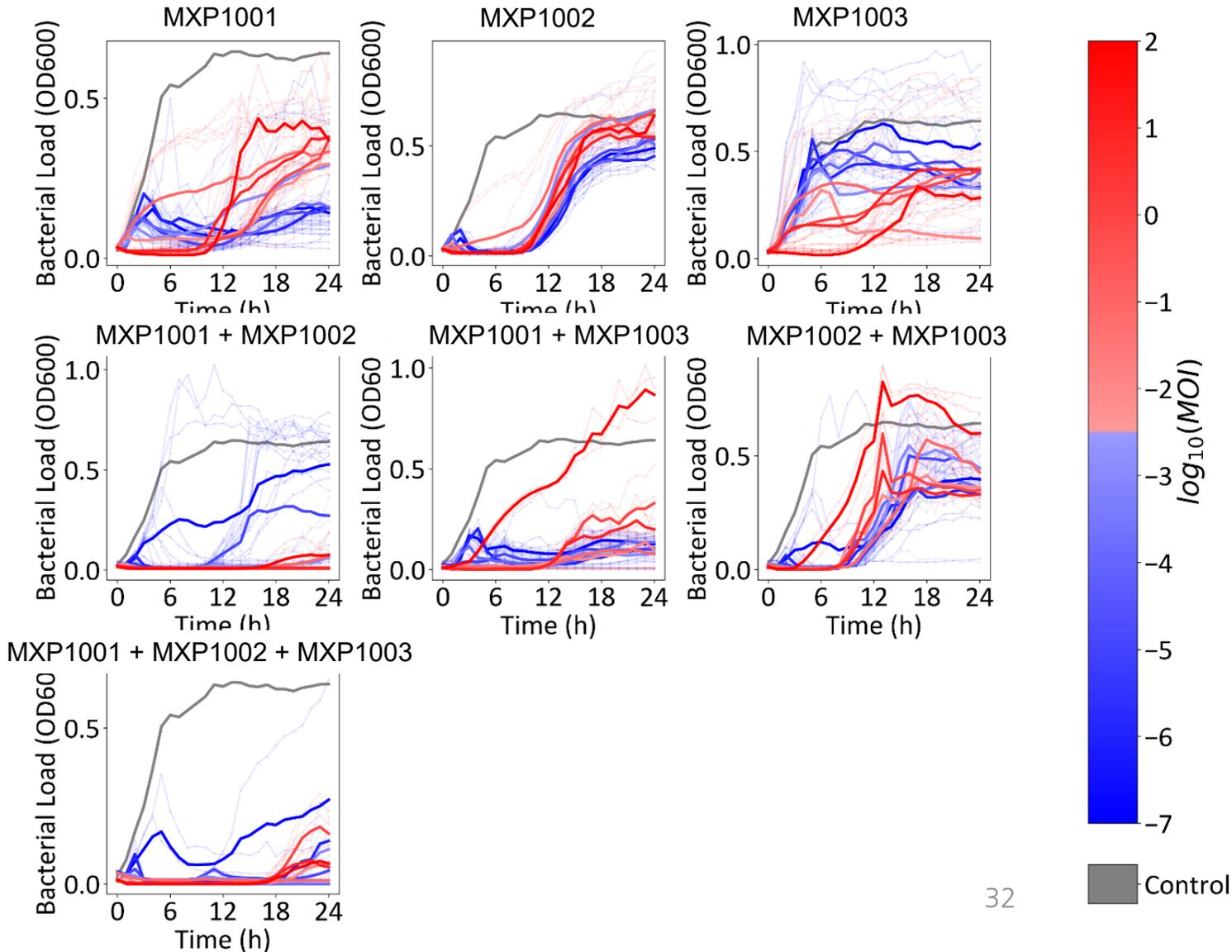
# One step growth curve



Infected bacteria 는 특정한 latent period 이후 phage 를 방출

Discrete time delay 를 도입한 모델을 통해 latent period 와 burst size 를 추정

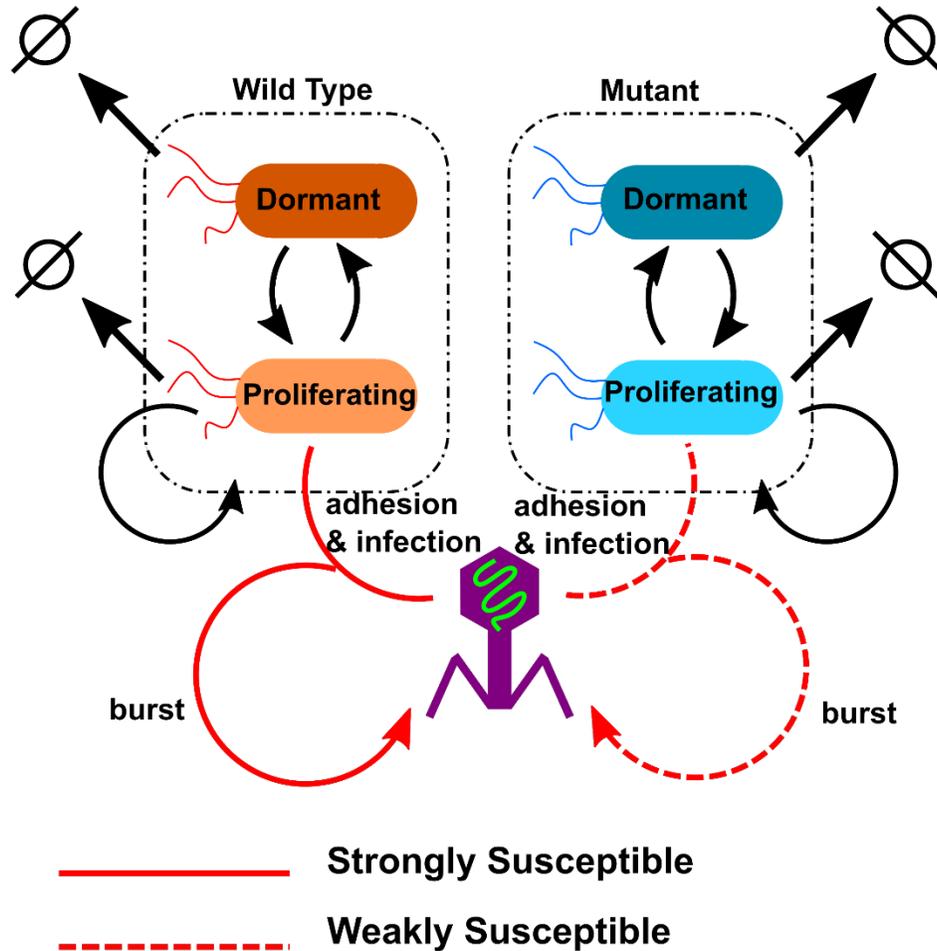
# In Vitro Mono- and Cocktail Treatments



