Introduction

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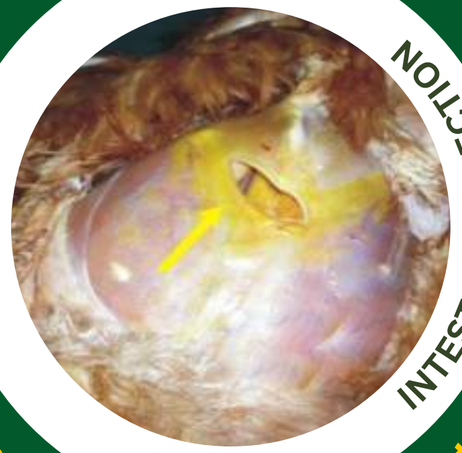
Poultry mortality



INTESTINAL INFECTION

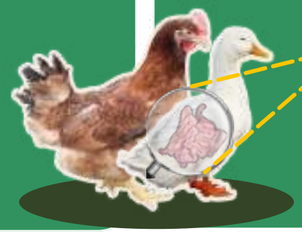
Vietnamese livestock industry

PROBLEM

01 **Antibiotic overuse**

- Antibiotic-resistant bacteria
- Consumer and environmental health

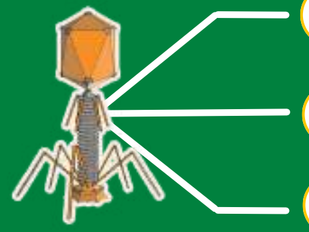
02 **E.coli APEC O78:H9**



- High virulence
- Multidrug resistance (MDR)
- Potential risk of colibacillosis outbreaks

SOLUTION

03 **Phage therapy**



Bacteriophage

- Natural origin
- Specific lytic ability against pathogenic bacterial cells
- Safety for animals and humans

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Isolation and screening bacteriophage strains with strong lytic activity specific to

Escherichia coli O78:H9

causing disease in chickens and ducks in Hai Phong, aiming to replace antibiotics in livestock production.

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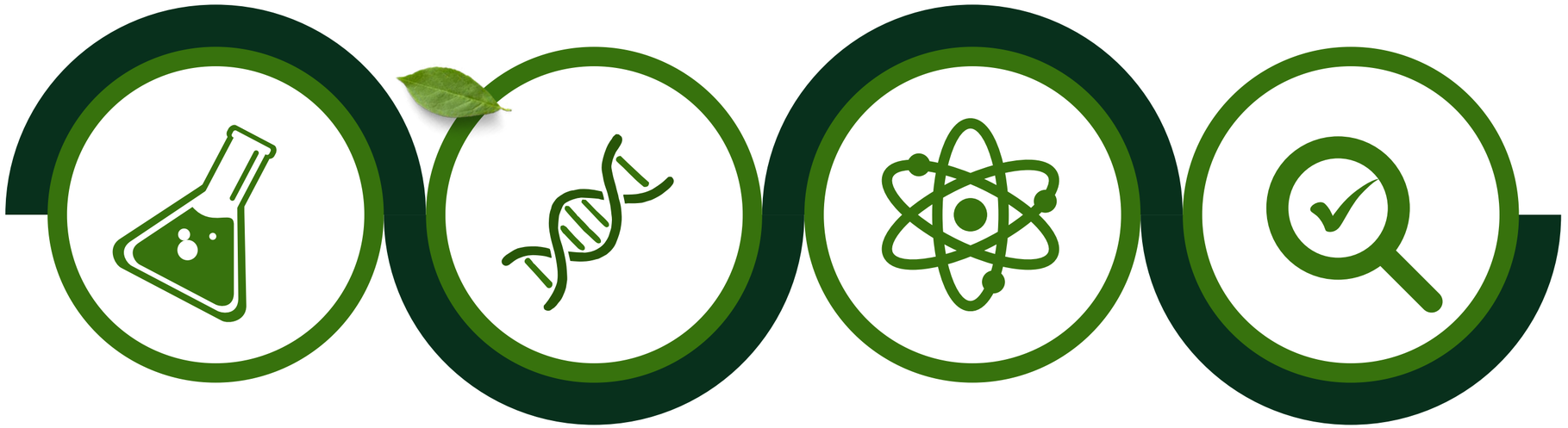
Objectives

01 | ***Isolate bacteriophages*** *from poultry farm wastewater in Hai Phong capable of lysing E. coli O78:H9.*

