

# General Microbiology Laboratory

## Lab Session 9 - Isolation and Titration of Bacteriophages

### Introduction

Viruses are obligate intracellular parasites of cells. They must, therefore, be grown in cell culture if one wishes to study them in the laboratory. Cell culture of plant or animal tissues is tedious and expensive, which makes study of plant or animal viruses difficult. An alternative is to study bacterial viruses because they can be grown in cells that are easy to prepare and maintain. The purpose of this lab is to isolate a bacterial virus from a natural source (e.g. *E. coli* phage from raw sewage) or, alternatively, to purchase virus from the phage store, and then use routine techniques to determine the concentration of virus in suspension.

### A. Review the following for reference:

1. Lab Manual: Exercise # 37 - Pages 225 to 228.
2. Text: Chap 16-Pgs 417-423 & Fig 16.12. Chap 17-Pgs 428-436 & Figs 17.3, and 17.12.
3. Use your BEST ASEPTIC TECHNIQUES when conducting this lab!

### B. Do the following during lab:

1. Part 1: Preparation of Phages and *E. coli*

#### Part 1A - Preparation of phage suspension

- a. Add 1.0 ml of diluent (sterile water) to a preparation of purified phages (either T1, T2 or T4).

Note: This will be done for you as a demonstration.

#### Part 1B – Preparation of a 'dilution series' of the phage suspension

- a. Add 900ul of sterile water to each of 7 tubes numbered 1 through 7.
- b. Add 100ul of a phage suspension (from part 1A above) to tube #1. Mix. Discard pipet tip.
- c. Transfer 100ul of the suspension in tube #1 to tube #2. Mix. Discard pipet tip.
- d. Transfer 100ul of the suspension in tube #2 to tube #3. Mix. Discard pipet tip.
- e. Continue this process to tube #7.

Note: Use micropipets: P1000 for 900ul, and P100 for 100ul and sterile pipet tips.

#### Part 1C - Preparation of *E. coli* for infection

- a. Prepare a 24 hour culture of *E. coli* in TSB.

Note: This has already been done.

2. Part 2: Titration of Bacteriophage Suspension

#### Part 2A - Titration using bacterial lawns

-Preparation of bacterial lawn cell cultures:

- a. Label 8 plates of TSA 1 through 8.
- b. Add 20 ul of *E. coli* to each of the 8 plates of TSA.
- c. Spread the *E. coli* evenly over the surface using a swab to make a lawn of growth.
- d. Allow the suspension to thoroughly dry! Incubate at 37 C if necessary.

- Infection of cells:

- a. Transfer 100ul of phage suspension from tube #7 to plate #7. Spread with swab.
- b. Transfer 100ul of phage suspension from tube #6 to plate #6. Spread with swab.
- c. Continue this process to plate #1.

Note: Only one micropipet tip and one swab is needed for the entire process.

- d. Plate #8 will be a control (i.e. there is no virus on this plate).
- e. Incubate plates for 24 hours. Count the number of plaques per plate.

#### Part 2B - Titration Using a broth clearing assay

-Preparation of bacterial broth cell cultures:

- a. Label 8 tubes of TSB 1 through 8.
- b. Add 100 ul of *E. coli* to each of the 8 tubes of TSB.

- Infection of cells:

- a. Transfer 100ul of phage suspension from tube #7 to TSB tube #7.
- b. Transfer 100ul of phage suspension from tube #6 to TSB tube #6.
- c. Continue this process to tube #1.

Note: Only one micropipet tip is needed for the entire process.

- d. TSB tube #8 will be a control (i.e. there is no virus in this tube).

e. Incubate tubes for 4-6 hours. Note at which phage dilutions there is growth of *E. coli*.

Part 2C - Titration using soft (i.e. top) agar:

-Preparation of plates:

a. Label 8 plates of "Bottom Agar" 1 through 8

-Preparation of bacterial soft agar cell cultures:

a. Label 8 tubes of TSA "Soft Agar" 1 through 8

b. Add 20ul of *E. coli* to each of the 8 tubes of TSA.

Note: Do not add 0.3 ml as the diagram shows!

Note: You will do this one tube at a time: See below.

- Infection of cells:

a. Transfer 100ul of phage dilution from Tube #7 to the *E. coli* soft agar tube #7.

Mix well and immediately pour into Plate #7. Gently swirl to disperse soft agar.

b. Transfer 100ul of phage dilution from Tube #6 and place in the *E. coli* soft agar tube #6.

Mix well and immediately pour into Plate #6. Gently swirl to disperse soft agar.

c. Continue this process to plate #1. Allow the soft agar to harden.

Note: Only one pipet tip is needed for the entire process.

d. Plate #8 will be a control (i.e. there is no virus on this plate).

e. Incubate plates for 24 hours. Count the number of plaques per plate.

**C. Results:**

1. Part 2B: Estimation of phage concentration:

a. Record the greatest phage dilution that inhibited growth of *E. coli*.

b. What is the minimum number of phages that are present in the original sample?

2. Part 2A and 2C: Calculation of actual phage concentration

a. Count the number of plaques on each plate in Part 2A (if possible) and 2C.

Note: Only count plaques on plates when there are 30 to 300 plaques total.

b. Calculate the number of viruses per ml in the original vial of virus (Part 1A above):

e.g. 48 plaques on plate #3 would =  $48 \times 10^3$  viruses per 100ul or 480,000/ml.

c. Correlate these data to the results of the broth clearing assay in 2B.

3. Are phages expensive?

a. Calculate the cost per phage at \$35.00 per vial of phage suspension.

b. Calculate the cost per kilogram of phages. Assume each phage weighs  $10^{-15}$  gram.

**D. Techniques:**

The following diagrams describe the dilution series and the experimental approaches.

Note: Don't confuse ul (microliters) with ml as used in the diagrams.

**E. Results and Calculations:**

Part 2A: Plaques	Plate #1	Plaques _____	Plate #2	Plaques _____
	Plate #3	Plaques _____	Plate #4	Plaques _____
	Plate #5	Plaques _____	Plate #6	Plaques _____
	Plate #7	Plaques _____	Plate #8	Plaques _____

Number of phages in original sample:

Part 2B: Broth	Tube #1	Result _____	Tube #2	Result _____
	Tube #3	Result _____	Tube #4	Result _____
	Tube #5	Result _____	Tube #6	Result _____
	Tube #7	Result _____	Tube #8	Result _____

Minimal number of phages in original sample:

Part 2C: Plaques	Plate #1	Plaques _____	Plate #2	Plaques _____
	Plate #3	Plaques _____	Plate #4	Plaques _____
	Plate #5	Plaques _____	Plate #6	Plaques _____
	Plate #7	Plaques _____	Plate #8	Plaques _____

Number of phages in original sample:

Part C3: Cost of Phages (Show the math)

Cost per phage:

Cost per kilo of phages:

## **F. Discussion and Conclusions:**