- **MOI (=용량)이 증가함에 따라 초기 균감소 증가**

- **10시간 이후 내성균** 성장은 도리어 촉진되는 현상 관찰

# In vitro phage dynamics 모델



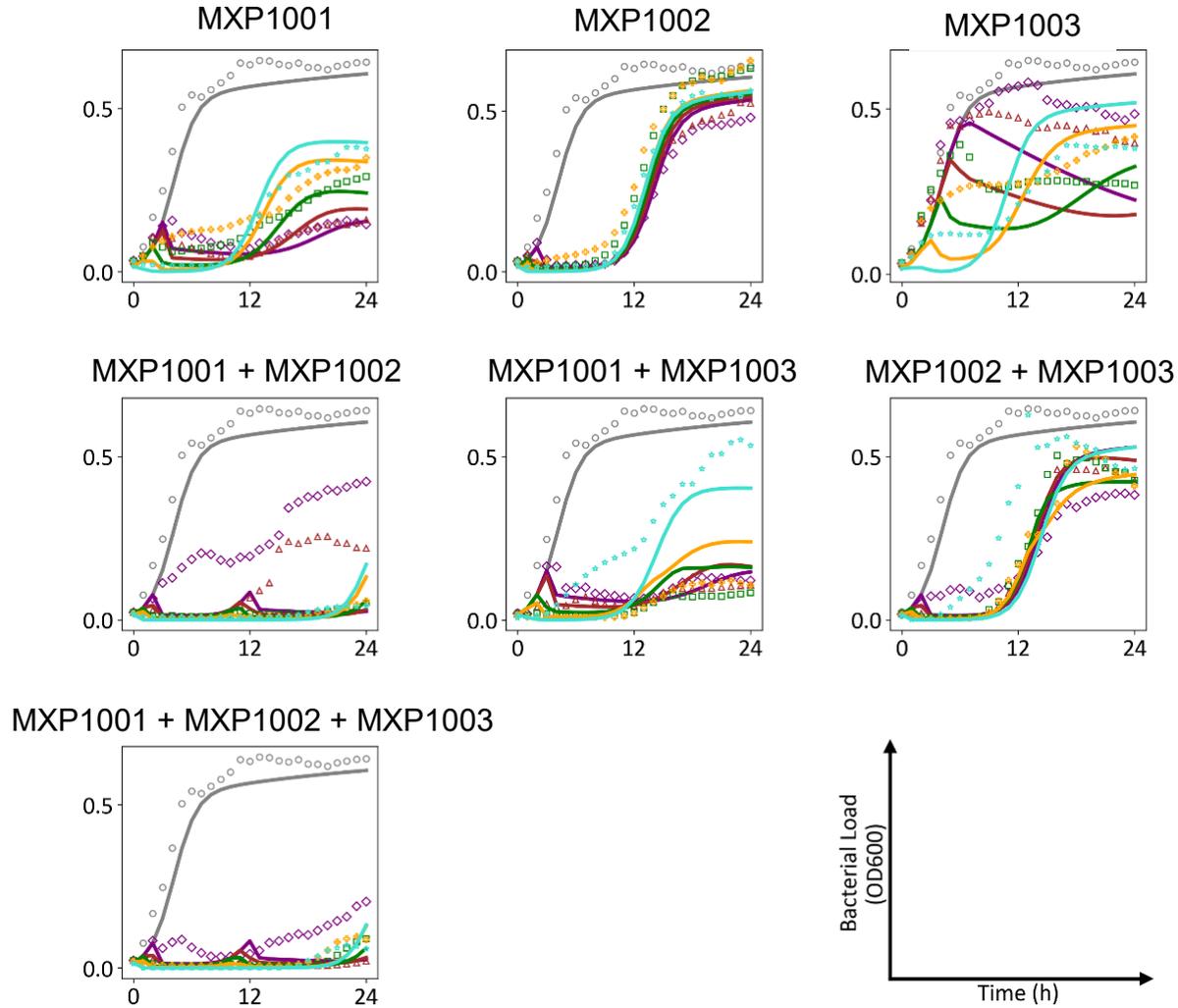
Mutant 내성균은 파지 투여 전부터 적은 빈도로 존재한다고 가정

모델의 데이터 접합을 통해 initial mutant fraction 을 추정

치료 중에도 mutation 이 발생할 수 있으나 24시간 time frame 이내에서 그 정도가 미미할 것으로 가정

3개 phage 에 대한 모든 가능한 subpopulation(예. WWW, WWM, WMW, WMM, ...; W: 감수성, M: 내성)의 initial fraction 추정

# 모델 예측과 관측치의 접합도



(Typical) population prediction

Monolix를 통해 모델 접합

Random effect : Inter-plate variability



# 표적군 내성빈도 추정

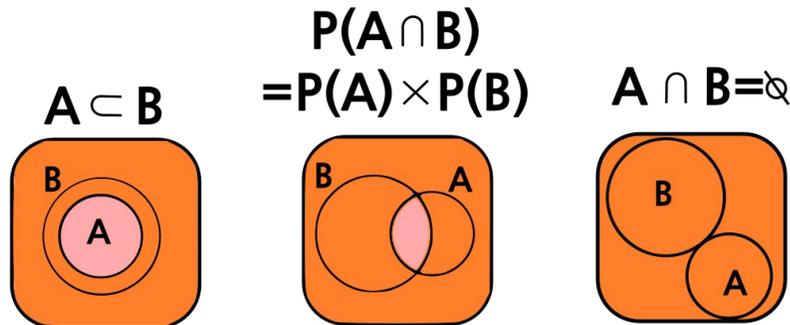
## • Subpopulation Structure

Parameter	Unit	Estimate (RSE (%))
Subpopulation Structure		
$\phi_{WWW}$	W: Wild type	0 fixed
$\phi_{WWM}$	M: Mutant	-4.9 (3.21)
$\phi_{WMW}$	Fraction = $\frac{\phi_{xyz}}{\sum_{i,j,k \in \{W,M\}} \phi_{ijk}}$	-7.81 (2.03)
$\phi_{WMM}$	The digits x, y, and z in the subscript of $\phi_{xyz}$ correspond to	-7.98 (2.4)
$\phi_{MWW}$	MXP1001, MXP1002, respectively	-6.83 (1.59)
$\phi_{MWM}$	MXP1003	-7.31 (2.99)
$\phi_{MMW}$		-17.1 (2.76)
$\phi_{MMM}$		-16.3 (2.77)

