REFINING METHODS FOR ISOLATING, PURIFYING, AND CHARACTERIZING *ENTEROBACTER* BACTERIOPHAGES

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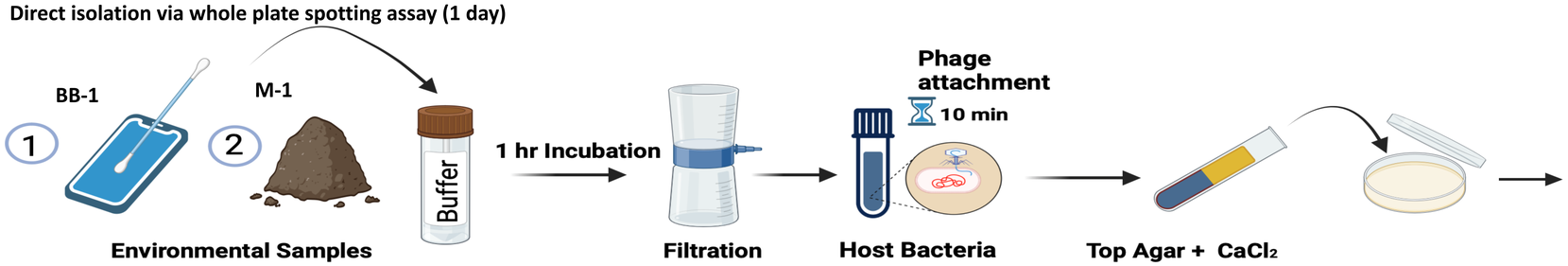
I. Introduction

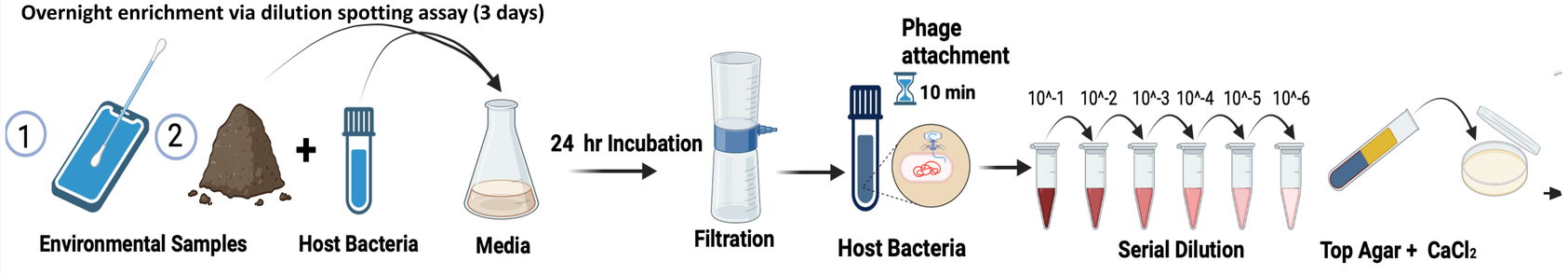
Bacteriophages infect bacteria and hijack the cellular machinery of their bacterial hosts to replicate, which ultimately leads to the host's destruction. Recent success in emergency phage therapy to treat antibiotic-resistant infections highlights the potential for this as an alternative or in addition to antibiotic. However, this requires the generation of a diverse phage library as most bacteriophage are highly specific in which bacterial strains they can infect. The first step in this process is to isolate new bacteriophages. To do this efficiently, it is important to optimize the protocol for isolation, purification, and characterization of the phages. Many academic groups have described protocols to obtain phages; however, there is not a defined consensus among the academic community. In this study, we refined methods for isolating and studying bacteriophages against *Enterobacter aerogenes*, a critical ESKAPE pathogen contributing to antibiotic resistance. We isolated three phages using different environmental samples and evaluated two isolation techniques: the overnight enrichment assay and direct isolation via the whole plate spotting assay. We also compared commercial DNA extraction kits versus zinc chloride precipitation to identify the more effective and time efficient procedure.

