

# Phages for Food Safety: phage application against *Yersinia*

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and Technology

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## Oyster

- ❖ Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), marine bivalve
- ❖ Cultured all over the world including Korea
- ❖ Valuable seafood
  - 1) Fresh taste
  - 2) Nutritionally perfect
  - 3) Commercially-important



## Infectious Agent

- Curved rod shape
- Gram-negative
- Single flagellum

## Symptoms

- 24 hours incubation period
- Watery diarrhea, nausea, vomiting, abdominal cramps, fever
- Resolves in 72 hours

## Risk Groups

- Along coasts
- People who eat shellfish
- **4,500 cases in US/year**

## Treatment

- Fluid and electrolyte replacement
- Antibiotics (immunocompromised patients)

## Transmission

- Water-borne
- Fecal-oral
- Food-borne in **shellfish**
- Wound infections after exposure to contaminated water

## Epidemiology

- **Pandemic strains (O3:K6 serotype)**
- India, Russia, Southeast Asia, Japan, Korea, North America



Study areaSeafood samplingSeafood samples

Black tiger shrimp (*Penaeus monodon*)  
 Corb shell (*Cyclina sinensis*)  
 Kuruma prawn (*Marsupenaeus japonicas*)  
 Razor clam (*Solen strictus*)  
 Short neck clam (*Venerupis philippinarum*)  
 Sea cucumber (*Stichopus japonicas*)  
 Sea mussel (*Mytilus coruscus*)  
 Pacific oyster (*Crassostrea gigas*)  
 Charm abalone (*Haliotis discushannai*)  
 White leg shrimp (*Litopenaeus vannamei*)

Environmental samples

Seafood-related Seawater and Water Sediment



Black tiger shrimp



Corb shell



Razor clam



Short neck



Sea mussel

Charm  
abalone

White leg shrimp



Isolation of strains

Fisheries wholesale market



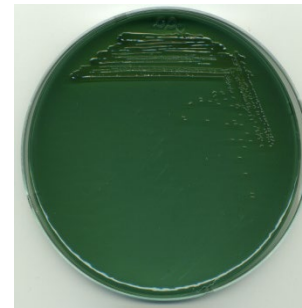
Seafood Samples



Stomacher homogenizer



APW containing 3% NaCl



TCBS agar



CHROMagar™ Vibrio



Pure cultures

### Molecular analysis

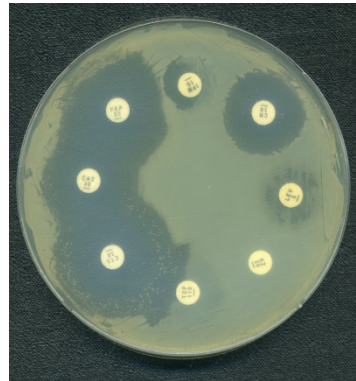
#### ❖ Multiplex-PCR

Primer	Target gene	Expected product	Reference
<i>Vp. flaE</i>	<i>flaE</i> sequence of <i>V. parahaemolyticus</i>	897 bp	Tarr et al., 2007. <i>J. Clin. Microbiol.</i>
<i>tl</i>	thermolabile hemolysin	450 bp	Bej et al., 1999. <i>J. Microbiol. Methods</i>
<i>tdh</i>	thermostable direct hemolysin	269 bp	Bej et al., 1999. <i>J. Microbiol. Methods</i>
<i>trh</i>	thermostable direct hemolysin-related hemolysin	500 bp	Bej et al., 1999. <i>J. Microbiol. Methods</i>
<i>toxR</i>	<i>V. parahaemolyticus toxR</i> gene	368 bp	Kim et al., 1999. <i>J. Clin. Microbiol.</i>
ORF8	open reading frame 8 of pandemic <i>V. parahaemolyticus</i> O3:K6 strain	369 bp	Myers et al., 2003. <i>Appl. Environ. Microbiol.</i>
<i>toxRS/old</i>	<i>toxRS</i> sequence of the O3:K6 clone isolated before 1995	651 bp	Okura et al., 2003. <i>J. Clin. Microbiol.</i>
<i>toxRS/new</i>	<i>toxRS</i> sequence of the O3:K6 clone isolated since 1996	651 bp	Matsumoto et al., 2000. <i>J. Clin. Microbiol.</i>

#### ❖ 16S rRNA gene sequencing analysis

### Antibiotic susceptibility test

- ❖ Antibiotic susceptibility test via the standard disk diffusion method using 22 antibiotic disks



CLSI (Clinical and Laboratory Standards Institute), 2005

- ❖ Multiplex-PCR to detect antibiotic resistant genes

Primer	Expected product	Primer	Expected product
SXT integrase	1035 bp	<i>sul2</i>	625 bp
<i>floR</i>	526 bp	<i>dfr18</i>	389 bp
<i>TetA</i>	950 bp	<i>strB</i>	470 bp
<i>dfrA1</i>	372 bp		

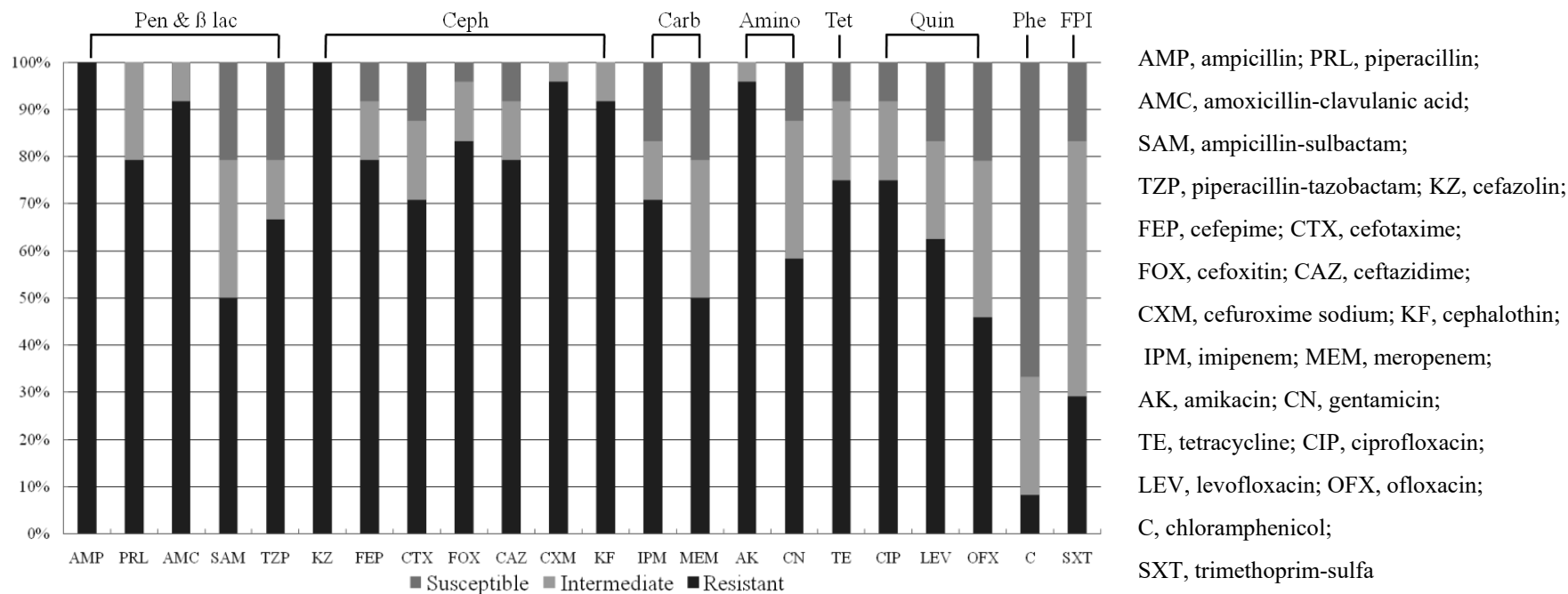


Isolation/ characterization of strains

Strains (Number)	Positive sample of PCR	
	ORF8	<i>toxRS/new</i>
<b>Reference strains</b>		
<i>V. parahaemolyticus</i> (3)	-	-
<b>Seafood isolates</b>		
<i>V. parahaemolyticus</i> (19)	-	-
<b>Environmental isolates</b>		
<i>V. parahaemolyticus</i> (3)	-	-
<b>Clinical isolates</b>		
<i>V. parahaemolyticus</i> (2)	<u>2/2</u> <b>O3:K6 serotype</b>	1/2
<b>Positive sample/total</b>	2/27	1/27

### Antibiotic susceptibility profile

❖ The antibiotic resistance patterns of 22 commercial antibiotics



❖ All isolates evidenced **multiple resistance** (resistance to > 2 antibiotics) and were resistant to more than 4 antibiotics.

## Publication

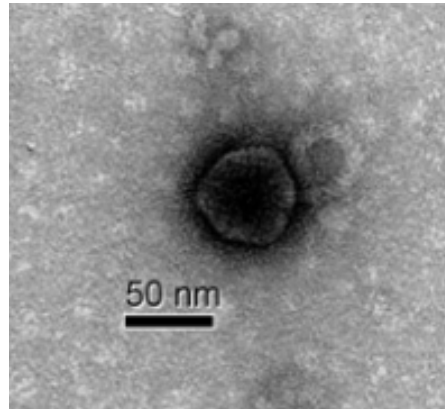
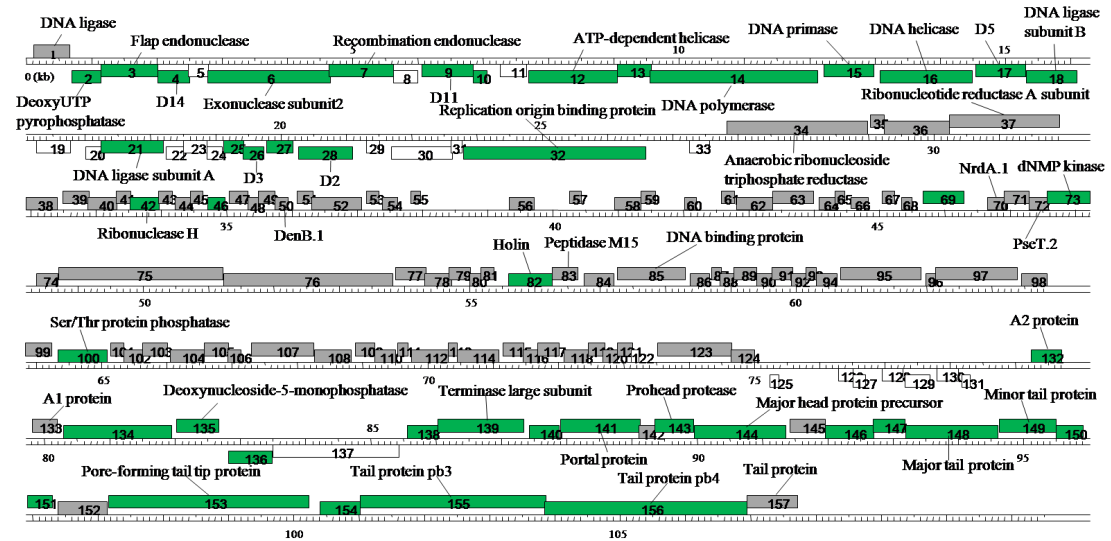
FOODBORNE PATHOGENS AND DISEASE  
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DOI: 10.1089/fpd.2011.1018

# Isolation, Molecular Characterization, and Antibiotic Susceptibility of *Vibrio parahaemolyticus* in Korean Seafood

Jin Woo Jun<sup>1</sup>, Ji Hyung Kim<sup>1</sup>, Casiano H. Choresca, Jr.<sup>1</sup>, Sang Phil Shin<sup>1</sup>, Jee Eun Han<sup>1</sup>,  
Sang Yoon Han<sup>1</sup>, Ji Young Chai<sup>2</sup>, and Se Chang Park<sup>1</sup>