# 교차 내성 분석

## • Cross-Resistance

maximum cross-resistance ← → minimum cross-resistance

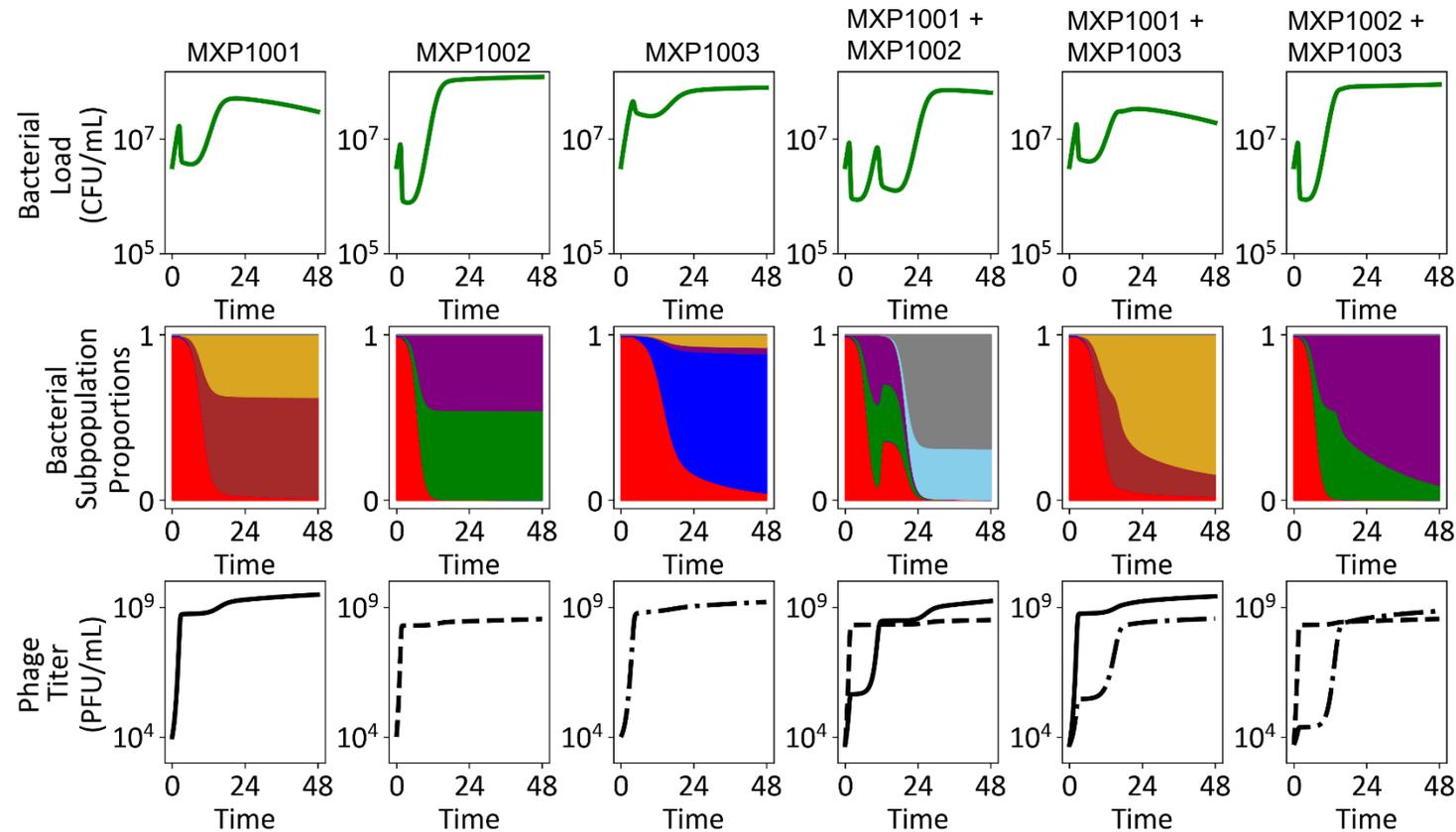


■ : Susceptible   ■ : Resistant

Resistance	Estimated Value	Expected Value Under Independent Mutations
MXP1001	$1.73 \times 10^{-3}$	-
MXP1002	$7.39 \times 10^{-4}$	-
MXP1003	$8.38 \times 10^{-3}$	-
MXP1001 + MXP1002	$1.18 \times 10^{-7}$	$1.28 \times 10^{-6}$ $((1.73 \times 10^{-3}) \times (7.39 \times 10^{-4}))$
MXP1001 + MXP1003	$6.63 \times 10^{-4}$	$1.45 \times 10^{-5}$ $((1.73 \times 10^{-3}) \times (8.38 \times 10^{-3}))$
MXP1002 + MXP1003	$3.38 \times 10^{-4}$	$6.19 \times 10^{-6}$ $((7.39 \times 10^{-4}) \times (8.38 \times 10^{-3}))$
MXP1001 + MXP1002 + MXP1003	$8.14 \times 10^{-8}$	$1.07 \times 10^{-8}$ $((1.73 \times 10^{-3}) \times (7.39 \times 10^{-4}) \times (8.38 \times 10^{-3}))$

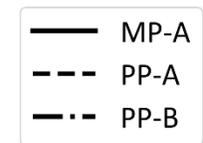
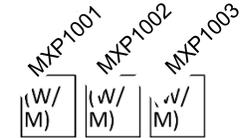
# 시뮬레이션 결과

## • Simulation of Bacterial Selection Dynamics



Coding for Subpopulations:

