ONE-STEP PHAGE GROWTH CURVE

I. OBJECTIVES

- To construct the growth curve of a phage from laboratory data and determine its burst size.
- To identify the phases of a phage growth curve.
- To define the events involved in phage:bacterium interaction resulting in phage replication and release.

II. INTRODUCTION

Viruses that attack bacteria are called bacteriophages or simply phages. Phages, like other viruses, cannot exist without a suitable host. In 1939, Ellis and Delbruck (J. Gen. Physiol. 22:365-385) proposed a technique to quantitate and monitor the growth of phage in a specific host. The bacteria are mixed with phage and incubated for a short period of time. The mixture is then diluted to drastically reduce the number of bacteria available for phage adsorption. Samples are removed at specified intervals and plated to quantitate the phage present in the culture.

At the start of the experiment, the plaque count is relatively constant over a time period because each infected bacterium will yield only one plaque. A rise in plaque forming units (pfu) to a plateau level occurs as bacteria are lysed and the newly synthesized phage are released into the medium. These phage particles fail to meet susceptible bacteria (due to the dilution of the adsorption mixture) and thus remain free in the culture fluid. The average number of phage released per bacterium is called the burst size and this value may be calculated from the data. The burst size varies in accordance with the specific virus, and may range from 10 to 100 for the DNA transducing phages to approximately 20,000 pfu for the RNA viruses.

To replicate, a virus should induce its host to synthesize components that are necessary for the assembly of new virus particles. The virus accomplishes this process by first attaching to the host (adsorption) and then injecting its nucleic acid into the cell (injection or penetration). The viral DNA can stay free in the cell and be replicated as such, or it can be incorporated into the host chromosome and be replicated simultaneously with it. Viral proteins are next synthesized with the host's machinery under the direction of viral DNA and the new virus particles are assembled mechanically. These particles can find their way out of the cell or lyse the cell and be released into the medium, ready to infect new cells.

If the number of phage particles was monitored during growth, a growth curve could be drawn which would be similar to that of the bacterial growth curve except in the

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last stage. The phage growth curve starts with a latent or eclipse period (similar to the bacterial lag phase). During this phase, the infection, adsorption, injection and syntheses of new viral DNA and protein coat occur. The next phase is called the maturation or release stage (similar to the log phase in bacteria) when new phage particles are assembled and released. The cycle can then start over with the infection of new cells. In this manner, the shape of the curve would look step-wise and that is why the process is called "one-step phage growth curve".

The single-step growth experiment of Ellis and Delbruck demonstrates the cyclic replication of the phage. These authors devised a method to demonstrate only a single step of the many steps of phage replication. Essentially they drastically diluted the mixture after attachment of phage to bacteria, so when the infected cells lysed, no new host cells could be found for a second round of infection. A number of modifications have been introduced since the original experiment was reported. For instance, instead of diluting the initial bacterium:phage mixture, antibodies specific for the phage attachment apparatus may be added to the mixture to 'neutralize' and thus render all of the unadsorbed phage unable to adsorb to any bacterium.

In this laboratory experiment, we shall attempt to repeat the Ellis-Delbruck experiment. There are a number of steps and manipulations to perform and you are cautioned to be deliberate in your technique.

III. LABORATORY SUPPLIES

Bacterial culture or host suspension	5 ml/group
Phage lysate, 0.1 ml in small tt	1 tt/group
Phage base plates	8/group
Phage soft agar tubes	8/group
Phage broth (9.9 ml/tube)	2 tubes/group
Sterile 1.0 ml pipettes	as needed
Water bath, 37°C	2/lab
Water bath, 50°C	2/lab

IV. PROCEDURE (The experiment will be performed by students at each table forming a group.)

Note: First Session

1. The bacterial culture or the host suspension that we will be using is *E. coli* B and our phage is T4. You will find the host suspension (2×10^8 cfu/ml) and the phage lysate (4×10^6 pfu/ml) in the 37°C water bath in the lab. Keep them at this temperature all the time. To the "Adsorption Tube" which already contains 0.1 ml of the phage lysate, add 0.9 ml of host culture, mix well, record the exact time and return the tubes to the 37°C water bath.

2. Obtain 2 large test tubes containing 9.9 ml of phage broth and label them 10^{-2} and 10^{-4} and place them at 37°C.

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3. At precisely 10 min after starting the incubation of the Adsorption Tube, remove 0.1 ml of adsorption mixture and transfer to the tube marked 10^{-2}. Mix thoroughly by vortexing.

4. Use another sterile pipet to transfer 0.1 ml from tube 10^{-2} to tube 10^{-4}. Mix thoroughly by vortexing and place at 37°C.