## Abstract

The principal objective of this study was to investigate the incidence, risk assessment, antibiotic resistance, and genotyping of *Vibrio parahaemolyticus* in Korean seafood. The incidence of *V. parahaemolyticus* in seafood obtained from several fish markets in Korea was investigated from May to December of 2009, except between July and September. Two selective mediums (TCBS [thiosulfate, citrate, bile salts, and sucrose] agar and CHROMagar<sup>TM</sup> Vibrio) were used, and the *V. parahaemolyticus* strains were identified via polymerase chain reaction (PCR) amplification (*Vp. flaE*, *tl*, and *toxR*). 16S rRNA gene sequencing and their virulence were analyzed via the detection of *tdh*, *trh*, ORF8, *toxRS/old*, and *toxRS/new* genes. We collected 24 strains of *V. parahaemolyticus*: 19 seafood isolates, three environmental isolates, and two clinical (human) isolates. Among these strains, two *tdh*+ strains, two ORF8+ strains, 16 *toxRS/old*+ strains, and one *toxRS/new*+ strain were isolated. Twenty-two

*Vibrio* phage pVp-1Genome of pVp-1

## Morphology

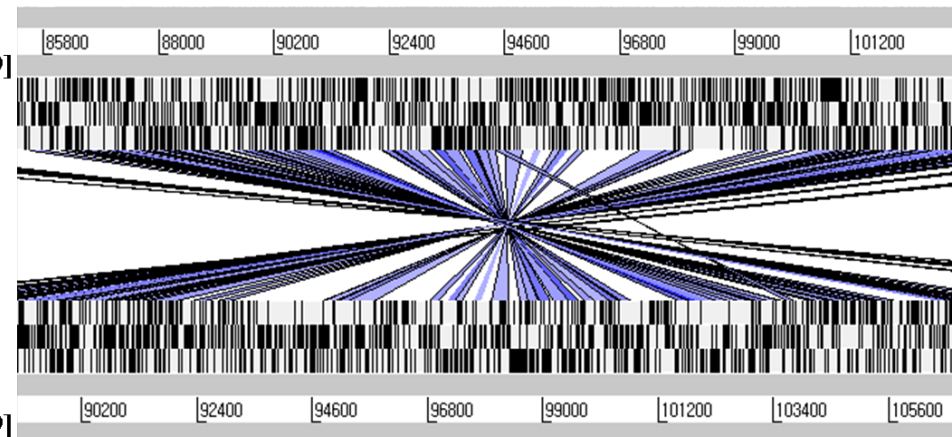
- *Siphovirus*

## Genome

- T5-like phage
- **No pathogenic factor**

pVp-1  
[NC\_019529]

T5  
[NC\_005859]



## Publication



GENOME ANNOUNCEMENT

# Complete Genome Sequence of a Novel Marine Siphovirus, pVp-1, Infecting *Vibrio parahaemolyticus*

Ji Hyung Kim,<sup>a,b</sup> Jin Woo Jun,<sup>a</sup> Casiano H. Choresca,<sup>a</sup> Sang Phil Shin,<sup>a</sup> Jee Eun Han,<sup>a</sup> and Se Chang Park<sup>a</sup>

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Among the abundant bacteriophages that belong to the order *Caudovirales* in the ocean, the genome sequences of marine siphoviruses are poorly investigated in comparison to those of myo- or podoviruses. Here we report the complete genome sequence of *Vibrio* phage pVP-1, which belongs to the family *Siphoviridae* and infects *Vibrio parahaemolyticus* ATCC 33844.

Marine viruses are the most abundant biological entities in the ocean (10), which makes the analysis of their genomes essential for a better understanding of their enormous genetic diversity (1). Most of the marine viruses reported to date are bacteriophages that belong to the order *Caudovirales*, which is divided into three families: *Myoviridae*, *Podoviridae*, and *Siphoviridae* (10). Among the marine phages whose genomes have been sequenced, siphoviruses are relatively poorly investigated (9) and only two of them, including phiHSIC (7) and SIO-2 (1), were studied and

*orf156*, and *orf157*), and lytic properties (*orf73*, *orf82*, and *orf83*). Interestingly, most of the ORFs containing DNA metabolism and viral morphogenesis genes were clustered together at each end of the sequenced genome by functional roles and were similar ( $\leq 79\%$ ) to those of T5 (11) or T5-like (3, 4) phages, thus indicating a close genetic relatedness between pVP-1 and those phages.

In contrast, there were no sequence similarities to marine *Vibrio* phages belonging to the family *Siphoviridae* (phiHSIC and SIO-2), and a large proportion of the genes in pVP-1 were not



1 h post treatment



3 h post treatment

12 h post treatment

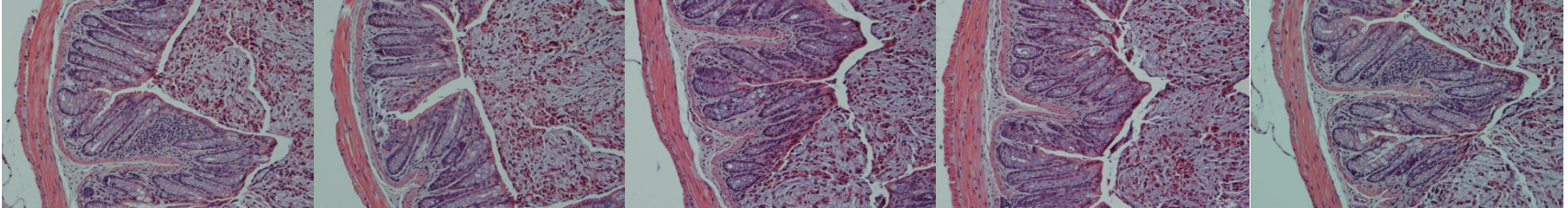


24 h post treatment

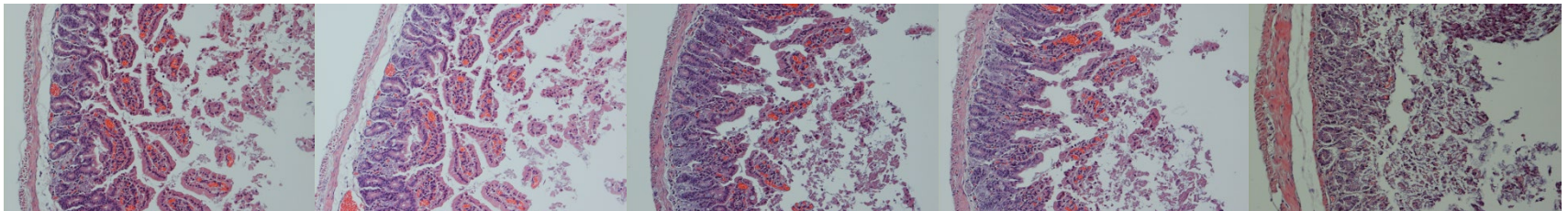


## Histopathology

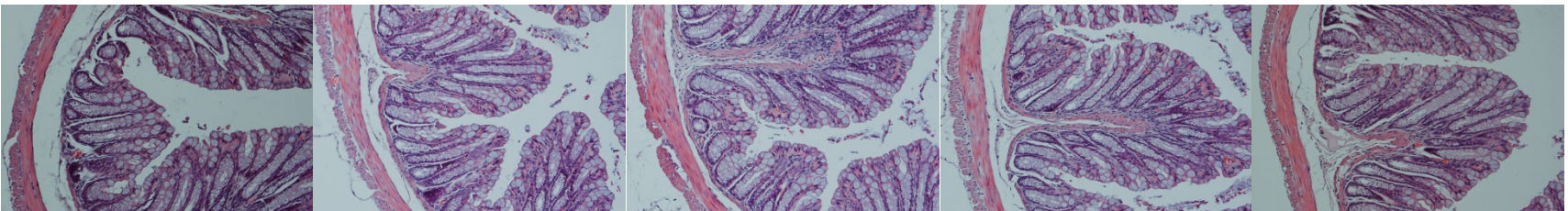
### Control



### Experiment



### Treated





Publication

## MAJOR ARTICLE

## Bacteriophage Therapy of a *Vibrio parahaemolyticus* Infection Caused by a Multiple-Antibiotic-Resistant O3:K6 Pandemic Clinical Strain

Jin Woo Jun,<sup>1,a</sup> Tae Hoon Shin,<sup>1</sup> Ji Hyung Kim,<sup>2,a</sup> Sang Phil Shin,<sup>1</sup> Jee Eun Han,<sup>1</sup> Gang Joon Heo,<sup>3</sup> Mahanama De Zoysa,<sup>4</sup> Gee Wook Shin,<sup>5</sup> Ji Young Chai,<sup>6</sup> and Se Chang Park<sup>1</sup>

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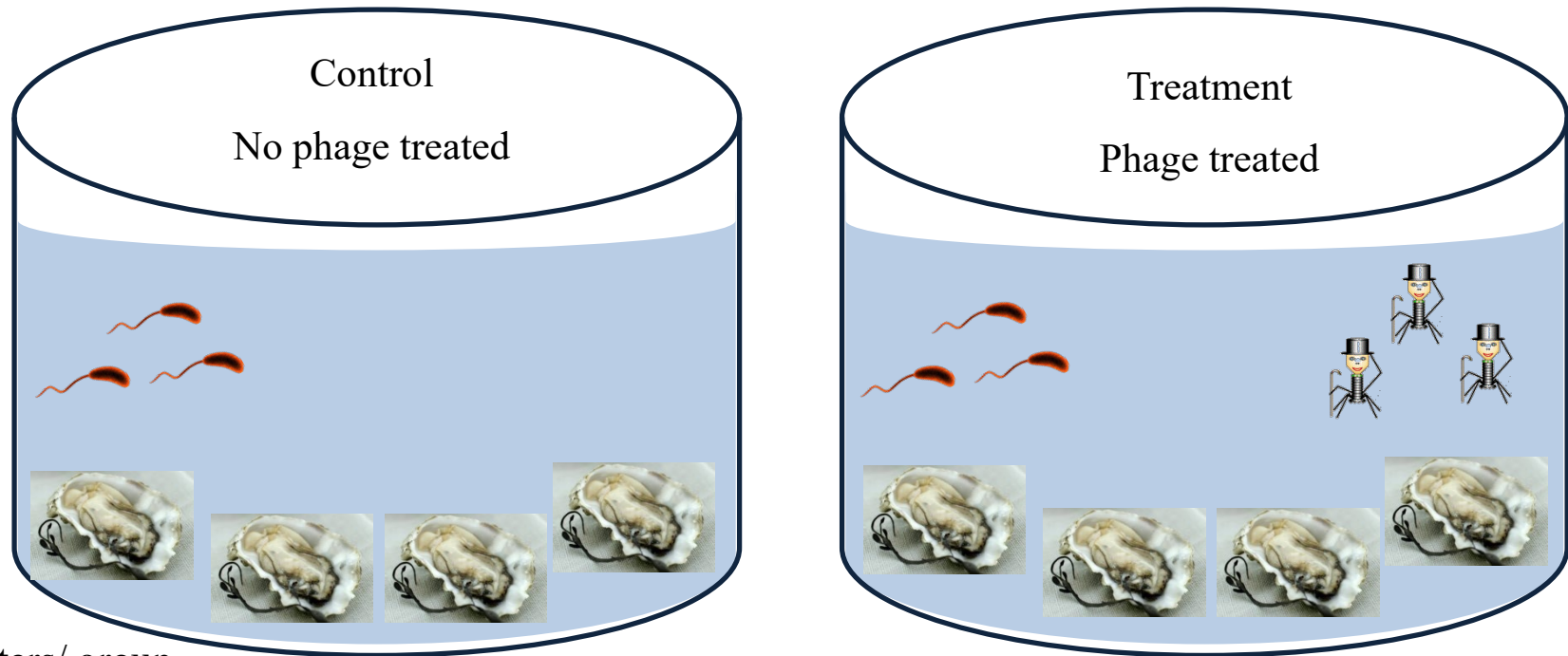
**Background.** Recently isolated *Vibrio parahaemolyticus* strains have displayed multiple antibiotic resistance. Alternatives to conventional antibiotics are needed, especially for the multiple-antibiotic-resistant *V. parahaemolyticus* pandemic strain.