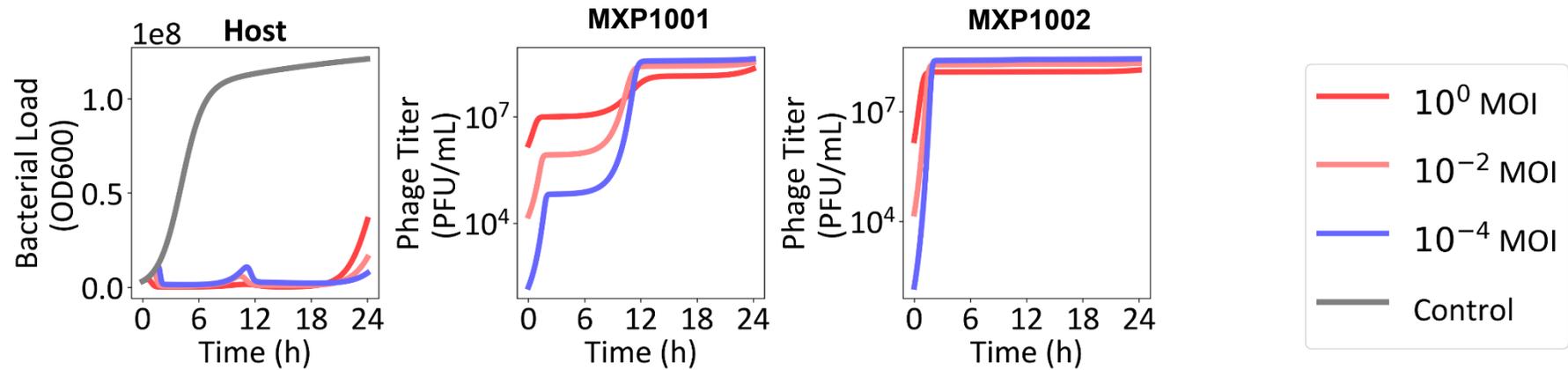
W: Wild Type  
M: Mutant



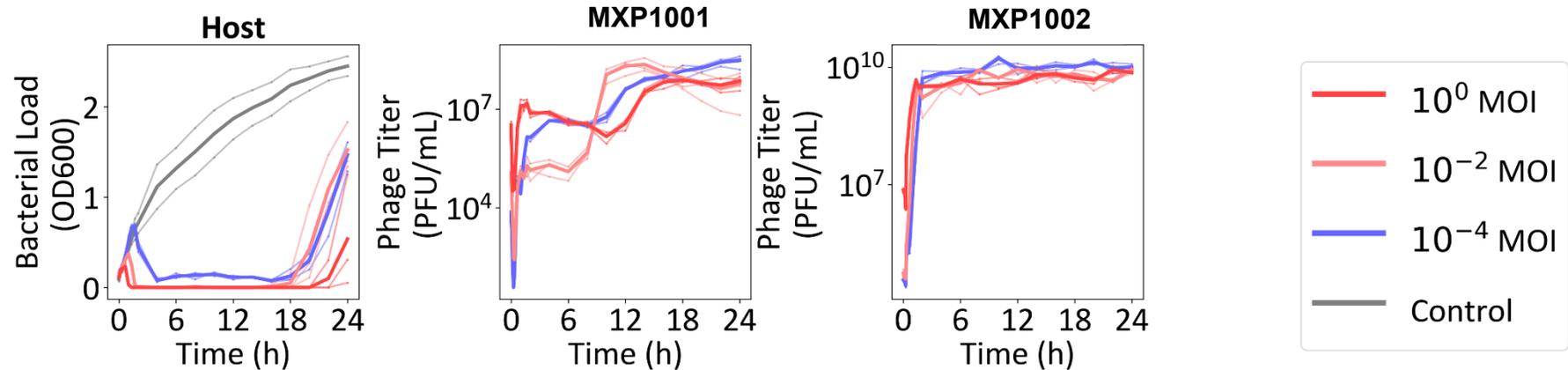
# MXP1001 + MXP1002 in vitro PKPD

## • In Vitro PKPD Experiments with MXP1001 + MXP1002 Cocktail

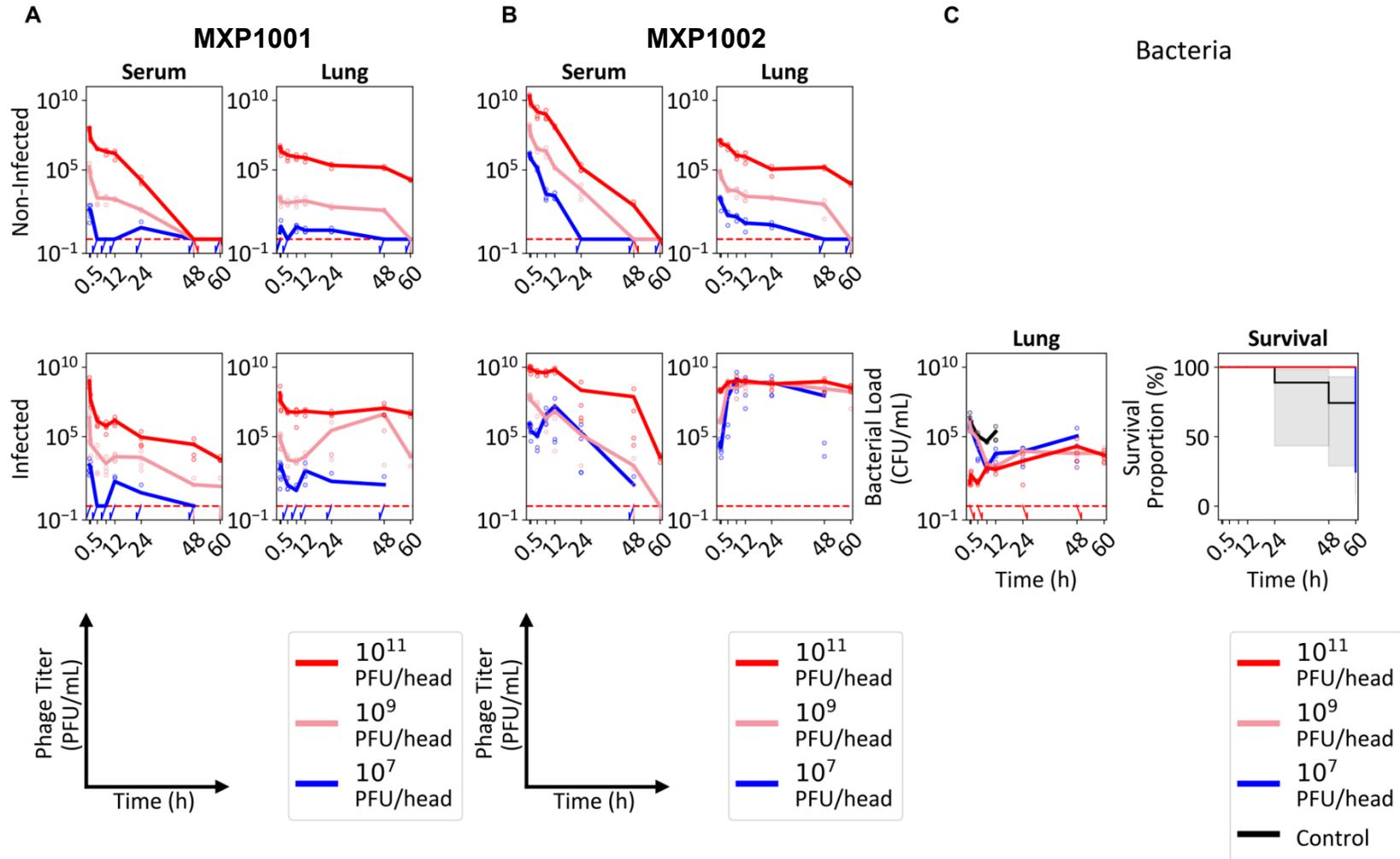
