Phage Isolation

Date: 2025-11-06 ~ 2025-11-22 | **Operator:** Yuhang Gong | **Status:** Completed

Objective

Isolate bacteriophages infecting EcAZ-1 (W strain) and EcAZ-2-OVA (R strain) from wastewater.

Materials

Bacterial Strains:

Name	Code	Source	Notes
EcAZ-1	W	Amir Zarrinar Lab	Wild type
EcAZ-2-OVA	R	Amir Zarrinar Lab	OVA-expressing

Reagents: 2x LB, SM Buffer, 0.22um filter, Soft agar (0.7%), LB agar plates (1.5%)
Equipment: Centrifuge, 0.22um filtration apparatus, 37°C shaker, 37°C incubator, Micropipette

Protocol

Sample Processing: Centrifuge wastewater → Filter supernatant (0.22um)
Phage Enrichment: Overnight host culture + 2xLB + wastewater supernatant → 37°C shaking overnight → Centrifuge & filter
Screening & Purification: Spot Test → Plaque Assay (double-layer) → Single clone isolation (5-6 rounds)

Experimental Records

Phase 1: Enrichment & Initial Screening (11-06 ~ 11-08)

Date	Operation	Results
11-06	Phage enrichment from wastewater; Spot test	
11-07	Spot test results; Plaque assay (10^{-9}~10^{-11})	Phage presence confirmed
11-08	Plaque assay results; Single clone selection	R: 4 types; W: 2 types

Phase 2: First Purification (11-09 ~ 11-10)

Date	Operation	Results
11-09	Enrichment; Plaque assay (10^{-9}~10^{-12})	
11-10	Results	R: failed (too concentrated); W: succeeded

Phase 3: Continued Purification (11-11 ~ 11-16)

Date	Operation	Results
11-11	R: Plaque assay (10^{-10}, 10^{-15}); W: Clone selection	R: still contaminated
11-12	W: Clone selection; R: Filtered & stored	W: Small plaques disappeared
11-13	W: 4th purification; R: 3rd purification	
11-14	Post-purification enrichment	W: 2 types; R: 3 types retained
11-15	W: 5th purification; R: 4th purification	

Date	Operation	Results
11-16	Results; Enrichment	Essentially pure; Pipette tips > inoculation loops

Phase 4: Final Purification (11-17 ~ 11-22)

Date	Operation	Results
11-17~18	Centrifuged; Prepared plates	
11-19~20	Plaque assay ($10^{-10/12/14}$)	R3: need higher dilution; W1 split into W1 & W2
11-21~22	Final plaque assay	Pure stocks prepared

Results

Successfully isolated 5 phage strains:

Phage	Host	Purification	Storage	Notes
R1	EcAZ-2-OVA	4-5 rounds	4°C	
R2	EcAZ-2-OVA	4-5 rounds	4°C	
R3	EcAZ-2-OVA	4-5 rounds	4°C	High titer
W1	EcAZ-1	5-6 rounds	4°C	
W2	EcAZ-1	5-6 rounds	4°C	Split from W1

Conclusions

1. Isolated 5 phages from wastewater (R: 3 strains; W: 2 strains)
2. R3 has highest titer (requires >10^{-14} dilution)
3. W2 separated from W1 during purification
4. One unstable small-plaque phage was lost

Notes & Improvements

- High-titer phages require higher dilutions ($10^{-14/16/18}$)
- **Use pipette tips instead of inoculation loops** for clone picking
- Replace bacterial cultures weekly

Recorded: 2025-01-14