**Methods.** A bacteriophage, designated pVp-1, showed effective infectivity for multiple-antibiotic-resistant *V. parahaemolyticus* and *V. vulnificus*, including *V. parahaemolyticus* pandemic strains. The therapeutic potential of the phage was studied in a mouse model of experimental infection using a multiple-antibiotic-resistant *V. parahaemolyticus* pandemic strain. We monitored the survivability and histopathological changes, quantified the bacte-

## Application to oyster processing

Infection method: bath immersion ( $10^6$ CFU/ ml )

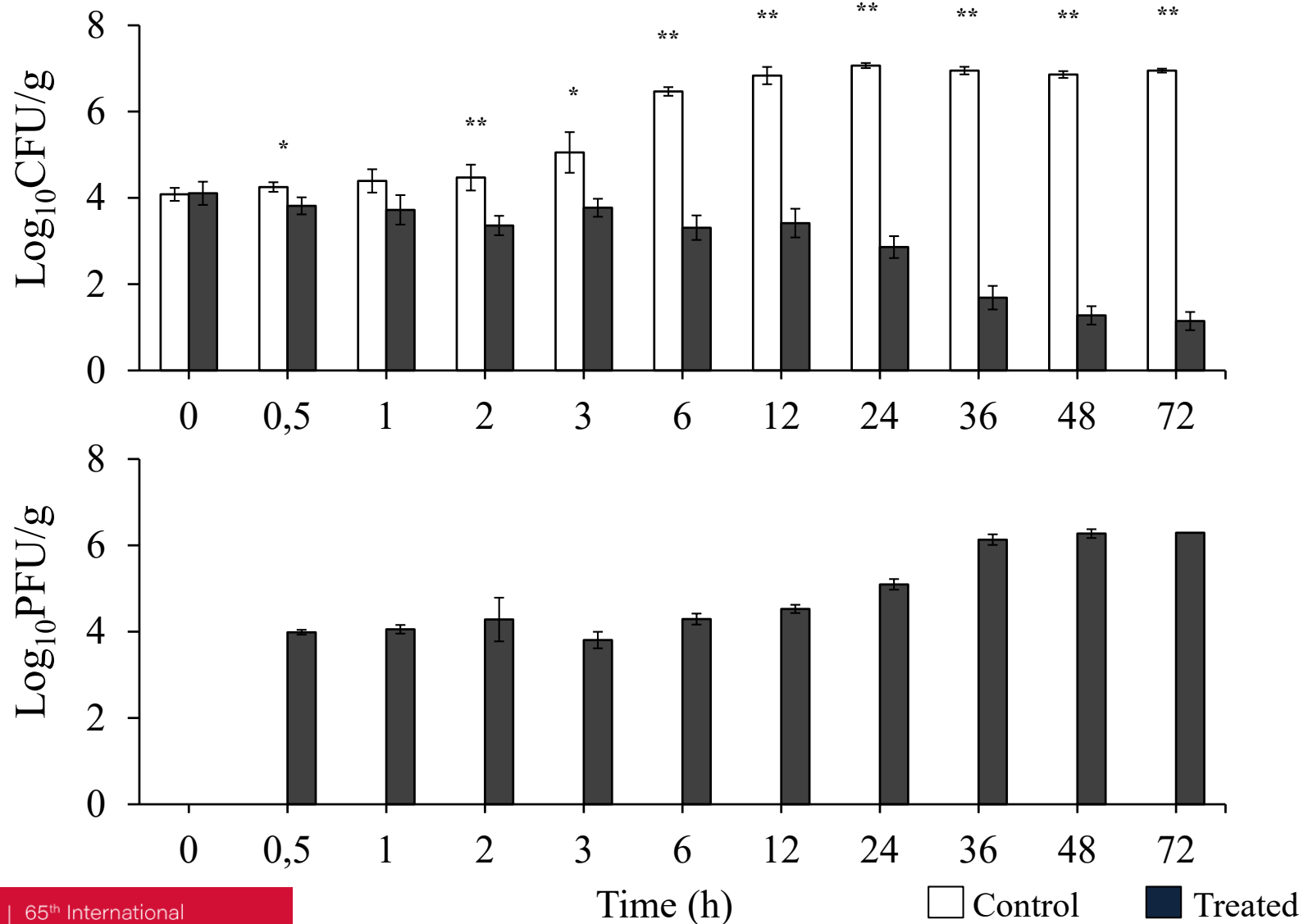
Treatment method: bath immersion ( $10^7$ PFU/ ml )



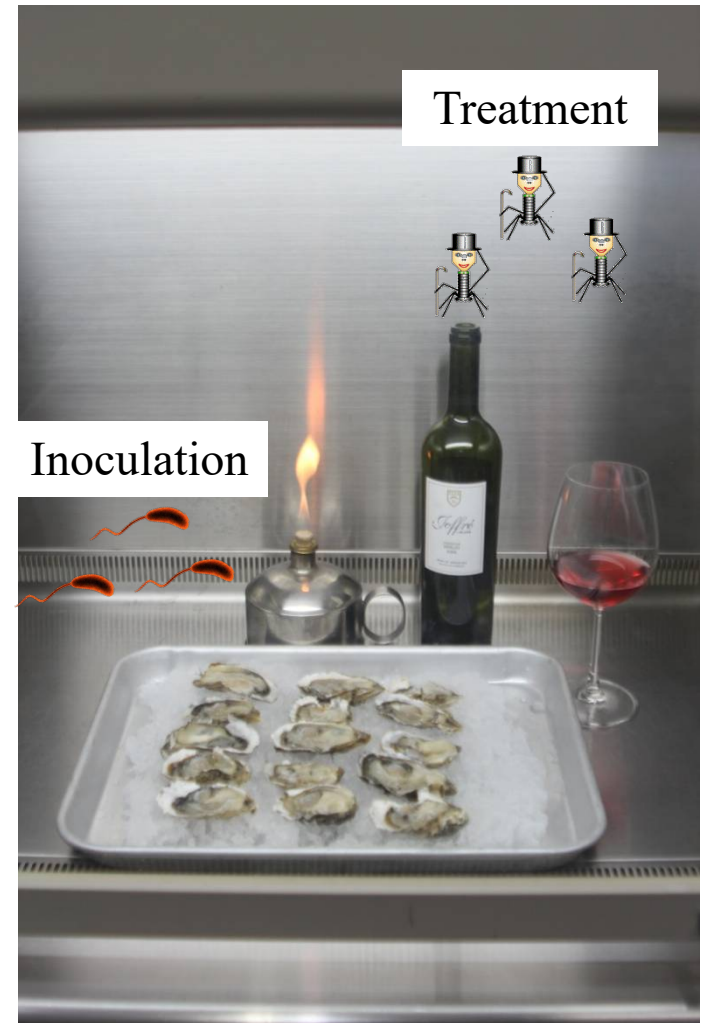
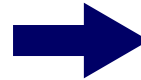
40 oysters/ group

Temp.:  $18 \pm 0.3$  °C

Volume of water: 35 L (artificial seawater)

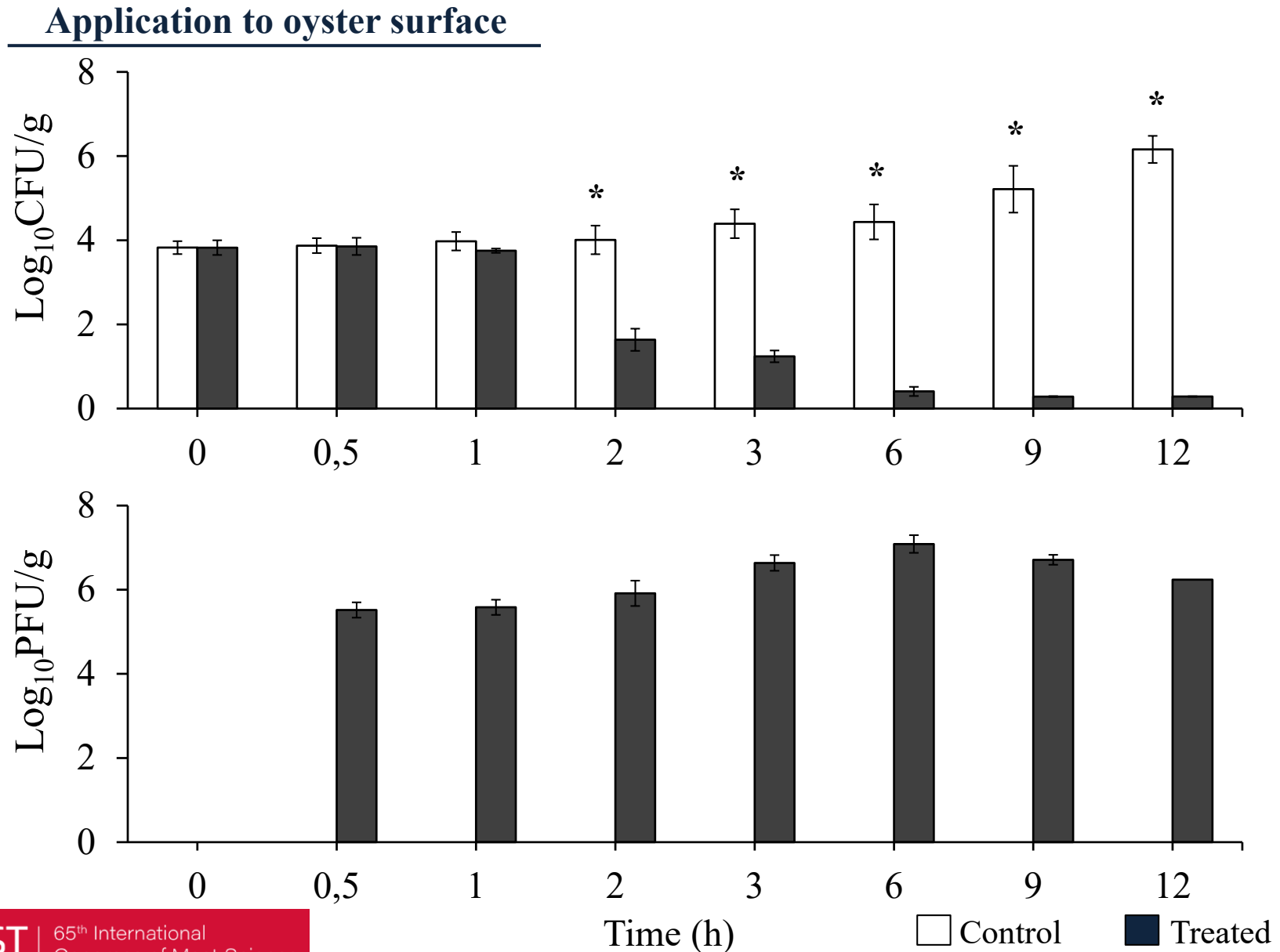
Application to oyster processing

### Application to oyster surface



Inoculation: bacteria spread on surface  
( $10^6$ CFU/ oyster )

Treatment: phage application on surface  
( $10^7$ PFU/ oyster )





## Publication

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journal homepage: [www.elsevier.com/locate/ijfoodmicro](http://www.elsevier.com/locate/ijfoodmicro)



Short communication

### Eating oysters without risk of vibriosis: Application of a bacteriophage against *Vibrio parahaemolyticus* in oysters



Jin Woo Jun <sup>a,b</sup>, Hyoun Joong Kim <sup>b</sup>, Sae Kil Yun <sup>b</sup>, Ji Young Chai <sup>c</sup>, Se Chang Park <sup>b,\*</sup>

