

# Screening of Bacteriophages from Environmental Samples and Multidrug-Resistant *Helicobacter pylori* Strains

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## BACKGROUND

The increasing prevalence of multidrug-resistant (MDR) *Helicobacter pylori* has significantly hindered eradication efforts (1), highlighting the need for alternative therapies. Phage therapy offers a promising strategy to overcome antibiotic resistance by utilising bacteriophages as targeted antibacterial agents (2). This study aimed to screen bacteriophages from environmental sources and MDR *H. pylori* strains.

## METHODS

### A. Environmental samples

- Type of samples: hospital sewage (n=1), residential sewage treatment plant, KLR 129 (n=6), household septic tank (n=1), seawater (n=1), river (n=1), pond (n=1), drain (n=1), and algae (n=4).
- Phage isolation and screening (3):
  - 10 ml samples were centrifuged for 20 min for 4000 rpm.
  - Supernatant was filtered through 0.22 µm syringe filter and lysates were kept at 4°C for later used.
  - Lysates were mixed with MDR *H. pylori* (n=25) in 96-well microplate and incubated at 37°C for 24 h in microaerophilic condition.
  - After incubation, OD<sub>600</sub> was measured.
  - Samples with OD value lower than the OD of positive control was considered to contain potential phages.
- Spot test:
  - H. pylori* suspensions (from OD screening) were lawn on CBA medium.
  - 10 µl of the corresponding lysates were dropped on the bacterial lawn.
  - Plates were incubated at 37°C for 72 h in microaerophilic condition.
  - Lysis zone was observed after incubation.

### B. UV induction of *H. pylori* prophage (4)

- MDR *H. pylori* (n=25) suspensions (McFarland 3) were centrifuged at 4000 rpm for 15 min.
- Pellet were mixed with 0.1 M MgSO<sub>4</sub> and transferred to 12-well microplate.
- Plates were irradiated with UV 245 nm lamp for 30, 60, 90 and 120 sec.
- After irradiation, bacterial suspension was mixed with BHI broth and incubated 37°C for 24 h in microaerophilic condition.
- Suspensions were then centrifuged at 4000 rpm, 15 min, 4°C.
- Supernatants were filtered using 0.22 µm membrane and lysates were used for spot test.

#### References:

- Gau et al. 2025. *Frontiers in Microbiology*. DOI: 10.3389/fmicb.2025.1626930
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## RESULTS

### Environmental samples

Strain no.	Spot test positive	Diameter of lysis, mm
HP18024	Household septic tank	4
	KLR 129 (crude sample)	9
	KLR 129 (final effluent)	9
HP19021	Hospital sewage	2
	Household septic tank	3
	River	15
HP20003	Pond	13
HP21043	Pond	10
HP22005	River	10
HP22027	Household septic	3
HP23017	KLR 129 (crude sample)	9
HP23018	Hospital sewage	2
	River	1
	Drain	3

### Prophage induction

Strain no.	Exposure time (spot test positive)	Diameter of lysis, mm
HP19005	120 s	2
HP19033	90 s	2
HP19046	30 s	5
HP19052	60 s	3
HP21041	30 s, 60 s, 90 s, 120 s	3-5
HP23017	90 s	4



Figure 2. Spot test showed lysis zone of HP23017 strain after 90 s exposure to UV irradiation

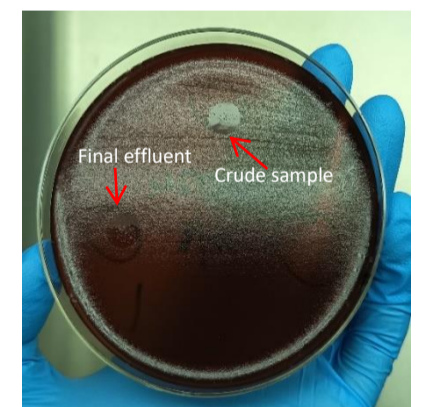


Figure 1. Spot test showed lysis zone of HP18024 strain towards lysates from crude sample and final effluent of sewage treatment plant, KLR 129

## DISCUSSION

- Environmental samples (pond, river, sewage and septic) yielded phage lysates with variable lytic activity against bacterial hosts.
- Lysates from pond and river showed the strongest lysis activity, followed by moderate activity for lysates from treatment plants, while lysates from hospital sewage, septic and drain showed weak lytic activity.
- Prophage induction revealed heterogenous responses; HP19046 displayed rapid and strong induction, while HP21041 showed consistent inducibility across multiple time points.
- In conclusion, identification of lytic phages from environmental samples and inducible prophages provide candidates for further confirmation, characterisation, genomic analysis and potential development in phage therapy for MDR *H. pylori* infection.

#### Acknowledgment:

This work was supported by Universiti Kebangsaan Malaysia through the research university grant scheme (grant no. GUP-2023-070).

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