02 | ***Select phage strains*** *showing strong lytic activity and high specificity toward multidrug-resistant E. coli O78:H9.*

03 | ***Characterize the selected strain*** *by morphology, host range, and in vitro bactericidal efficiency.*

04 | ***Evaluate its stability*** *under various* ***pH and temperature*** *conditions.*



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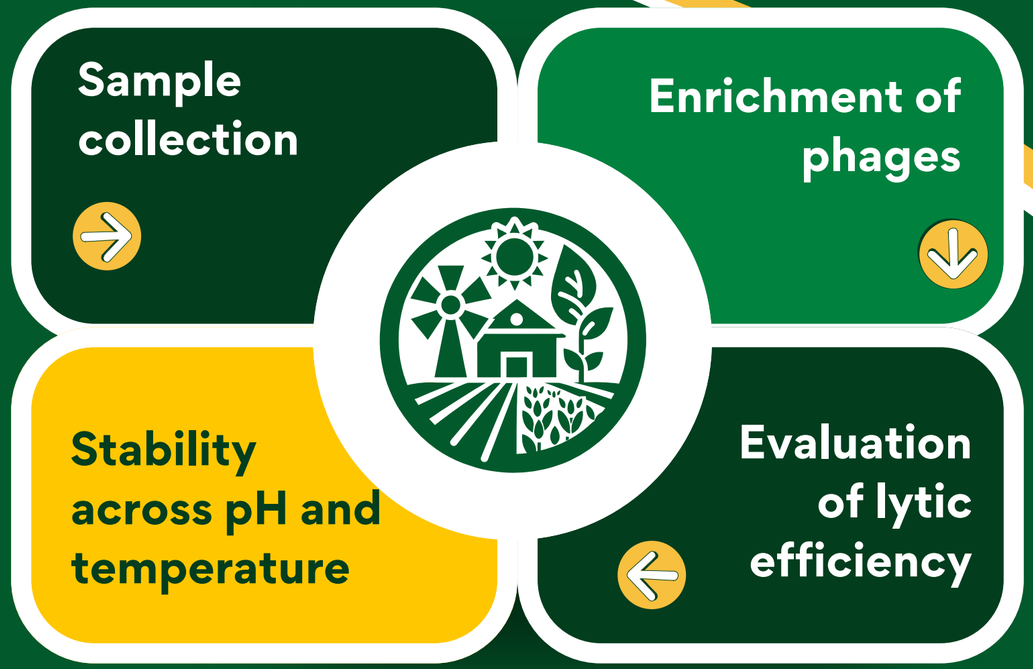
METHODOLOGY



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METHODOLOGY

Sample collection

Enrichment of phages

Evaluation of lytic efficiency

Stability across pH and temperature

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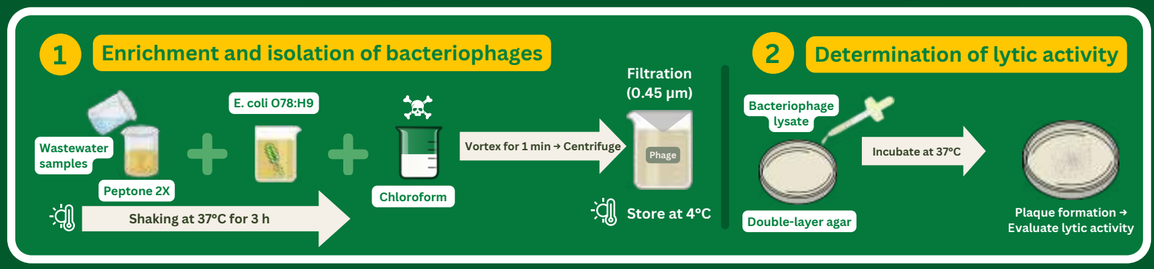