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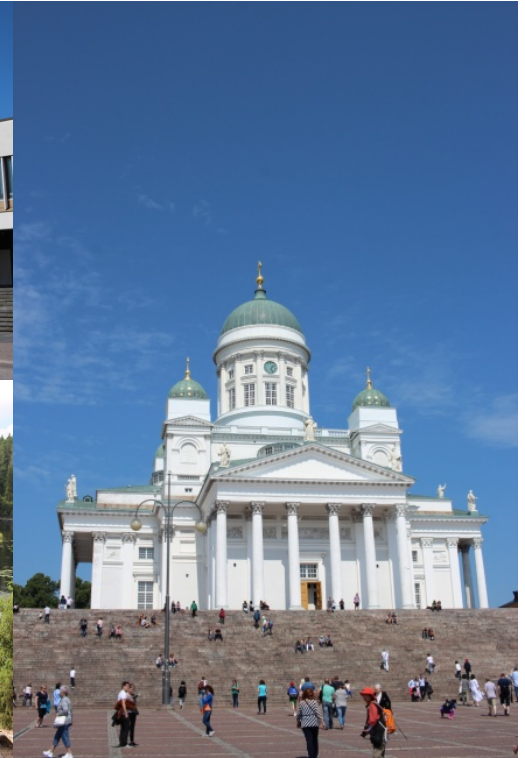
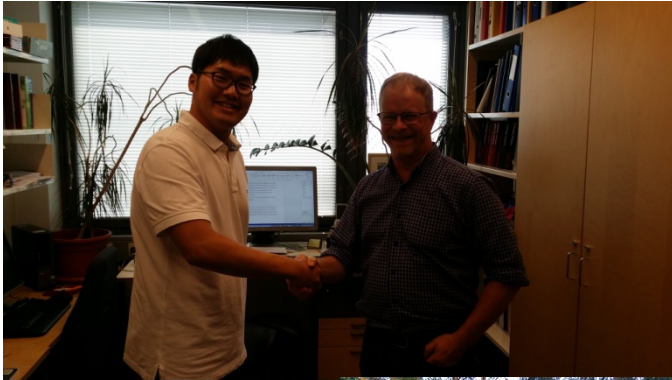
*Vibrio parahaemolyticus*

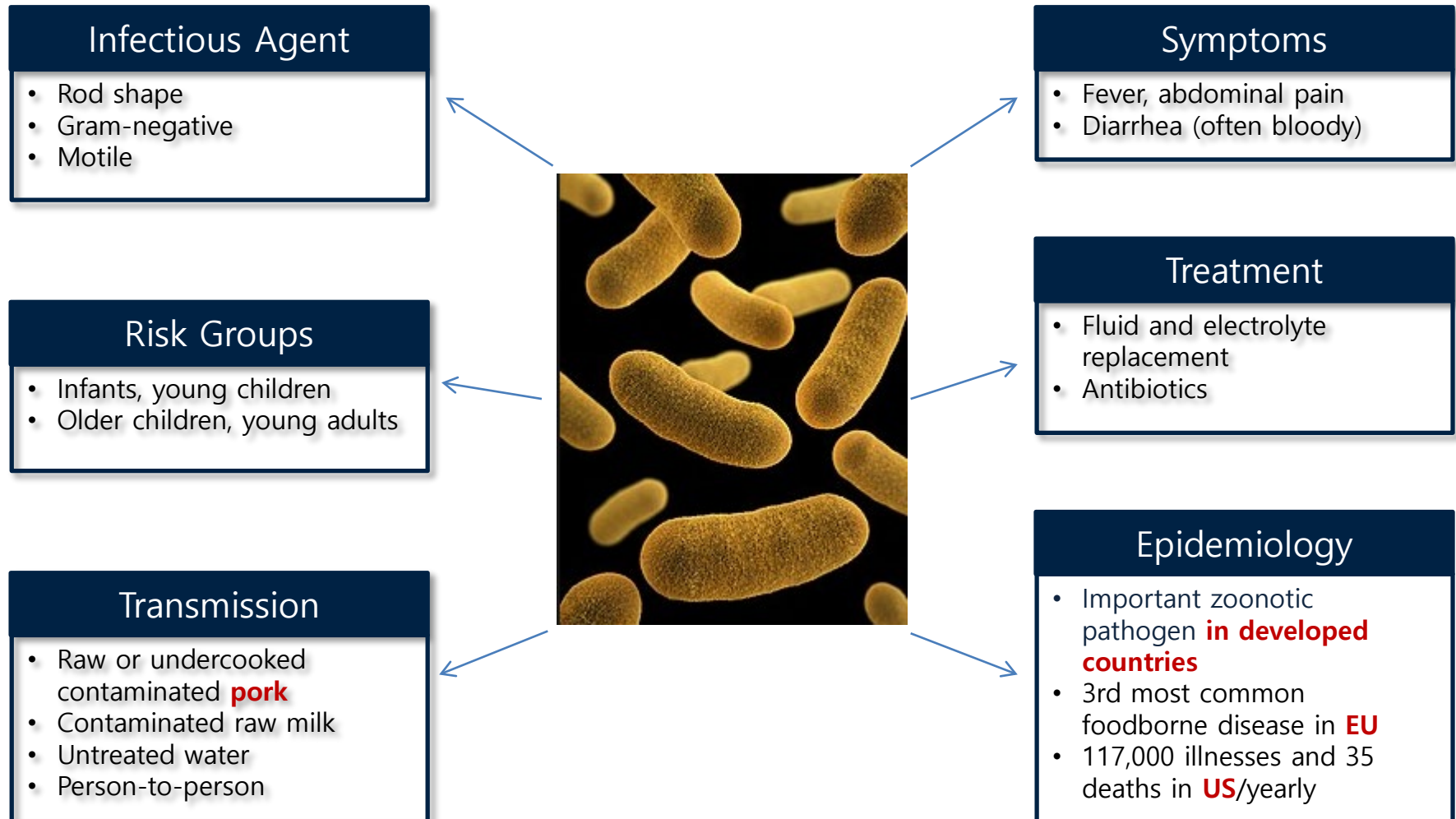
Oyster

#### ABSTRACT

*Vibrio parahaemolyticus* is a major cause of foodborne illness and related with the consumption of raw contaminated seafood, especially oysters. To evaluate the effectiveness of various applications of a bacteriophage (phage), pVp-1, against a multiple-antibiotic-resistant *V. parahaemolyticus* pandemic strain (CRS 09-17), we designed artificial contamination models that are most likely to be encountered during oyster processing. When live oysters were treated with bath immersion with pVp-1 after CRS 09-17 challenge, the growth of bacterial strain was significantly reduced. After 72 h of phage application with bath immersion, bacterial growth reduction was observed to be  $8.9 \times 10^6$  CFU/ml (control group) to  $1.4 \times 10$  CFU/ml (treatment group). When pVp-1 was surface-applied on the flesh of oysters after CRS 09-17 inoculation, bacterial growth was properly inhibited.

## University of Helsinki

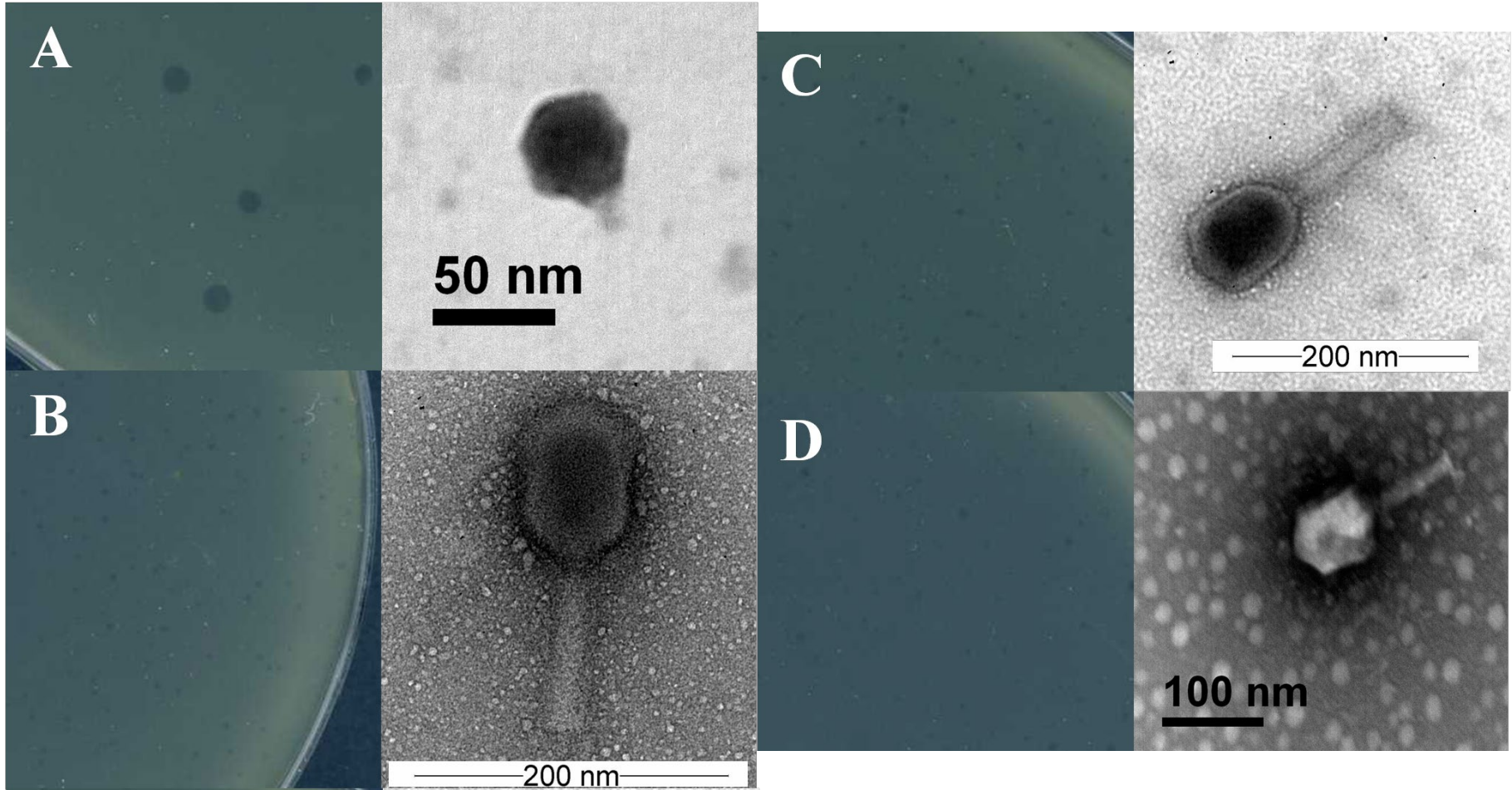






## Summer in 2016



Phage morphology

❖ *Podoviridae* : fHe-Yen3-01 (A)

❖ *Myoviridae* : fHe-Yen9-01 (B), fHe-Yen9-02 (C), fHe-Yen9-03 (D)



### Host range

- ❖ Infectivity of the four *Yersinia* phages (fHe-Yen3-01, fHe-Yen9-01, fHe-Yen9-02, and fHe-Yen9-03)
  
- ❖ 106 *Yersinia* strains, 10 *Yersinia* species
  - Y. enterocolitica* ( $n = 81$ ), *Y. frederiksenii* ( $n = 6$ ), *Y. kristensenii* ( $n = 4$ ),
  - Y. pseudotuberculosis* ( $n = 4$ ), *Y. bercovieri* ( $n = 3$ ), *Y. aleksiciae* ( $n = 2$ ),
  - Y. intermedia* ( $n = 2$ ), *Y. mollaretii* ( $n = 2$ ), *Y. nurmii* ( $n = 1$ ), and *Y. pekkanenii* ( $n = 1$ )

## Host range

Table 1  
Bacterial strains used in this study and infectivity of the *Yersinia* phages fHe-Yen3-01, fHe-Yen9-01, fHe-Yen9-02, and fHe-Yen9-03.

