

LABORATORY

Bacteriophage Burst

OBJECTIVES

- To understand the bacteriophage burst that occurs in a culture of sensitive bacteria
- To perform the bacteriophage plaque assay to demonstrate a viral growth curve

BACKGROUND

When phages are mixed with a bacterial culture, viruses must attach and enter the cell, take over the cellular machinery to produce nucleic acid and protein for more phages, assemble the phages, and then lyse the cell to release the new viruses. During the attachment, entry, and release phases of the cycle, there is viable phage in the broth culture. However, during the synthesis and assembly phase, there are little to no phage particles present in the broth. After addition of phage to the broth in this experiment, viral attachment happens very rapidly, while the viral reproduction cycle takes time. So shortly after phage addition, there will be few free phages in the culture and few intact viruses present. After the new viral assembly and subsequent lysis of many of the cells, or the phage burst, there are many free phages present in the culture.

This experiment involves the class performing a phage titer at sequential time points during a bacteriophage infection of *E. coli*. As samples are taken from the original culture, they are treated with chloroform to lyse all bacterial cells present and release phage. You will then add back in fresh bacteria so that the only cells available for viral replication in plaque assay are the seed bacteria on the plate. All of the phage will begin attachment at the same time.

The number of plaques at each time point is determined by one group, and class data is collected and graphed in order to determine the course of phage infection and burst in the culture.

MATERIALS

(For each pair of students)

- 4 lambda plates
- 4 tubes of suspension medium
- 4 soft agar overlays (PICK UP FROM WATER BATH JUST BEFORE USE)
- Broth culture of sensitive bacteria
- Sample of T4 phage (1×10^{-2} dilution)

PROCEDURE

1. Clearly label your 4 dilution tubes and 4 plates for your series of dilutions. Make sure you also record your time point in your lab notes.
2. Make 4 serial tenfold dilutions of your time point sample of phage in the suspension medium. Remember your starting tube is already diluted at 1:100, or 10^{-2} .
3. Make dilutions of 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} . This is done for the first tube by adding 1 ml of the 10^{-2} dilution to a 9 ml dilution tube of suspension medium (labeled 10^{-3}) and mixing well.
4. Pipette 1 ml of the 10^{-3} dilution into a 9 ml tube labeled 10^{-4} and mix well.
5. Repeat two more times in a series to complete the dilutions.
6. Get one tube of soft agar, kept at a temperature just above solidification. If this is obtained too early, it will harden before pouring.
7. Add 2 drops of bacterial host culture and 1 ml of your bacteriophage 10^{-6} dilution to the soft agar tube. Mix by rolling between your hands, flame the mouth of the tube, and pour it onto the 10^{-6} plate immediately. Swirl the plate to spread the top agar over the entire plate.
8. Repeat Step 7 for each of your other three dilutions—4 plates total.
9. When soft agar is completely set, invert plates, and incubate for 24 hours at 37°C .
10. Observe plaques on each plate and count the number of plaques on the plate that contains between 30 and 300. Calculate the concentration in your original suspension by multiplying your count by the inverse of that plate's dilution (e.g., if 65 plaques are on your 10^{-5} plate, then the count, or titer, is 65×10^5 , or 6.5×10^6 phages per ml). Collect class data for the burst time course.

DISCUSSION

1. What was the titer of the bacteriophage at your time point?
2. Graph the class data to get a curve of virus replication for the culture.
3. Explain what is happening in each portion of the curve.
4. What is the difference in the data obtained for virus-infected cultures when samples are collected with or without chloroform? In other words, why is chloroform used?