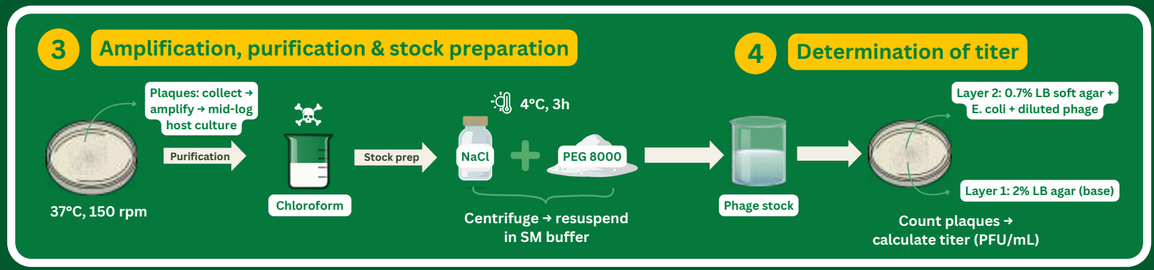

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METHODOLOGY

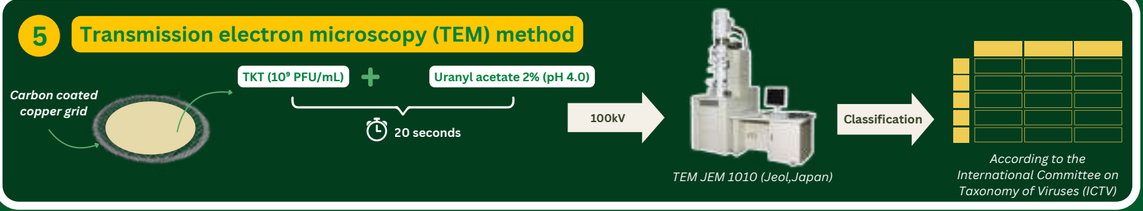
Sample collection

Enrichment of phages

Stability across pH and temperature

Evaluation of lytic efficiency

5 Transmission electron microscopy (TEM) method



6 Evaluation of lytic efficiency

7 Evaluation of lytic efficiency

Layer 2: 0.7% LB soft agar + E. coli + diluted phage

Layer 1: 2% LB agar (base)

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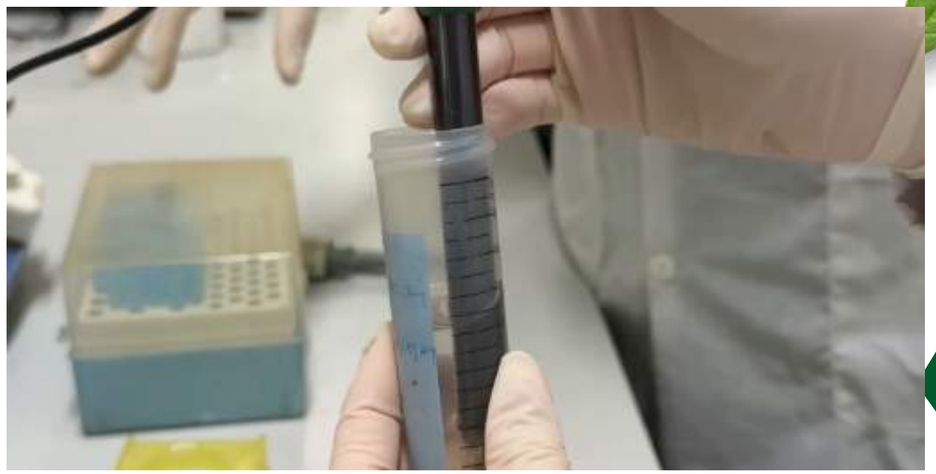
METHODOLOGY