Bacterial species (n)	Strain	Sensory ph <sup>a</sup>	Host range <sup>b</sup> (ICPF <sup>c</sup> )				Reference
			fHe-Yen3-01	fHe-Yen9-01	fHe-Yen9-02	fHe-Yen9-03	
<i>Y. enterocolitica</i> (81)	6471/76	Cr3	++ (1.00)	++ (0.47 ± 0.10)	++ (0.94 ± 0.13)	++ (0.48 ± 0.13)	Skaerlik, 1984
	Rankin/71	Cr9	—	++ (1.00)	++ (1.00)	++ (1.00)	Skaerlik, 1984
	10/84	NT	—	++ (0.64 ± 0.06)	++ (0.77 ± 0.06)	—	Leco-Velarde et al., 2016
	153/9	NT	—	—	—	—	Leco-Velarde et al., 2016
	8333/94	NT	++ (0.34 ± 0.10)	++ (0.88 ± 0.06)	++ (0.35 ± 0.08)	—	Leco-Velarde et al., 2016
659/83	K1, NT	—	++ (0.78 ± 0.12)	++ (0.89 ± 0.06)	—	—	Leco-Velarde et al., 2016
	K1, NT	—	—	—	—	—	Leco-Velarde et al., 2016
436/7/83	Cr3	—	++ (0.62 ± 0.08)	++ (0.77 ± 0.07)	++ (0.54 ± 0.06)	—	Leco-Velarde et al., 2016

Table 1 (continued)

Bacterial species (n)	Strain	Sensory ph <sup>a</sup>	Host range <sup>b</sup> (ICPF <sup>c</sup> )				Reference
			fHe-Yen3-01	fHe-Yen9-01	fHe-Yen9-02	fHe-Yen9-03	
<i>Y. enterocolitica</i> (81)	6471/76	Cr3	++ (1.00)	++ (0.47 ± 0.10)	++ (0.94 ± 0.13)	++ (0.48 ± 0.13)	Skaerlik, 1984
	Rankin/71	Cr9	—	++ (1.00)	++ (1.00)	++ (1.00)	Skaerlik, 1984
	10/84	NT	—	++ (0.64 ± 0.06)	++ (0.77 ± 0.06)	—	Leco-Velarde et al., 2016
	153/9	NT	—	—	—	—	Leco-Velarde et al., 2016
	8333/94	NT	++ (0.34 ± 0.10)	++ (0.88 ± 0.06)	++ (0.35 ± 0.08)	—	Leco-Velarde et al., 2016
659/83	K1, NT	—	++ (0.78 ± 0.12)	++ (0.89 ± 0.06)	—	—	Leco-Velarde et al., 2016
	K1, NT	—	—	—	—	—	Leco-Velarde et al., 2016
436/7/83	Cr3	—	++ (0.62 ± 0.08)	++ (0.77 ± 0.07)	++ (0.54 ± 0.06)	—	Leco-Velarde et al., 2016

- ❖ fHe-Yen9-01 had the broadest host range, infecting 61.3% (65/106 strains).
- ❖ fHe-Yen9-02 exhibited the second broadest host range, infecting 42.5% (45/106).
- ❖ Followed by fHe-Yen3-01 (29.2%, 31/106), and fHe-Yen9-03 (25.5%, 27/106)

296/94	Cr6	++ (0.21 ± 0.06)	++ (0.15 ± 0.07)	—	++ (0.11 ± 0.06)	Leco-Velarde et al., 2016
189/90	Cr6,30	—	++ (0.13 ± 0.08)	—	—	Skaerlik, 1984
6737/90	Cr6,30	++ (0.41 ± 0.07)	—	—	—	Skaerlik, 1984
2604/90	Cr6,30	++ (0.44 ± 0.10)	—	—	—	Skaerlik, 1984
436/90	Cr6,31	++ (0.63 ± 0.09)	++ (0.79 ± 0.05)	—	—	Skaerlik, 1984
1304/90	Cr6,31	++ (0.78 ± 0.03)	++ (0.75 ± 0.10)	++ (0.63 ± 0.07)	—	Skaerlik, 1984
22848/79	Cr7,8	—	++ (0.51 ± 0.07)	++ (0.39 ± 0.08)	—	Skaerlik, 1984
17869/83	Cr7,8	++ (0.69 ± 0.11)	++ (0.62 ± 0.09)	—	—	Leco-Velarde et al., 2016
p310	Cr8	—	—	—	—	Leco-Velarde et al., 2016
CD242/85	Cr8	—	—	—	++ (0.67 ± 0.10)	Genotek et al., 1980
TAMU-75	Cr8	—	—	—	++ (0.75 ± 0.09)	Genotek et al., 1980
98A	Cr8	—	—	—	—	Genotek et al., 1980
JD 801	Cr8	—	—	—	++ (0.61 ± 0.08)	Schlesinger and Deventer, 1982
8081	Cr8	—	—	—	—	Rooney et al., 1981
8081-82	Cr8	—	—	—	—	Zhang et al., 1987
8081-82	Cr8	++ (0.17 ± 0.07)	—	—	—	This study
Y408-ctWbctG2	Cr8	—	—	—	—	Bergoch et al., 2004
Y408-ctWbctG2	Cr8	—	—	—	—	Bergoch et al., 2004

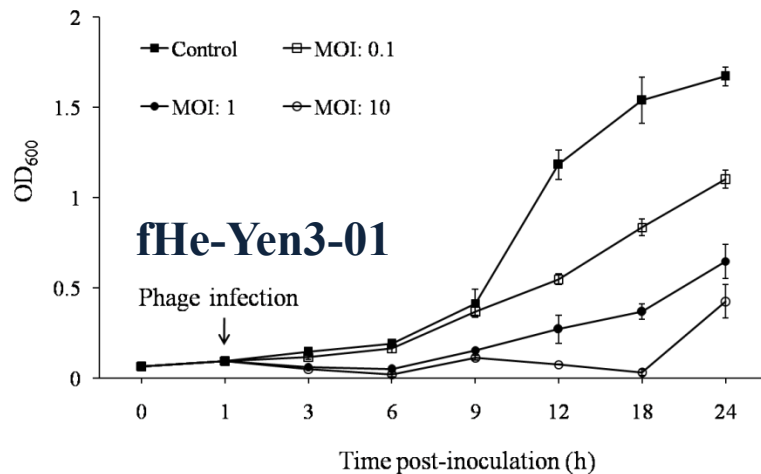
(continued on next page)

gc 2139-72	Cr34	—	—	—	—	—	—
240/94	Cr35,32	—	—	—	—	—	—
7104/83	Cr35,36	Y. berkeleyi (3)	127/94	NT	++ (0.41 ± 0.06)	++ (0.51 ± 0.10)	—
264/85	Cr41,43	—	—	—	—	—	++ (0.52 ± 0.07)
626/83	Cr41 (27)	—	3016/94	Cr58,36	++ (0.61 ± 0.06)	++ (0.42 ± 0.06)	—
42	Cr41 (27)	—	3884/94	Cr58,36	++ (0.52 ± 0.10)	++ (0.51 ± 0.07)	++ (0.62 ± 0.07)
9613/83	Cr41 (27)	—	404/81	Cr16	—	++ (0.34 ± 0.07)	—
19442/83	Cr41 (27)	—	317/82	Cr16	—	++ (0.82 ± 0.12)	++ (0.75 ± 0.08)
142, 81	Cr41 (27)	Y. moshkovii (2)	9/85	Cr16,21	—	—	++ (0.71 ± 0.07)
3229	Cr50	—	821/94	Cr52,54	++ (0.17 ± 0.07)	++ (0.93 ± 0.10)	++ (0.85 ± 0.06)
498/85	NT	Y. mollaretii (2)	57 22404	Cr3	++ (0.25 ± 0.08)	—	—
28/85	K1, NT	—	92/84	Cr59 (20-36,7)	++ (0.35 ± 0.08)	++ (0.24 ± 0.05)	++ (0.32 ± 0.10)
57 23047	Cr3	—	DSM 22296	UT	—	—	—
340/83	Cr16	Y. ruckeri	Δ125 H3182	UT	—	—	—
3317/94	Cr35	Y. pseudotuberculosis	—	—	—	—	—
38/83	Cr48	—	—	—	—	—	—

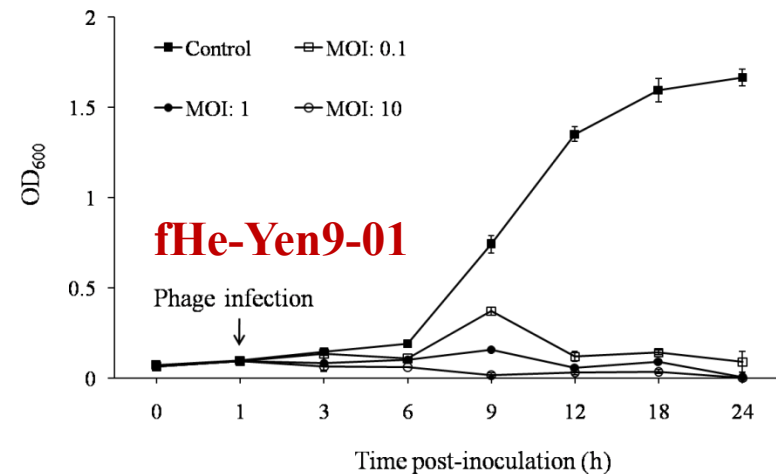
<sup>a</sup> NT, non-typable; UT, untyped.<sup>b</sup> ++, clear plaques; +, turbid plaques; —, no plaques.<sup>c</sup> The IOP (efficiency of plating) values were shown as the mean of observations at three different occasions.

Bacteriolytic effect (*in vitro*)