Prediction



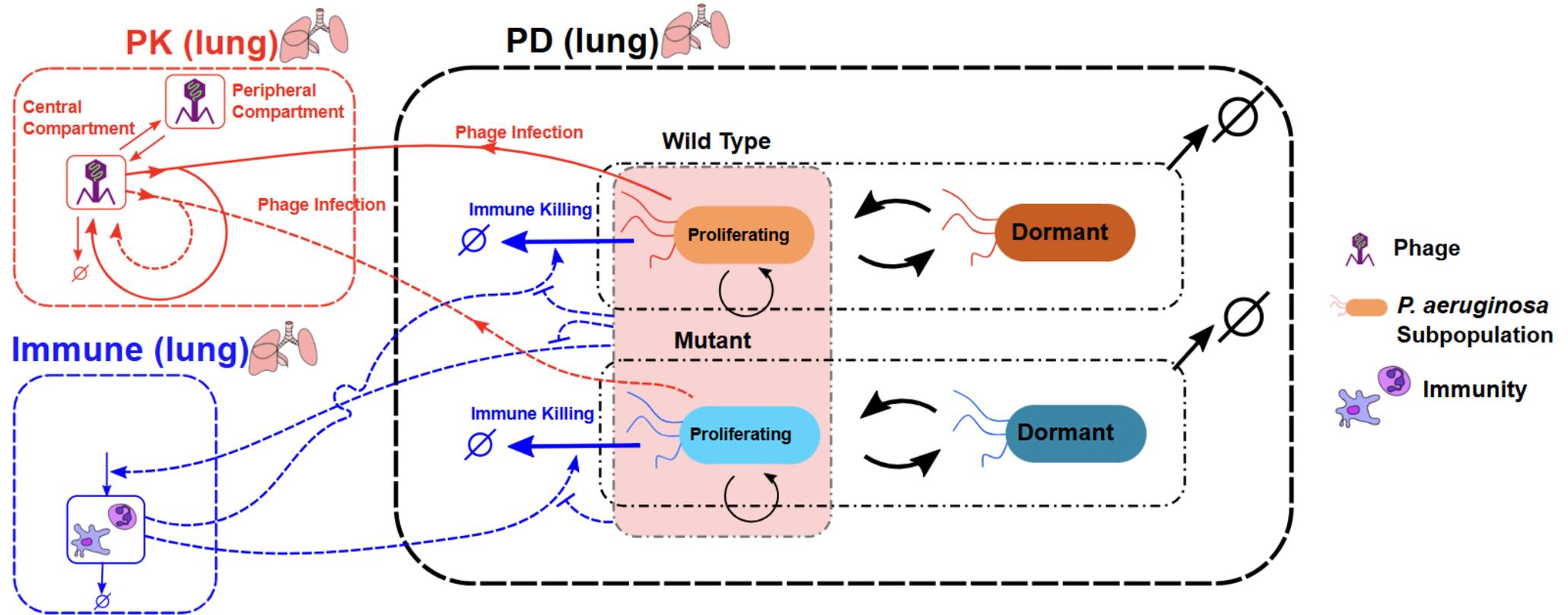
Observation



# In Vivo PKPD Evaluation

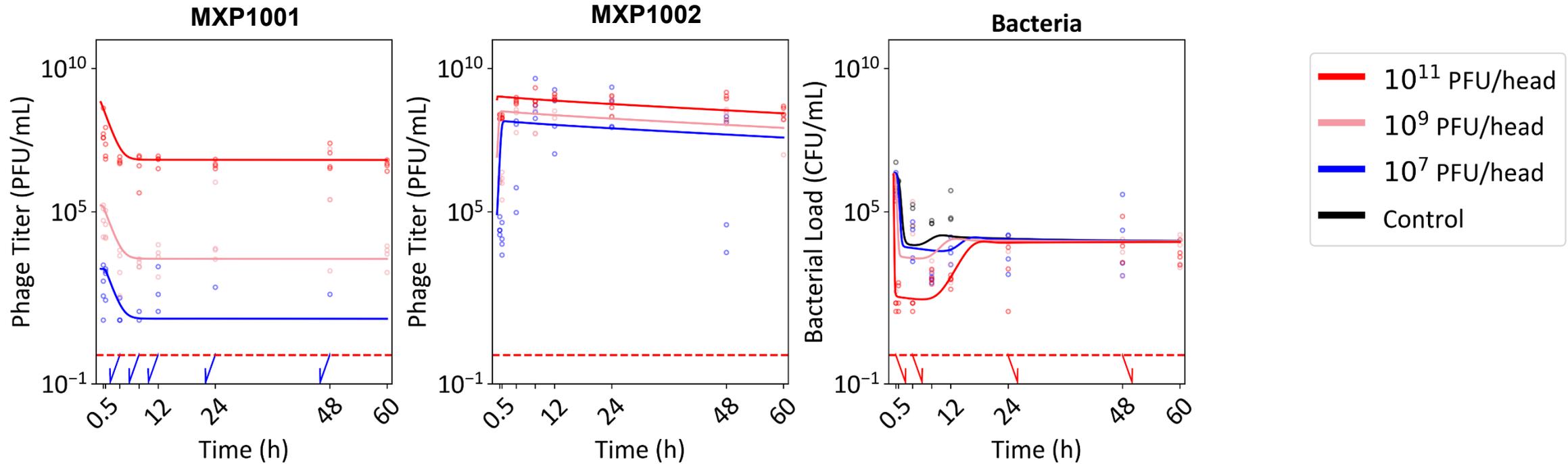


# In vivo 면역계 포함 확장 모델



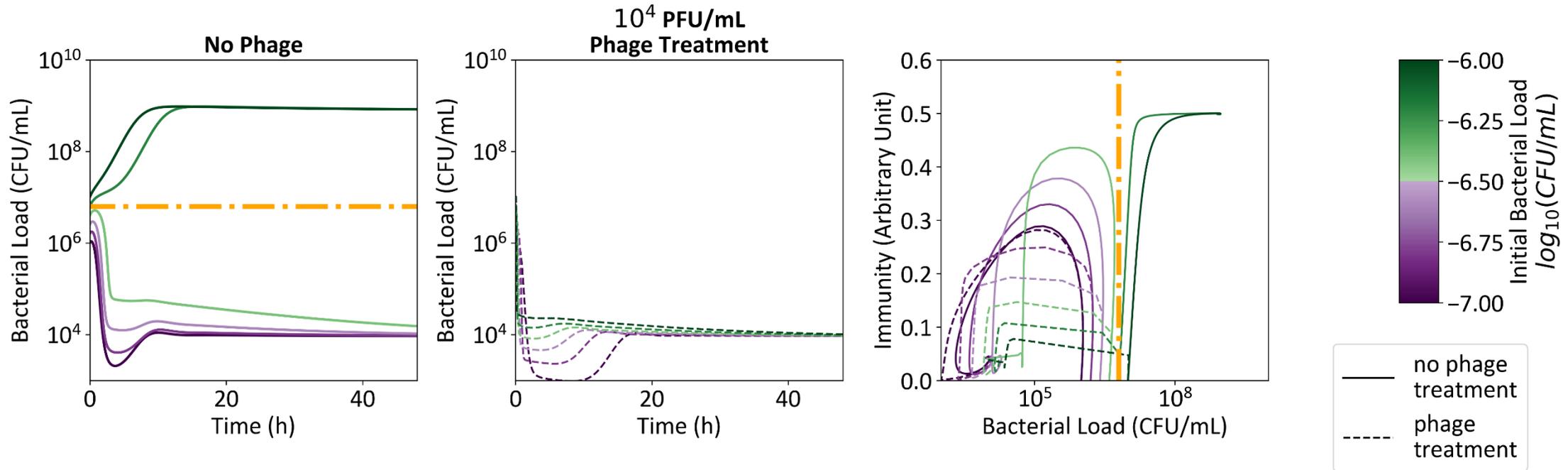