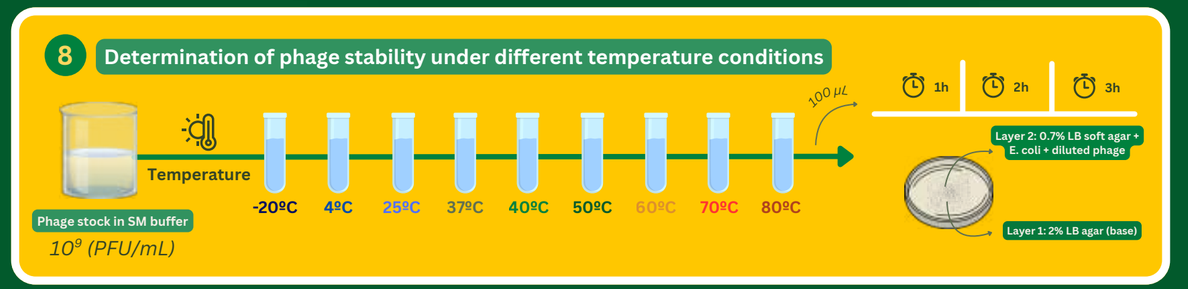


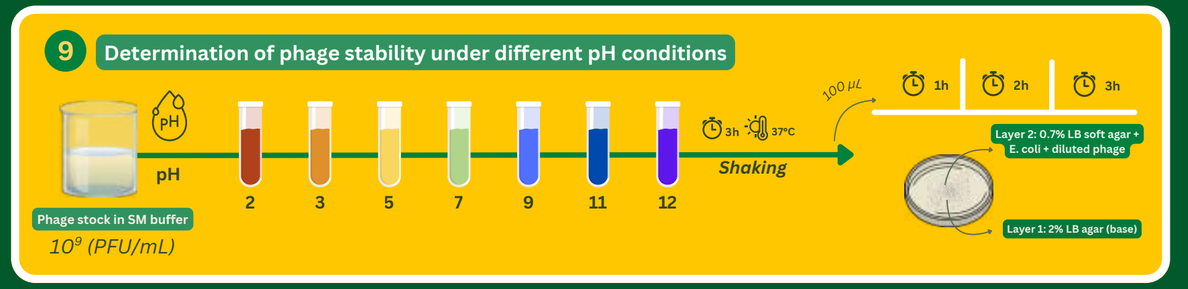
Sample collection

Enrichment of phages

Evaluation of lytic efficiency

Stability across pH and temperature





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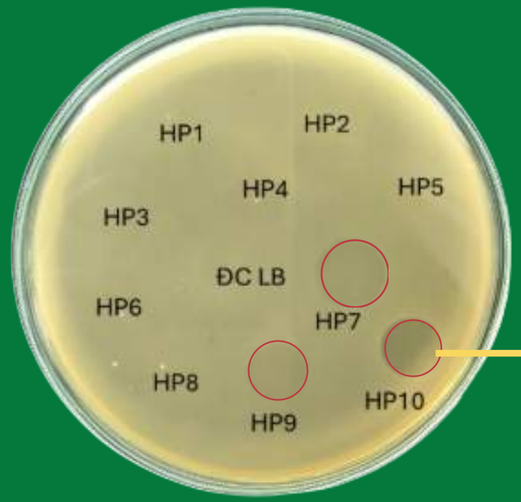
RESULTS AND DISCUSSION

Phage isolation



From 20 wastewater samples, **only three (HP7, HP9, HP10)** showed clear plaques, indicating the presence of bacteriophages.

Sample HP10 exhibited the **most distinct plaques** and was selected for amplification and purification, designated **PE1**.

Figure 1A. Plaques of bacteriophages on the double-layer agar plate.

- Round, transparent plaques

→ **Strong and specific lytic activity** against *E. coli* O78:H9.

- After amplification, the **phage titer** reached **1.3×10^9 PFU/mL.**

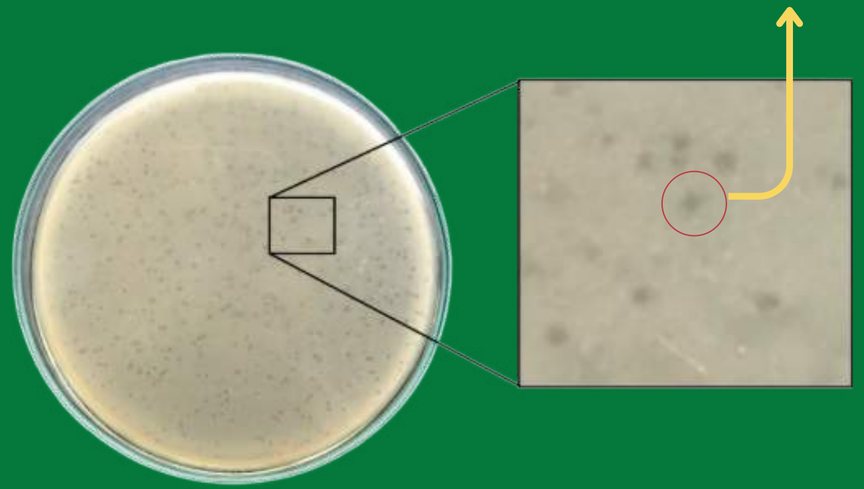
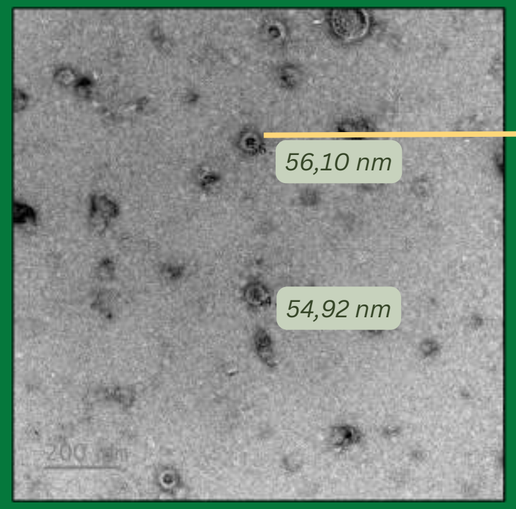


Figure 1B. Plaques of purified phage PE1 on E. coli O78:H9.