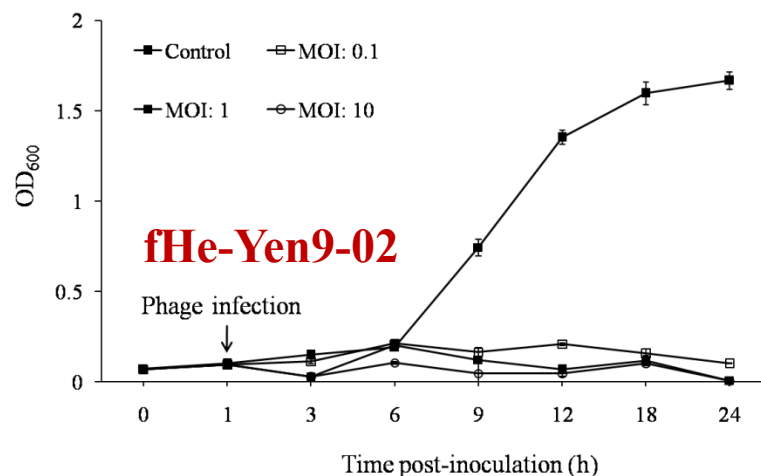
A



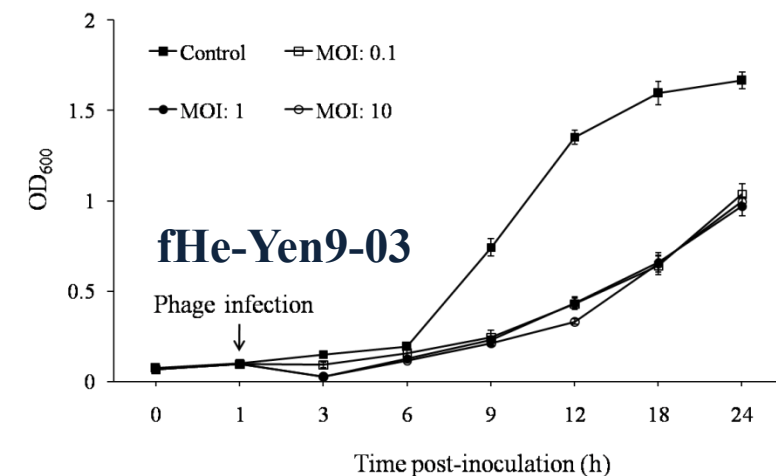
B



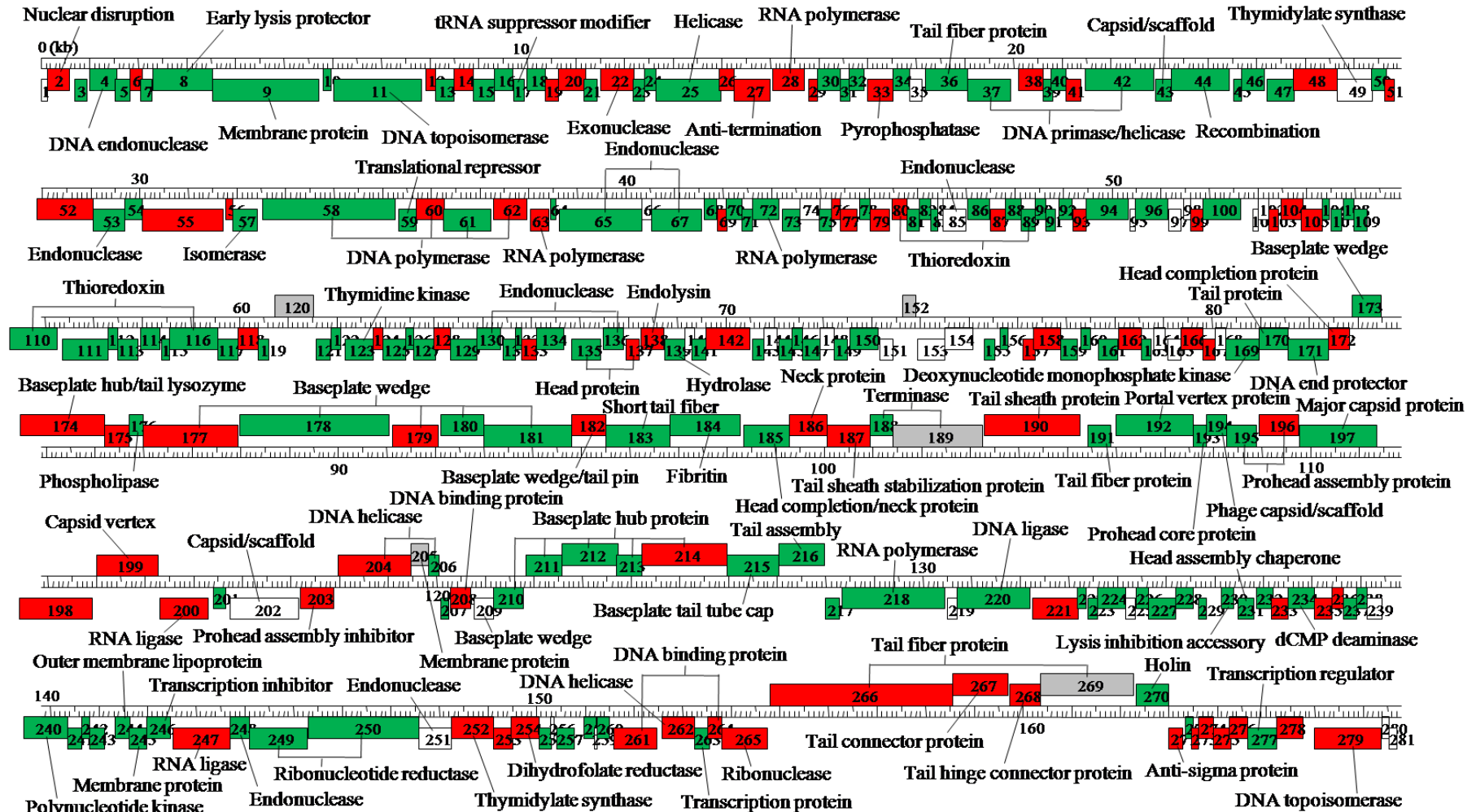
C



D

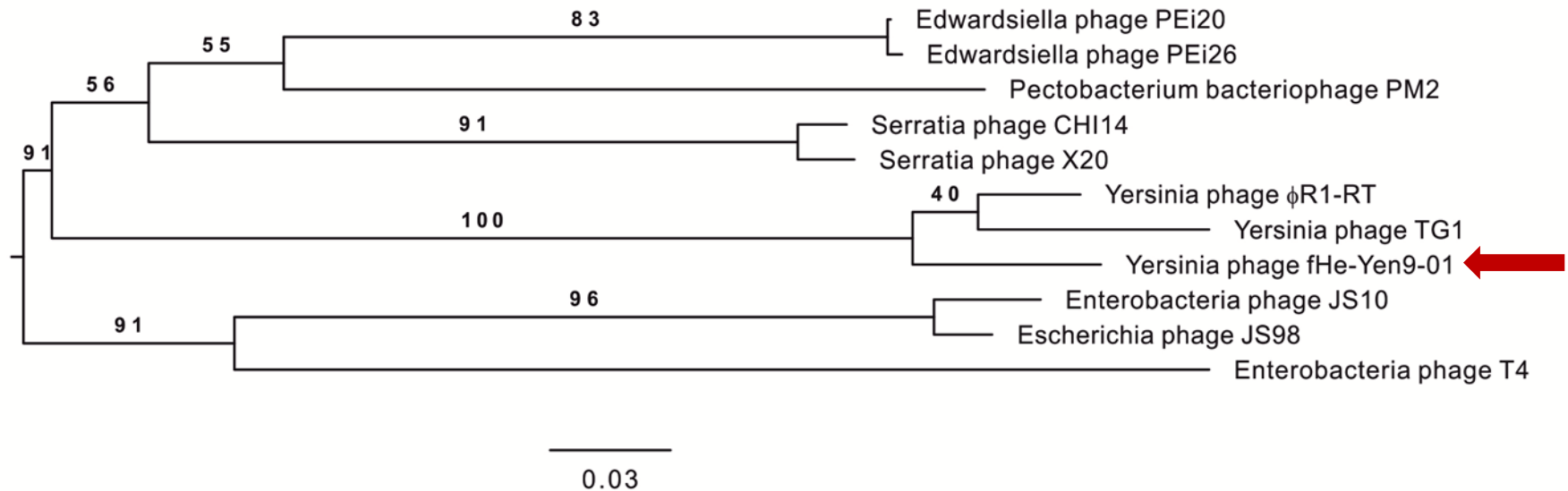


## Genomic map of fHe-Yen9-01



Green gene: shared with vB\_YenM\_φR1-RT

Red gene: shared with vB\_YenM\_TG1

Phylogeny of fHe-Yen9-01

**Genes related to lysogeny or virulence factors were not found in the genome.**





Centers for Disease Control and Prevention  
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4 °C



Avoid eating raw or undercooked pork >



Learn the safe minimum cooking temperature for pork >

Drink only pasteurized milk



Consume only pasteurized milk and milk products, such as soft cheese, ice cream, and yogurt. Find out about the dangers of raw milk >

Wash hands with soap and water



Wash hands with soap and water before eating and preparing food, after contact with animals, and after handling raw meat. See why handwashing is like a "do-it-yourself" vaccine >

Take care when preparing chitlins



After handling raw chitlins, clean hands and fingernails thoroughly with soap and water before touching infants or their toys, bottles, or pacifiers. Someone other than the person handling food should care for children while chitlins are being prepared. Find out how to stay safe while preparing chitlins >

Use separate cutting boards



Use separate cutting boards for meat and other foods and carefully clean all cutting boards, countertops, and utensils with soap and hot water after preparing raw meat. Learn four simple steps to food safety >

Dispose of animal feces in a sanitary manner



Pick up and dispose of animal feces (poop), especially in areas where children might play. Wash your hands with soap and running water after contact with animal feces. Be sure to avoid children with handwashing. Get tips to help keep you and your pets healthy >



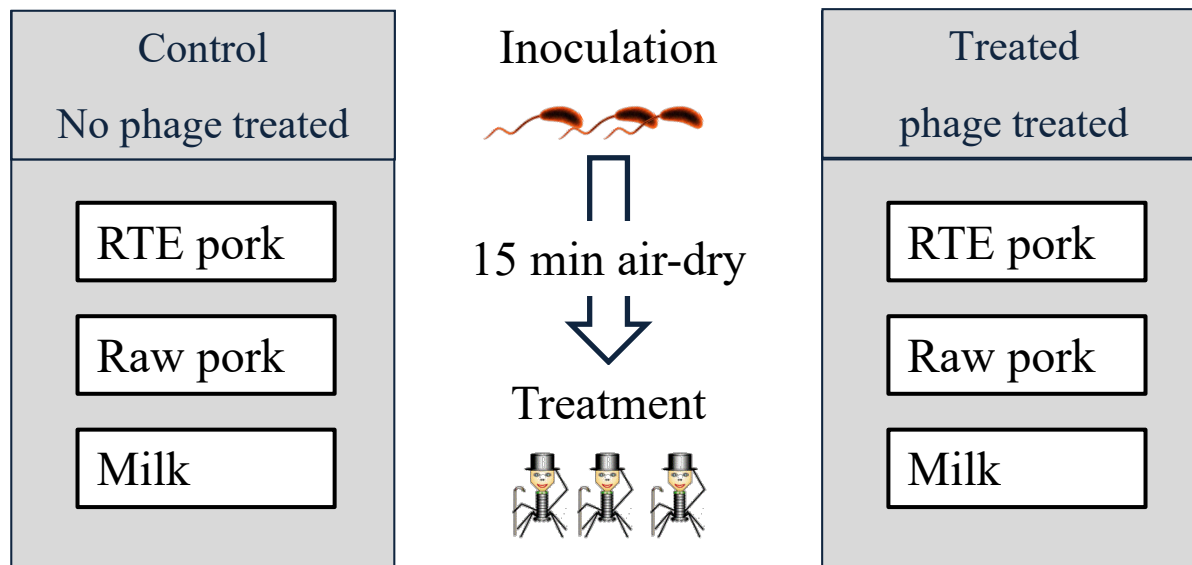
Ready-to-eat  
barbequed pork

Raw minced pork

### Food samples

Inoculation: *Yersinia* contamination ( $10^3$ CFU/ g or ml )

Treatment: phage application ( $10^8$ PFU/ g or ml )



In duplicate. 72 h (raw pork and milk), 12 h (RTE pork)

Temp.:  $26 \pm 1$  °C (RTE pork). 4 °C (raw pork and milk)

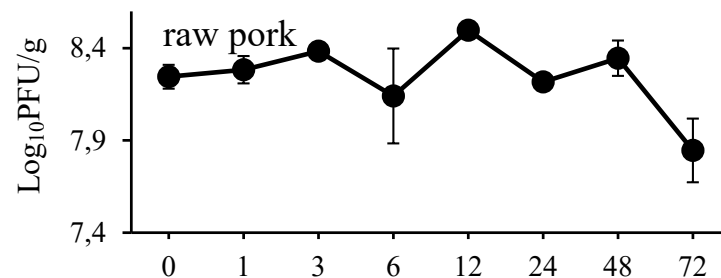
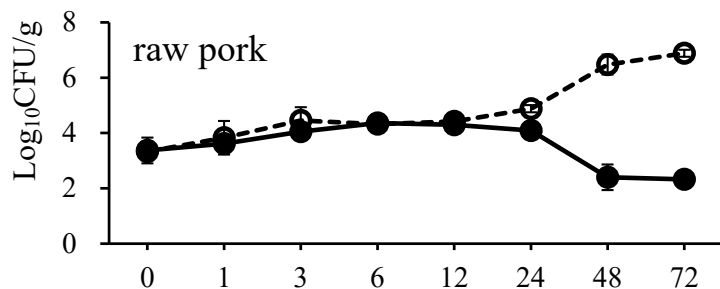
CFU count by selective (CIN) agar; PFU count by double-layer agar method