5. At exactly 20 minutes into the experiment, add 0.1 ml from the 10^{-4} tube and 0.1 ml of the host culture to a soft agar tube and mix thoroughly by rolling the tube in the palms of your hands.

   Note: Soft agar (molten) tubes are kept at the 50°C water bath. Remove only one tube at a time to use immediately. The soft agar solidifies in the tubes in a few minutes, if the tube is placed at room temperature. So, once again: DO NOT REMOVE ANY SOFT AGAR TUBE FROM 50°C BATH UNTIL NEEDED!

6. Pour the soft agar onto a base plate labeled "20 min" and rotate the plate to evenly spread the soft agar across the surface of the entire plate. Allow the agar to gel, invert the plate, and incubate at 37°C overnight.

7. Repeat steps 5 and 6 at precisely 25, 30, 40, 50, 60, 70 and 80 minutes into the experiment.

8 Your plates will be moved to a refrigerator after 24 hours of incubation at 37°C.

Second Session

1. Calculate the multiplicity ratio in the Adsorption Tube. This is done by dividing the number of phage by the number of bacteria in the Adsorption Tube at the start of the experiment. This ratio provides an estimate of the number of phage available to infect each bacterium.

2. Obtain your plates. Count the number of plaques (pfu) per plate and complete the table in the "Results" section. Plates with a large number of plaques can be divided into 2 or 4 sections and only one section counted and then the result multiplied by 2 or 4 to get the total plaque number per plate.

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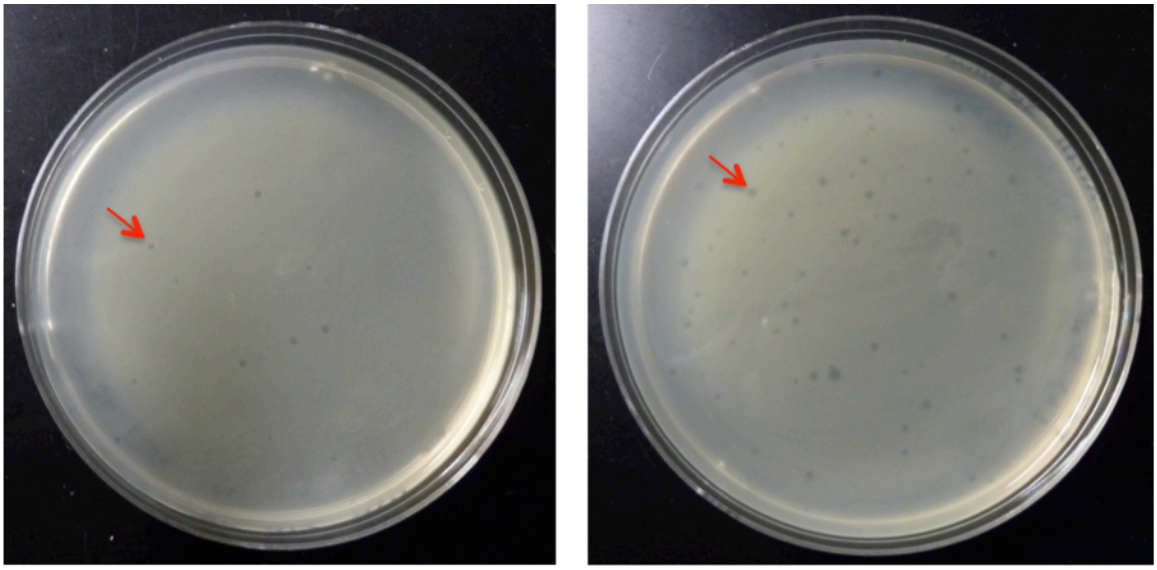
Figure: Plaques seen on a lawn of bacteria after 25 (picture on left) and 60 (picture on right) minutes into the experiment. Arrows show plaque forming units (pfu). Enlarge the pictures to see plaques better.



Results of the Phage Growth Curve Exercise

NAME _____DATE _____ GROUP NAME _____

PARTNER(S) _____

1. Calculate the multiplicity ratio in the Adsorption Tube (# of phage to # of bacteria):

2. How many bacteria are present for each phage in the Adsorption Tube?

3. Fill out the following table. Calculate the actual counts based for the number of plaques found on your plates multiplied by the dilution factor and the amount of inoculum used. Look at the actual counts and decide whether the very first or the average of the first few counts can be chosen as the base line (eclipse period). Divide all other counts by the base line to obtain the relative titers (last column in above table).

Incubation time (min)	No. of plaques observed on plate	Actual counts in Ads. Tube (pfu/ml)	Relative titers
20			
25			
30			
40			
50			
60			
70			
80			

4. The maximum relative titer would be the burst size for the phage under study. What was the burst size of your phage in this experiment?

4. Graph the relative titers (y-axis) over time (x-axis).

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