RESULTS AND DISCUSSION

Identification of the phage using transmission electron microscopy (TEM)



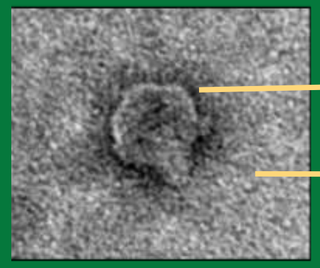


Figure 3. TEM image of phage PE1.

Head diameter: 55.51 ± 0.59 nm.

According to ICTV (2022): PE1:

- **Podoviridae** group.
- Class Caudoviricetes.

The head is icosahedral

The tail is short and non-contractile.

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RESULTS AND DISCUSSION

Bactericidal activity of phage PE1

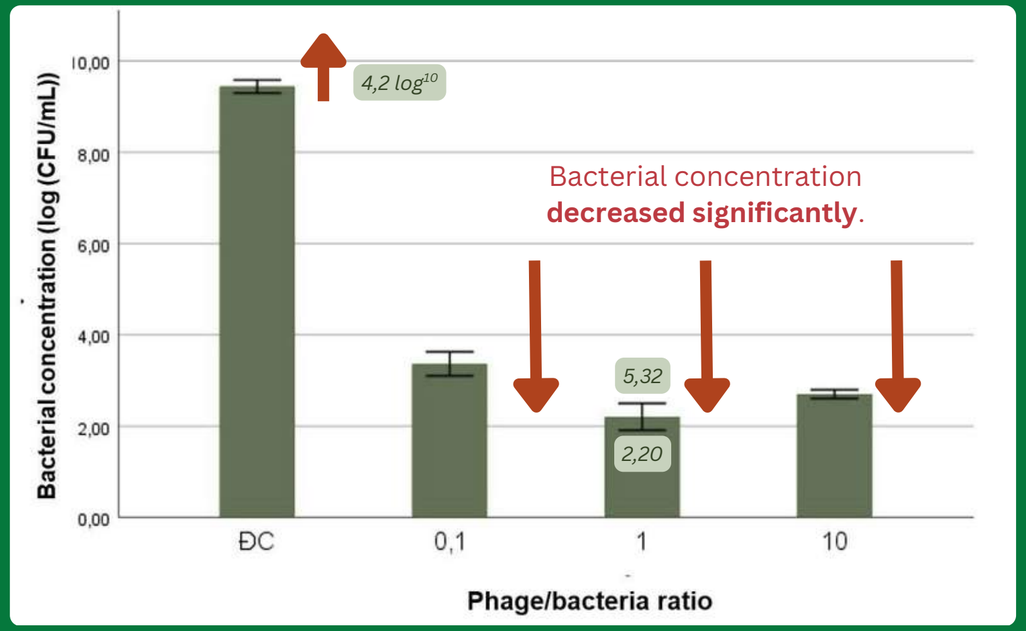

Figure 2. Bactericidal effect of phage PE1 against E. coli O78:H9

- The **bacterial reduction** ranged from **1,95 - 3,11 log CFU/mL** after 3 hours.
- The **1:1** ratio showed the **highest efficiency**, reducing the bacterial concentration from **5,32 - 2,20 log CFU/mL**.

The strong bactericidal activity of phage PE1 against E. coli O78:H9
→ **Potential** as a **biological agent** for controlling E. coli O78:H9.

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RESULTS AND DISCUSSION

Host range of phage PE1

Bacterial species	Strain	Source	Plaque formation
Escherichia coli	*E.coli* O78:H9 (HPVN24)	Diseased chicken and duck	++
	E1		++
	E2		+
	E3		+
	E4		-
	E5		-
Bacillus cereus	BC1	Beef	-
	BC2		-
Salmonella enterica	*Salmonella*_HaiDuong1	Diseased chicken	-
	*Salmonella*_HaiDuong2		-
Vibrio parahaemolyticus	Vp-HP1	Diseased shrimp	-
	Vp-HP2	Diseased shrimp	-
Lactobacillus kunkeei	LK_VN01	Honey	-
Staphylococcus aureus	SA 01	Beef	-
	SA 02	Beef	-

Table 1: Host range of phage PE1.