### Food samples

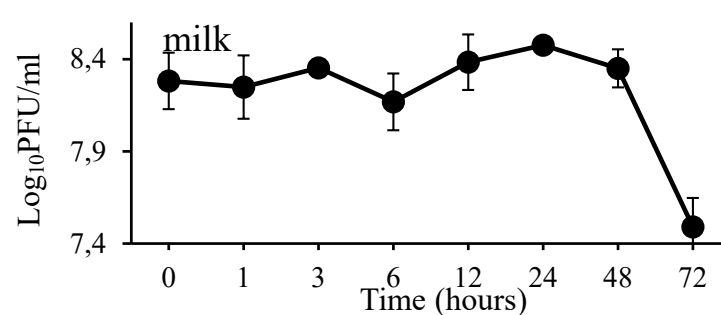
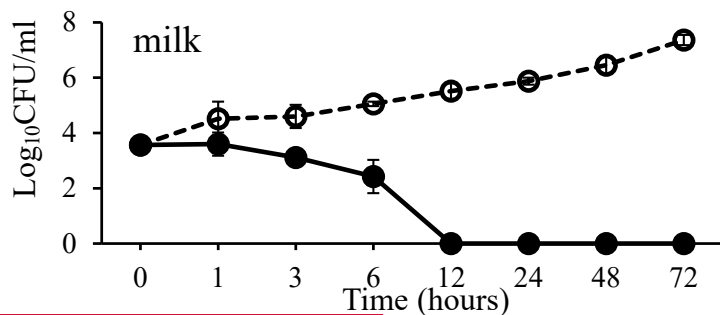
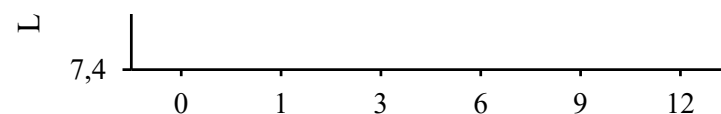
CFU, left; PFU, right

Control (non-treated) group: open circle

Phage-treated group: filled circle



**Bacterial counts decreased by 1-3 logs from the original levels.**





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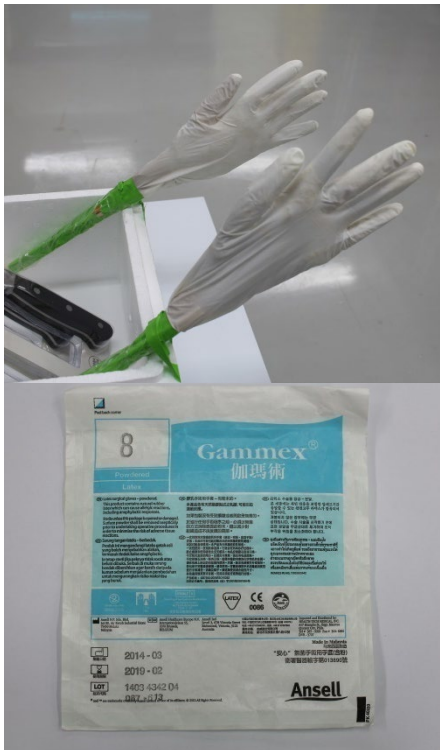
Cutting board (plastic)



Cutting board (wood)

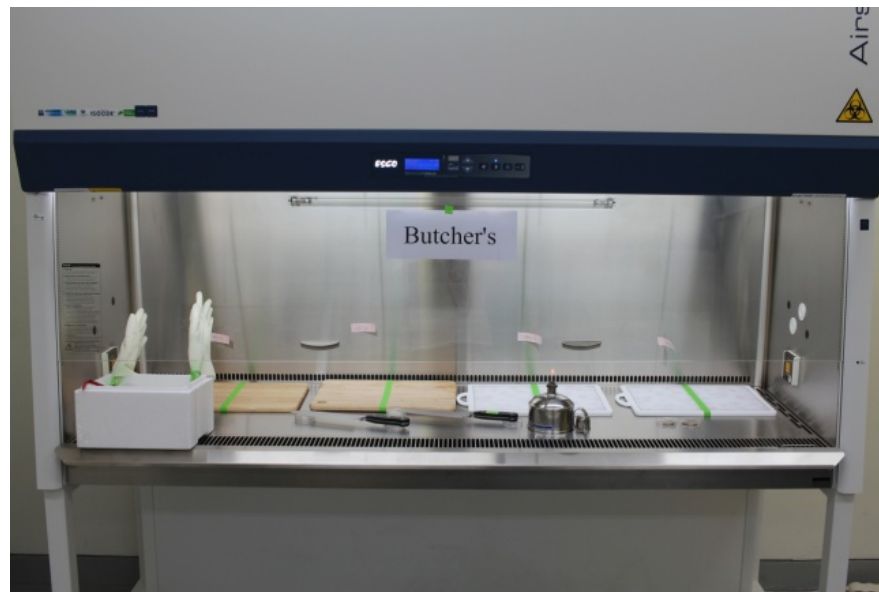


Knife

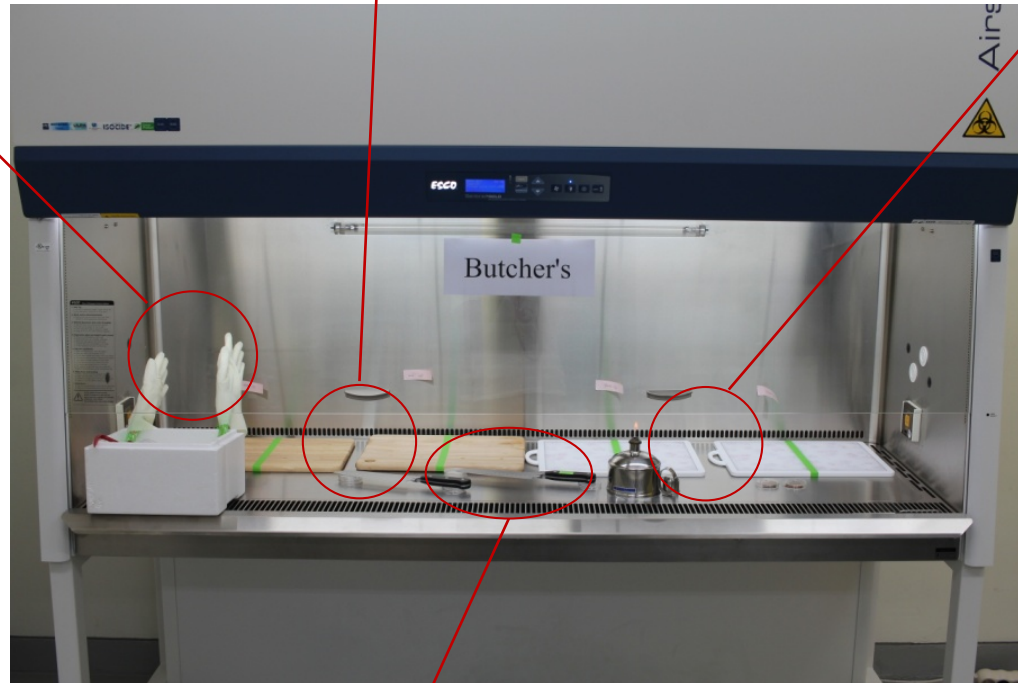
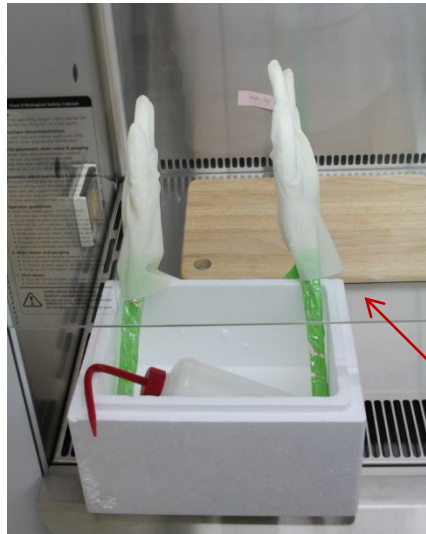


Artificial hands  
(surgical gloves)





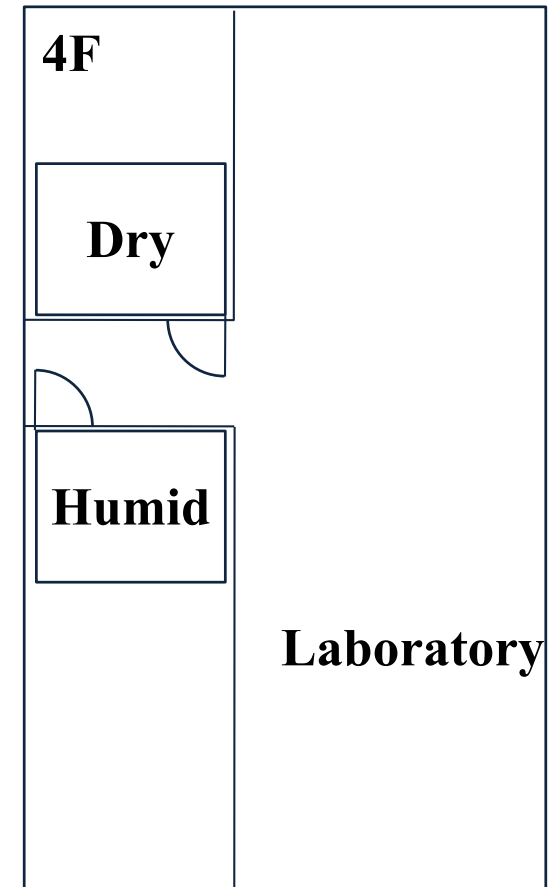




### Dry vs. humid

Dry condition: biosafety level-2 laminar flow hood

Humid condition: biosafety level-2 laminar flow hood  
(relative humidity,  $81 \pm 3 \%$ )



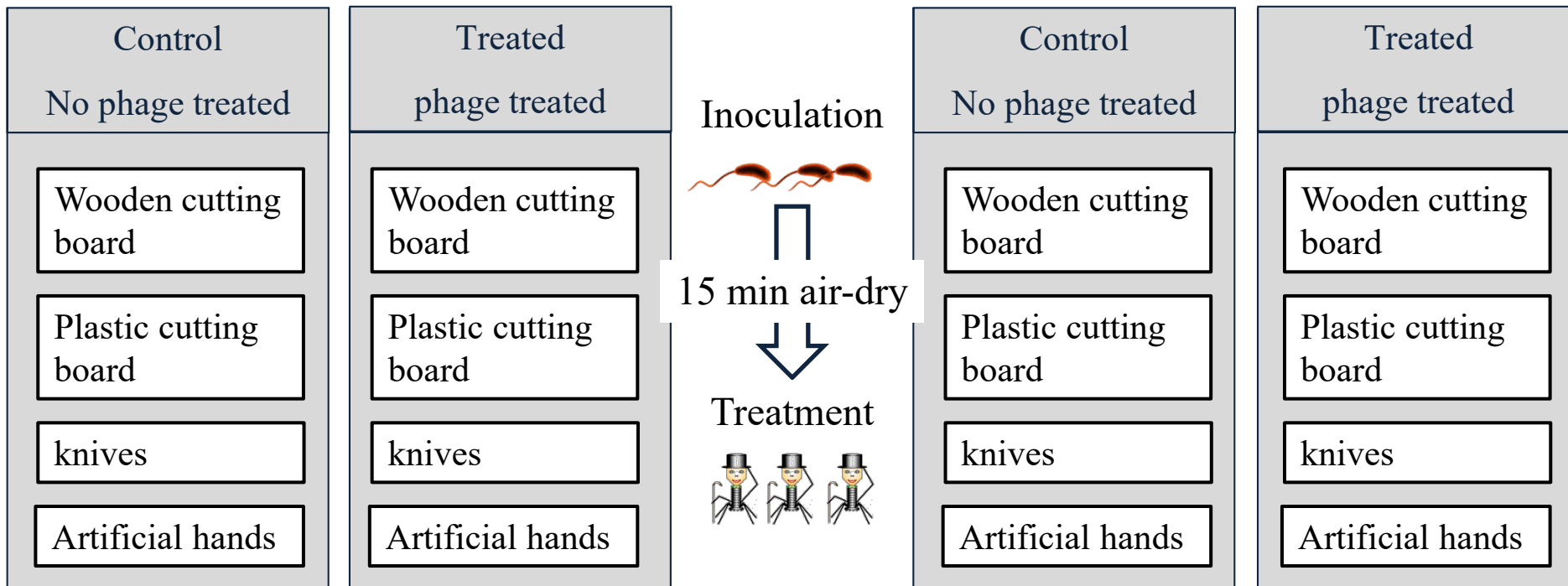
Kitchen utensils

Inoculation: *Yersinia* contamination ( $10^3$ CFU/ cm<sup>2</sup> or ml )