# 모델 예측치와 관측치 적합도

## • Goodness of Fit



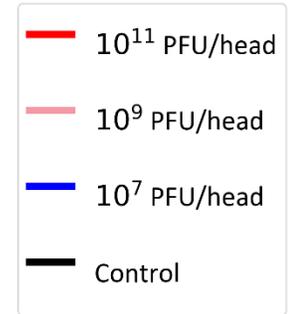
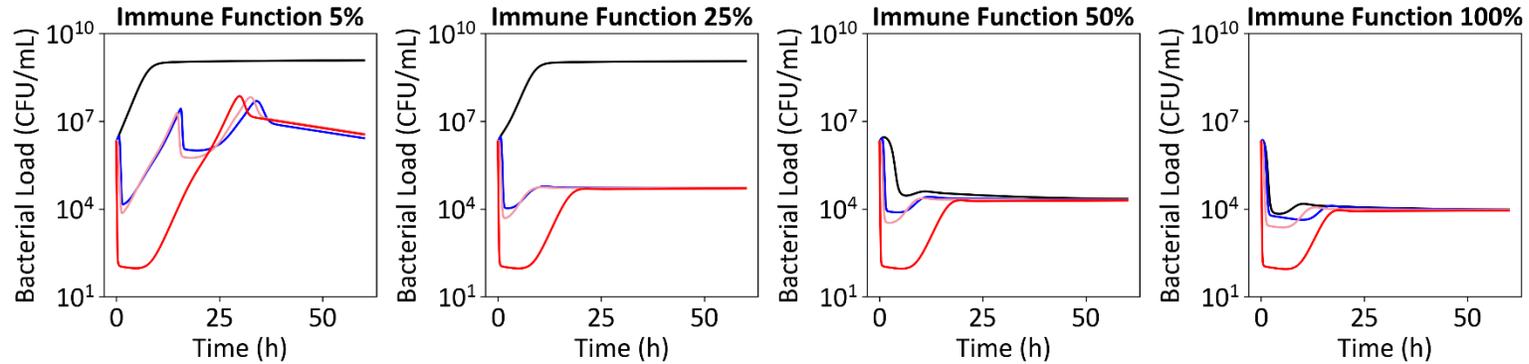
# 파지와 면역계의 시너지

- 서로 다른 감염균 용량별 파지의 효능 예측

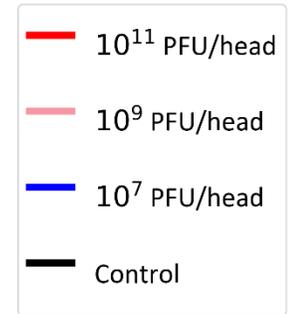
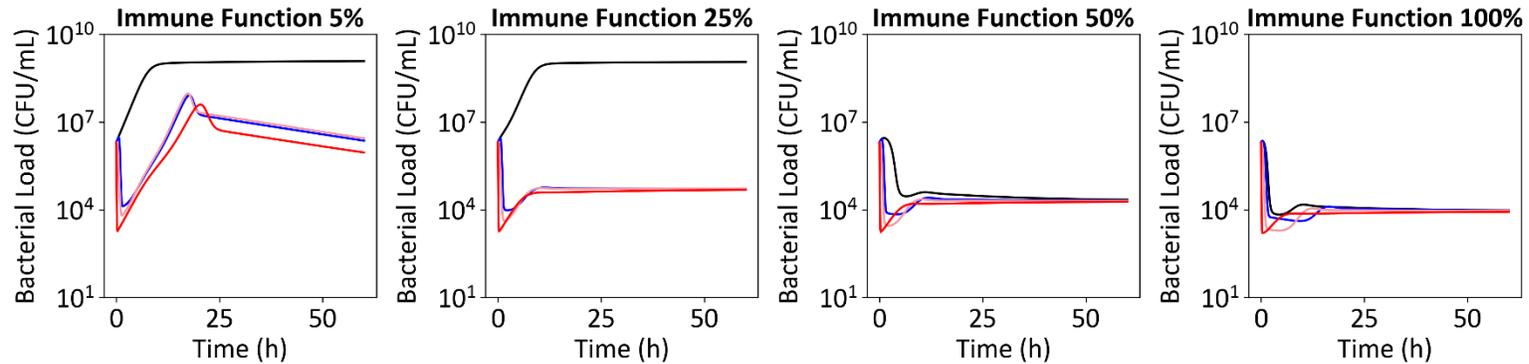