++ : Clear plaques + : Faint plaques - : No plaques formed

- **PE1** lysed **3/5** additional E.coli strains beyond its **main host**:
  - E.coli O78:H9 (HPVN24)
  - E1
  - E2
  - E3
- **Strong, specific activity** against E. coli strains.

Potential to **inhibit pathogenic E. coli** in poultry in Vietnam.

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RESULTS AND DISCUSSION

Evaluation of PE1 activity under different temperature and pH conditions

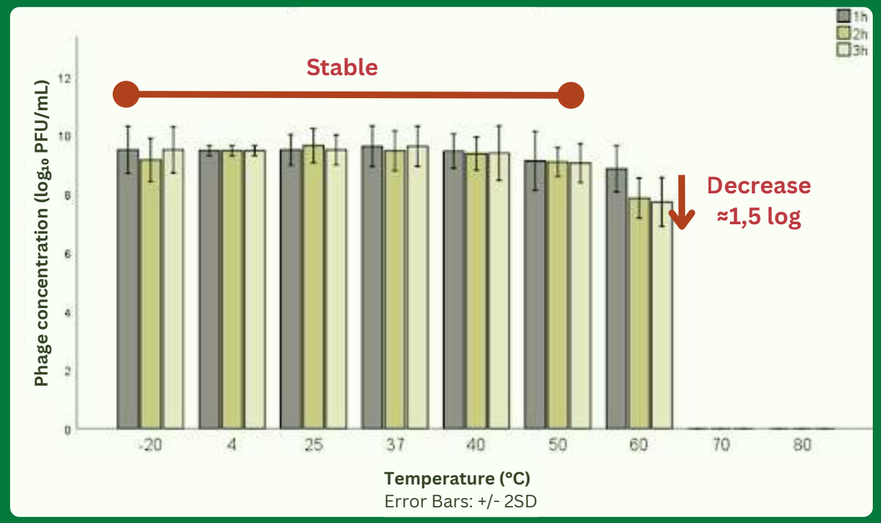

Figure 4. Phage PE1 titer after 1, 2, and 3 hours under different temperature conditions.

- **–20 - 50°C: Stable.**
- **60°C: Stable** after **1 hour**, but **decreased** by **≈1.5 log** after **3 hours.**
- **70 - 80°C: Inactivated,** with the phage titer dropping to ~0 PFU/mL.

Figure 5. Phage PE1 titer after 1, 2, and 3 hours under different pH conditions.

- **pH 5 - 12: Stable** across a wide pH range.
- **pH 3: Decreased by ≈3 log** (PFU/mL) after **1 hour and completely inactivated** after **2 hours.**

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Conclusion

The bacteriophage strain PE1 was successfully isolated

Showing strong lytic activity and **high specificity against Escherichia coli O78:H9 (HPVN24).**

Transmission electron microscopy (TEM) revealed that PE1:

- Has an **icosahedral head** measuring **55.51 ± 0.59 nm.**
- Has a **short, non-contractile tail.**
- Belongs to the **Podoviridae family, Caudoviricetes class.**

The host range of PE1

- Lysed **3 out of 5 E. coli isolates and E.coli O98:H9** with varying degrees of activity.
- No lytic activity was observed against other tested bacterial species.

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Conclusion

The bactericidal efficiency of PE1:

At a phage-to-bacterium ratio (MOI = 1), the bacterial count decreased from **5.32 $\log_{10}$ CFU/mL to 2.20 $\log_{10}$ CFU/mL.**

The stability of PE1 under temperature and pH:

- **Stable** within the temperature range of **–20°C to 50°C** for 3 hours.
- **Stable** within the pH range of **5 to 12** after 3 hours of testing.

The PE1 phage strain shows strong potential for developing phage therapy to prevent and treat E. coli infections in chickens and ducks.

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Future work

- **Perform a comprehensive evaluation of bactericidal efficacy**
  (Compare PE1 with other phages and with antibiotics).
- **Analyze and decode the PE1 genome**
  (Identify genes for lytic enzymes, structural proteins, and any virulence-related genes).
- **Assess stability and biosafety**
  (Test irritation potential and effects on beneficial microbiota in chickens and ducks).
- **Conduct on-farm field trials**
  (Apply PE1 in prevention and treatment models for E. coli–induced diarrhea in poultry).

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Safer

Sustainable

Environmentally friendly

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Thank You