II. Isolation assay

Direct isolation via whole plate spotting assay (1 day)



Overnight enrichment via dilution spotting assay (3 days)



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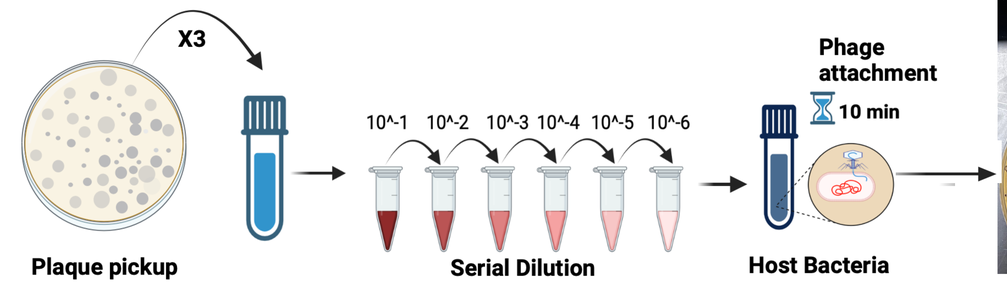
III. Purification assay



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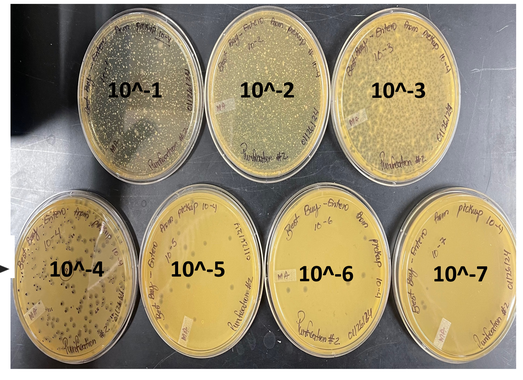
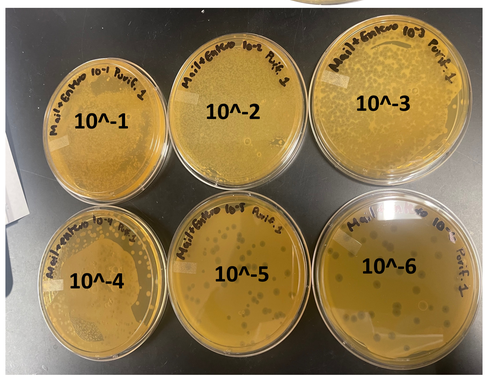


Incubation of BB-1 for 10 hrs.



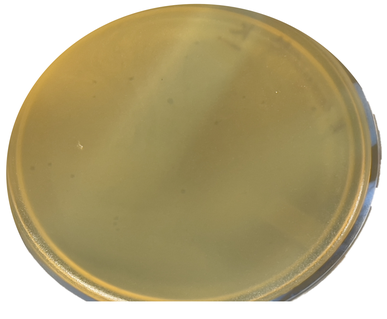
Incubation of M-1 for 24 hrs.

IV. DNA extraction and phage characterization

DNA extraction Protocol Comparison in BB-1 phage

	Concentration (ng/µL)	A260/ A280	Price per prep	Working time (min)
QIAamp Viral RNA Mini Kit	388.1	2.46	$7.30	45 -60
GRS Viral DNA/RNA Purification Kit	4.8	1.82	$12.86	30
ZnCl2 Precipitation	180.9	1.78	$0.91	120

Phage cross-infectivity



+ Plaques
K-1 plated with *K. aerogenes*

V. Conclusions

- Direct isolation is a more efficient method that gives accelerated results and minimize resource utilization.
- The incorporation of calcium chloride into the soft agar enhances plaque clarity and visibility.
- Zinc chloride precipitation offered the best DNA yield and purity for the lowest cost, although it was less time efficient than commercial kits.

VI. Future directions

- Comparing lysis time of the phages.
- Testing cross-infectivity with a CDC panel of *Enterobacter* strains.
- Sequencing and annotating the phage genomes.

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