# 면역계 기능의 영향

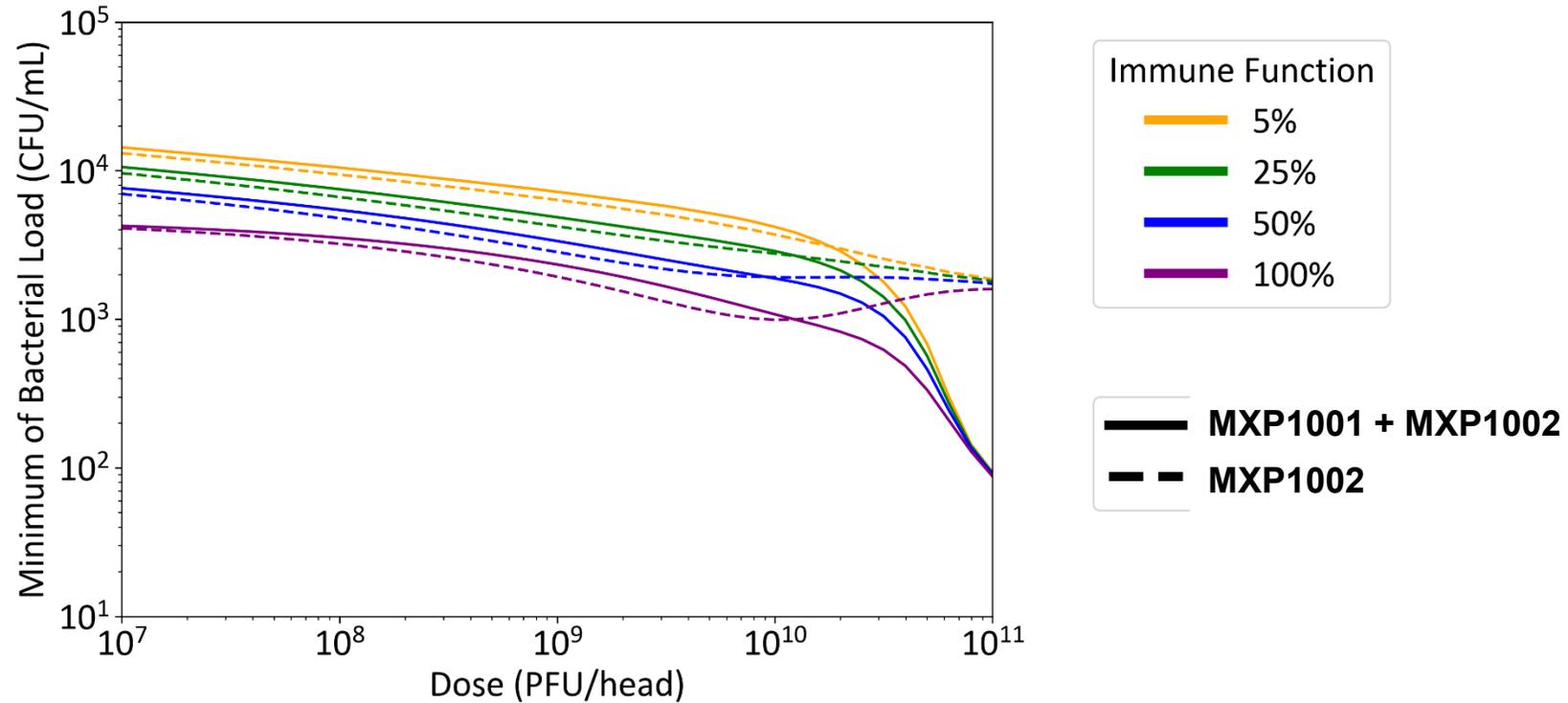
**MXP1001 +  
MXP1002**



**MXP1002  
only**

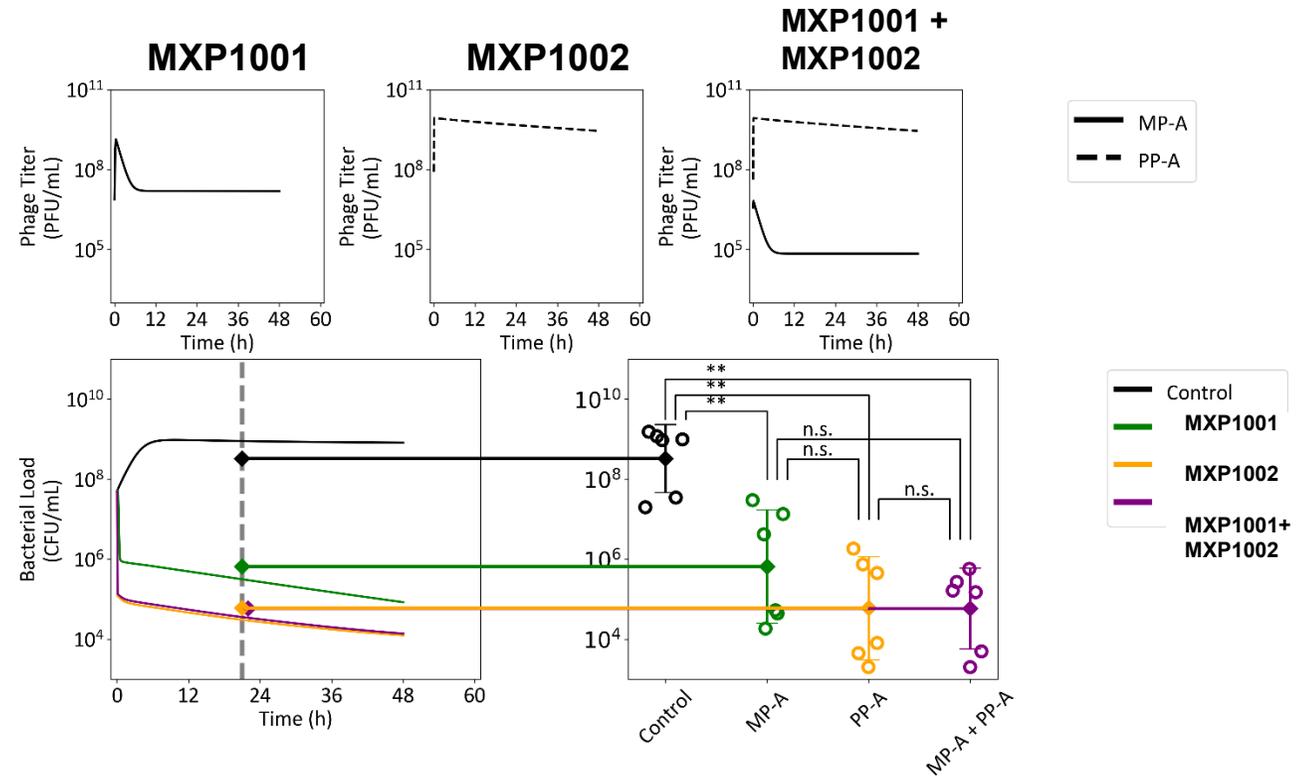
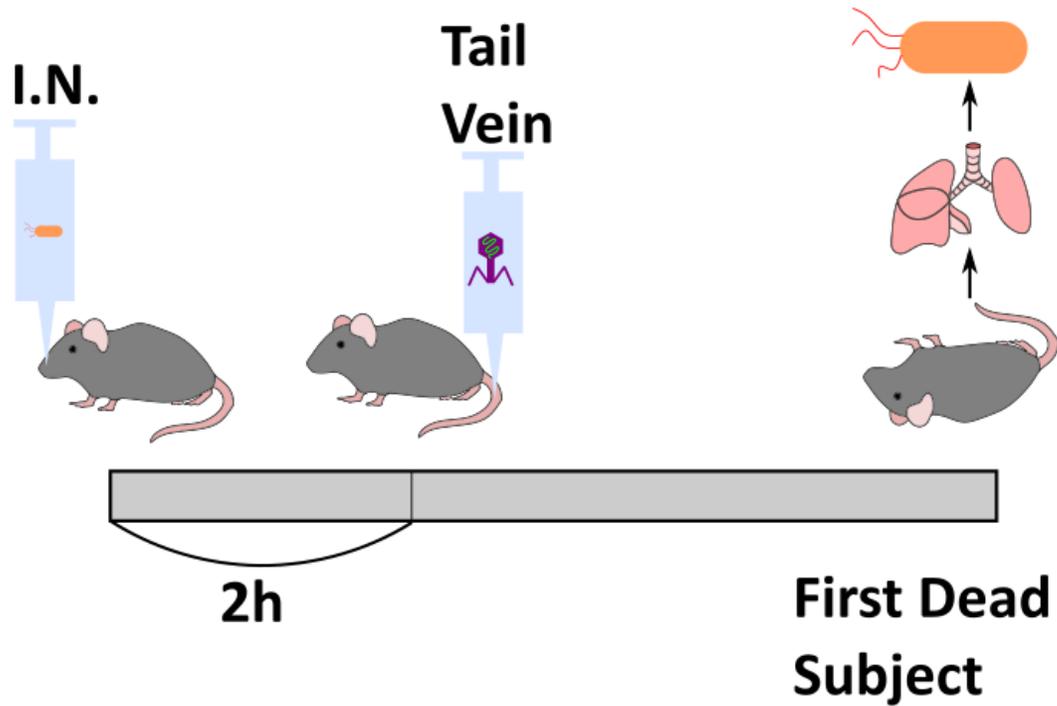