Treatment: phage application ( $10^8$ PFU/ cm<sup>2</sup> or ml )

## Dry condition

## Humid condition

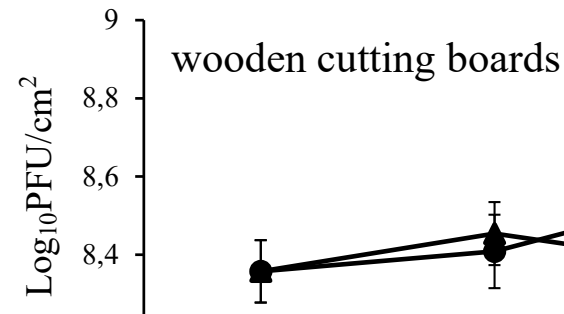
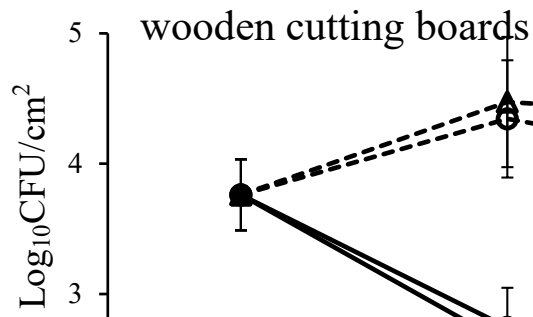


For up to 2 h

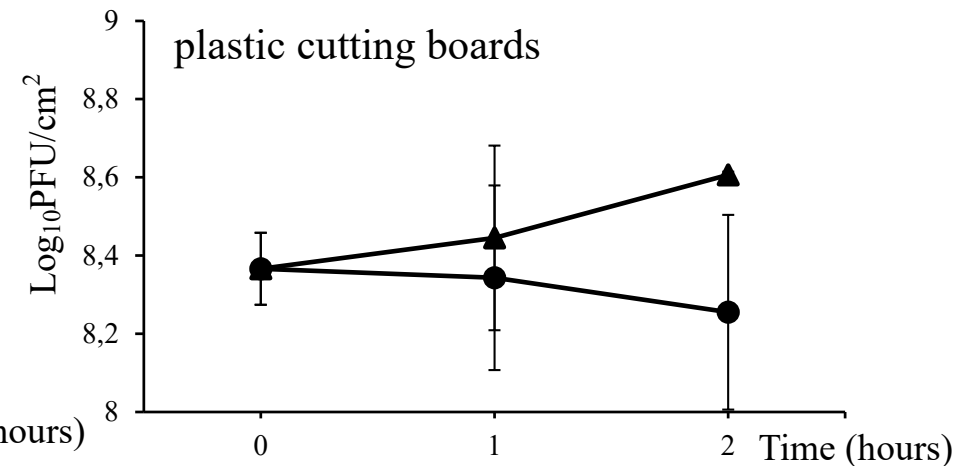
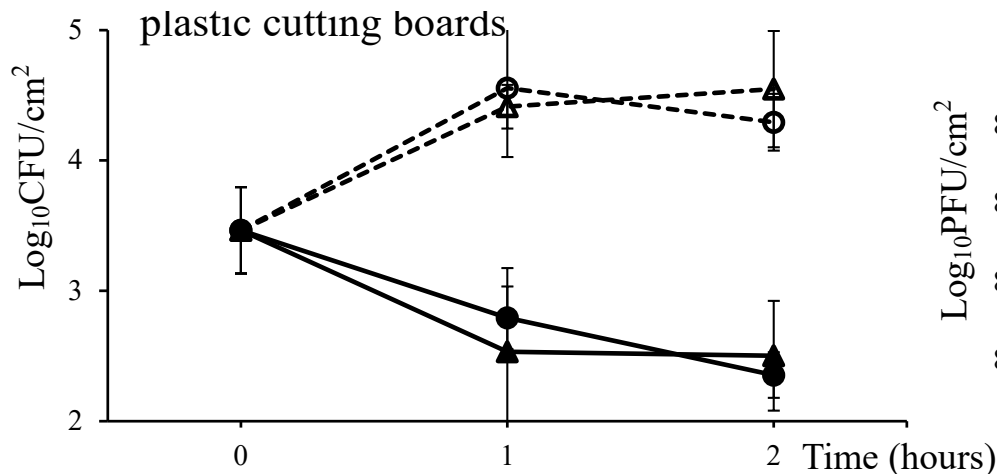
CFU, left; PFU, right

Treated group, filled symbols; control, open symbols

Dry, triangles; humid, circles

Kitchen utensils

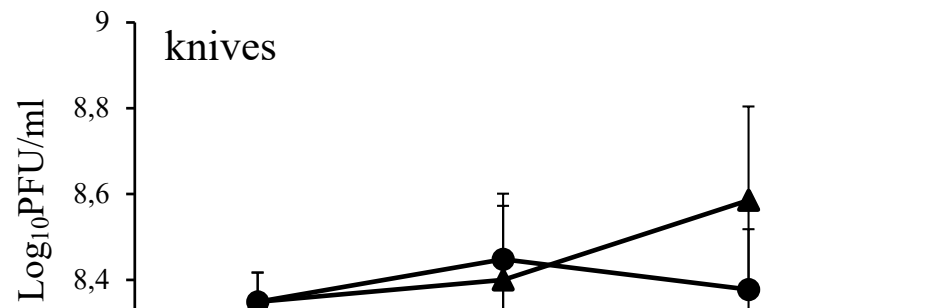
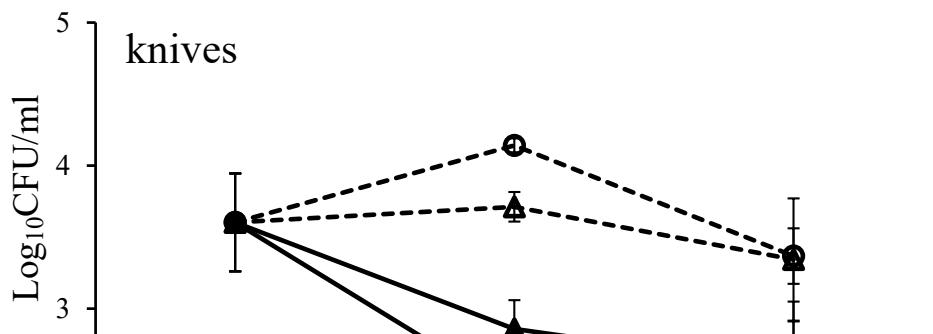
**Bacterial counts decreased by 1-2 logs from the original levels.**



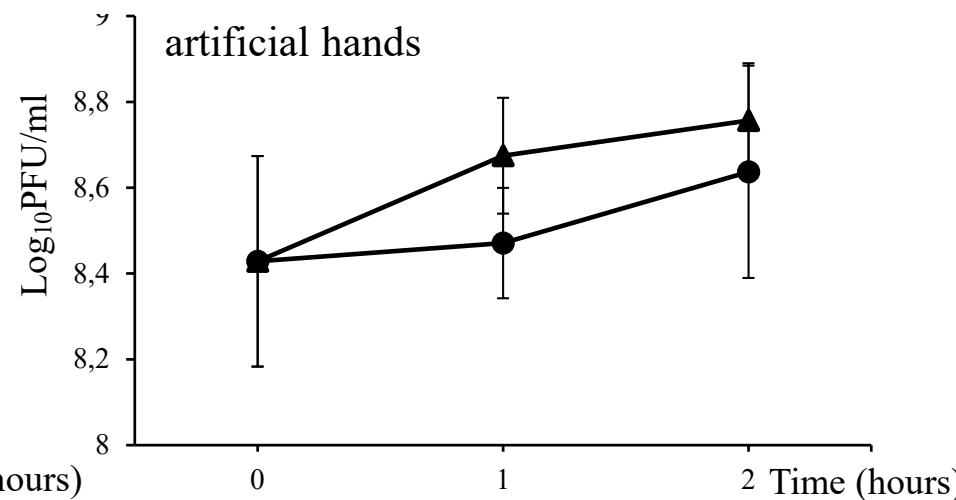
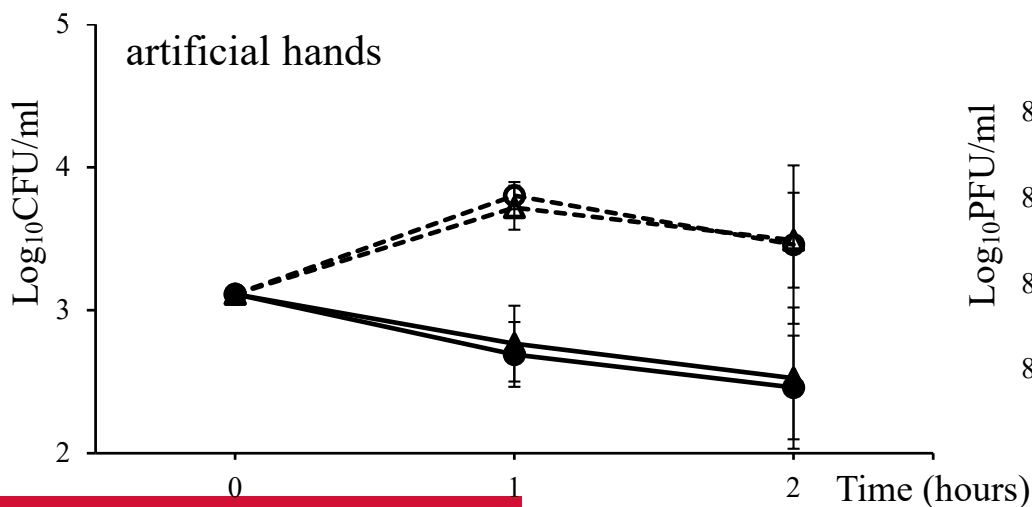
CFU, left; PFU, right

Treated group, filled symbols; control, open symbols

Dry, triangles; humid, circles

Kitchen utensils

**Bacterial counts decreased by 1-2 logs from the original levels.**





### Phage safety assessment in mice

- ❖ Ethics statement, SNU-170417-7
- ❖ Six-week-old SPF BALB/c mice
- ❖ Five groups of six mice
- ❖ Crude phage lysate,  $10^{10}$ PFU/ ml; highly purified preparation,  $10^{12}$ PFU/ ml
- ❖ Intragastrically administration
- ❖ The health of the mice was monitored for 28 days.
- ➡ Both phage preparations did not affect the physical condition or survival of the mice over the 28 days of observation.

- ❖ Keeping kitchen utensils under humid conditions were not recommended.
- ❖ It would be desirable to dry kitchen utensils as soon as possible.

➡ **This is hard to achieve in the kitchen environment.**

**The presence of food residues may increase the risk of *Yersinia* spreading.**

- ❖ Certain foods pose a yersiniosis risk.

**Mett** is a preparation of minced raw pork that is **popular in Germany**.



# Thank you for your attention.

