Lab Report 14 Bacteriophage Specificity

Course:

Professor's Name:

Institution :

Location of Institution :

Date :

Abstract

This experiment investigates the specificity of bacteriophages to their bacterial hosts. Bacteriophages, or phages, are viruses that infect bacteria with high host specificity, often limited to particular bacterial strains or species. The purpose of this study was to determine the host range of different bacteriophages isolated from environmental samples and analyze their specificity through plaque assays on various bacterial lawns. Results demonstrated distinct specificity patterns among phages, with some infecting multiple bacterial strains while others were highly specific. These findings have implications for phage therapy, bacterial detection, and microbial ecology.

Introduction

Background

Bacteriophages are viruses that specifically infect bacteria. They play a crucial role in microbial population control and gene transfer in natural environments. The interaction between a bacteriophage and its host bacterium is highly specific, generally determined by the recognition of receptors on the bacterial surface by phage attachment proteins. This specificity makes phages attractive tools for applications in bacterial detection, typing, and therapy, especially given the rise of antibiotic-resistant bacteria.

Bacteriophage Specificity

Phage specificity refers to the ability of a phage to infect certain bacterial strains or species but not others. This specificity depends on the interaction between phage adsorption structures (e.g., tail fibers) and bacterial surface receptors (e.g., lipopolysaccharides, membrane proteins). Determining the host range of a bacteriophage involves assessing its ability to lyse different bacterial strains, which can be tested through plaque assays.

Objectives

The primary objectives of this experiment were:

1. To isolate bacteriophages from environmental samples.
2. To test the specificity of isolated bacteriophages against different bacterial strains.
3. To analyze and interpret the host range patterns observed.

Materials and Methods

Materials

- Environmental samples (sewage, pond water)

- Bacterial strains: *Escherichia coli* (E. coli) strains A, B, and C; *Pseudomonas aeruginosa*; *Staphylococcus aureus*
- Nutrient agar plates
- Soft agar overlay medium (0.7% agar)
- Phage buffer (SM buffer)
- Sterile pipettes and micropipettes
- Incubator set at 37°C
- Sterile test tubes and flasks
- Sterile syringes and filters (0.22 µm)

Methods

Isolation of Bacteriophages

1. Environmental samples were collected from sewage and pond water.
2. Samples were centrifuged at 3000 rpm for 10 minutes to remove debris.
3. Supernatants were filtered through 0.22 µm filters to remove bacterial cells, retaining only phages.
4. Filtered samples were mixed with overnight cultures of *E. coli* strain A and incubated at 37°C for 4 hours to enrich for phages specific to *E. coli*.

Preparation of Bacterial Lawns

1. Overnight bacterial cultures were diluted 1:100 in fresh nutrient broth and grown to mid-log phase (OD600 ≈ 0.5).
2. 100 µL of bacterial culture was mixed with 3 mL of soft agar (cooled to 45°C).
3. The mixture was poured evenly onto nutrient agar plates and allowed to solidify.

Plaque Assay for Phage Detection and Specificity Testing

1. Serial dilutions of the phage-enriched samples were prepared in phage buffer.
2. 100 µL of each dilution was mixed with 100 µL of bacterial culture and 3 mL of soft agar.
3. The mixture was poured onto nutrient agar plates containing the corresponding bacterial lawn.
4. Plates were incubated overnight at 37°C.
5. Plaques (clear zones indicating bacterial lysis) were observed and counted.

Testing Phage Host Range

The same plaque assay procedure was repeated using different bacterial strains: *E. coli* strains A, B, C, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Plaque formation indicated phage infection specificity.

Results

Phage Isolation and Plaque Formation

Phages were successfully isolated from both sewage and pond water samples using *E. coli* strain A as the host for enrichment. Plaques formed on *E. coli* A lawns indicated active phage infection.

Host Range and Specificity

The host range of isolated phages was tested against multiple bacterial strains:

Bacteriophage Sample	***E. coli* A**	***E. coli* B**	***E. coli* C**	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
Phage 1	+ (clear plaques)	+ (smaller plaques)	- (no plaques)	-	-
Phage 2	+ (clear plaques)	-	-	-	-
Phage 3	-	-	-	+ (clear plaques)	-
Phage 4	+	+	+	-	-
Phage 5	-	-	-	-	-

(+ indicates plaque formation; - indicates no plaques)

Interpretation of Results

- Phage 1 showed moderate specificity, infecting *E. coli* A and B but not C.
- Phage 2 was highly specific to *E. coli* A.
- Phage 3 was specific to *Pseudomonas aeruginosa*, indicating a different host specificity.
- Phage 4 exhibited a broad host range within *E. coli* strains but did not infect other genera.
- Phage 5 did not form plaques on any tested strain, possibly indicating low phage concentration or non-infective phage particles.

Discussion

Phage Specificity Patterns

This experiment confirmed that bacteriophages exhibit a high degree of specificity toward their bacterial hosts. The observed variation in plaque formation across bacterial strains underscores the importance of receptor compatibility for phage adsorption and infection. Phage 4's ability to infect multiple *E. coli* strains suggests it recognizes a conserved receptor present on these strains, while Phage 2's strict specificity implies recognition of a more strain-specific receptor.

Factors Influencing Specificity

Several factors could explain the specificity patterns observed:

- **Receptor Recognition:** Phage tail fibers must bind to specific bacterial surface molecules such as lipopolysaccharides or proteins.
- **Bacterial Defense Mechanisms:** Some strains may possess resistance mechanisms like restriction-modification systems or CRISPR-Cas immunity.
- **Phage Mutations:** Genetic variability in phages could lead to differences in host range.

Comparison with Literature

These findings align with previous studies reporting narrow to broad host ranges for different phages. For instance, phages targeting *E. coli* commonly show specificity to certain serotypes, and some phages can infect multiple strains by recognizing common receptors. The inability of phages to infect *Staphylococcus aureus*, a Gram-positive bacterium, is expected due to significant differences in cell wall structure compared to Gram-negative bacteria like *E. coli* and *Pseudomonas*.

Applications of Phage Specificity

- **Phage Therapy:** Highly specific phages can be used to target pathogenic bacteria without affecting beneficial microbiota.
- **Bacterial Typing:** Phage typing is a classical method for bacterial strain differentiation.
- **Environmental and Clinical Diagnostics:** Phage specificity aids in rapid bacterial detection.

Limitations

- The experiment did not quantify phage titers precisely, which may affect interpretation of plaque absence.
- Only a limited number of bacterial strains were tested, restricting generalization of host range.
- Environmental phage isolates might include mixed populations, complicating specificity analysis.

Future Directions

- Genomic analysis of isolated phages to identify genes responsible for host recognition.
- Testing a broader range of bacterial strains and species.
- Investigating phage resistance mechanisms in bacteria.
- Evaluating therapeutic potential of isolated phages against pathogenic bacteria.

Conclusion

This study demonstrated that bacteriophages isolated from environmental samples show varying degrees of specificity to different bacterial strains. Specificity is governed by molecular interactions between phage proteins and bacterial receptors. Understanding these interactions enhances the potential application of phages in medicine, biotechnology, and microbial ecology.

References

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