# 파지 용량-반응 곡선



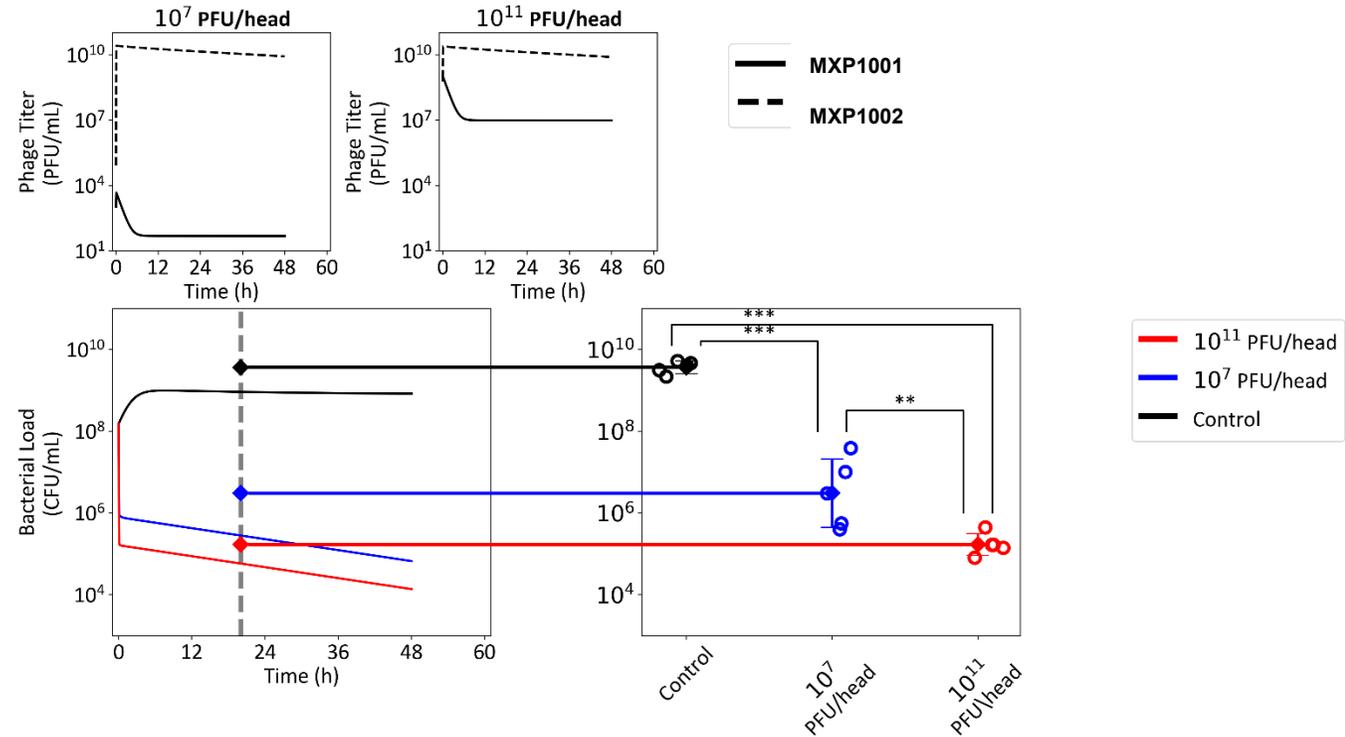
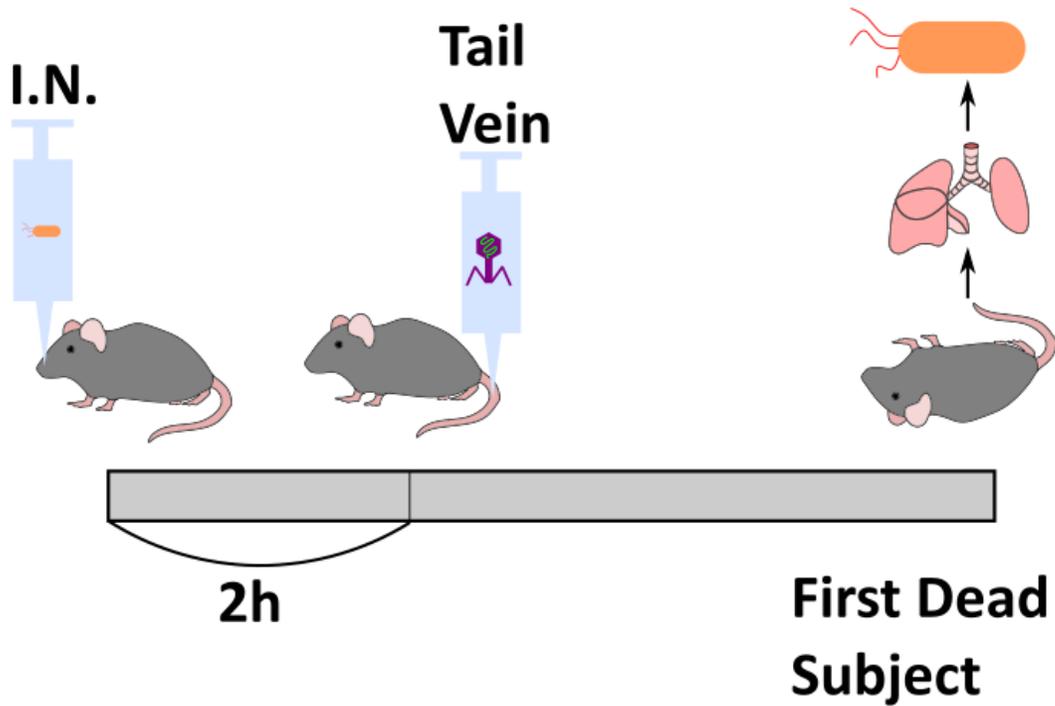
# In Vivo Model Validation

## • Regimen Composition



# In Vivo Model Validation

- MXP1001 + MXP1002





# 토의 및 결론

# Unique PKPD

- 종래의 항생제와 달리 **phage** 는 자가증식능에 의해 독특한 **PKPD** 양상을 보임
- **Phage exposure**는 **dose** 뿐 아니라 얼마나 증식할 수 있느냐에도 의존
- 얼마나 증식하는지는 **target pathogen** 의 농도, 감염 효율, 그리고 **burst size**에 의존

# Phage therapy의 핵심 작용 기전

- **Two stage process**
  - 1) **Early phage-induced bacterial reduction**
  - 2) **Late immune-mediated scavenging of phage-resistant bacteria**

# Further studies

- 숙주의 면역계 반응을 더 구체적으로 반영하는 것이 필요
- **Altered host physiology (e.g. chronic 감염 시 bacterial dormancy 확률 증가) 반영**
- **Time-dependent PK 및 anti-phage antibody 생성 고려**
- **Phage-antibiotics synergy 연구**



**THANK YOU**