CITIZEN SCIENCE
Bard College

Transduction of Bacteria: Plaque Assay

Viruses that attack bacteria are called bacteriophages, or simply phages. Phages can infect bacteria and insert their genes. The infected bacteria can then reproduce, carrying the copied bacteriophage genes to each successive generation. In some cases, the bacteriophage may contain antibiotic resistance genes, providing another avenue for transfer of resistance. This process is called lysogeny.

In other cases, the phage genes are transcribed and translated immediately, resulting in multiplication and maturation of the phage and breakdown of the bacteria, a process called lysis. Today we will use a lytic phage to infect bacteria and observe lysis. We will also determine the number of bacteriophages used to infect the bacteria.

Materials

- 37˚C incubator and 55˚C/100˚C water baths
- Tryptone Broth – 10 ml in white capped culture tube
- Tryptone base-layer agar plates (7/group)
- Tryptone top agar at 55º (7/group to be handed out one tube at a time)
- Sterile 5 ml culture tubes (blue cap) (7 per group)
- E.coli B- Overnight culture (0.5 ml per group)
- Phage aliquot (20 µl per group to be handed out right before use)

Protocol-Day 1

1. Melt top agar in the 100˚C water bath. Once melted, move to 55˚C water bath for at least 10 minutes (must cool from 100˚C to 55˚C or heat will kill bacteria). (*This has been done for you*)

2. Make serial dilutions of the phage stock (T4) as follows:

a) Label the sterile 5 ml culture tubes (blue cap) 1-7. Label the tube not the cap.

b) Add 990 µl of broth to tubes labeled 1-3, and 900 µl of broth to tubes labeled 4-6.

c) Add 10 µl of your phage stock to the tube 1. Mix thoroughly but GENTLY! Change tip.

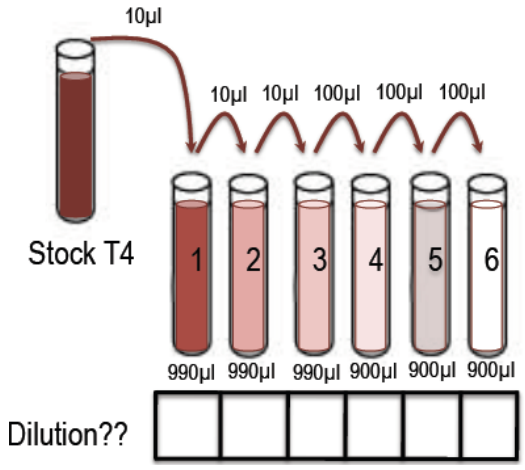
d) Remove 10 µl from the tube 1 and place it in tube 2. Mix gently. Change tip.

e) Remove 10 µl from the tube 2 and place it in tube 3. Mix gently. Change tip.

f) Remove **100 µl** from tube 3 and place it in tube 4. Mix gently. Change tip.

g) Remove **100 µl** from tube 4 and place it in tube 5. Mix gently. Change tip.

h) Remove **100 µl** from tube 5 and place it in tube 6. Mix gently. Change tip.



3. Prepare tube 7, a sample without phage, by adding only 1 ml of tryptone broth and no phage.

4. Add 30 µl of *E. coli* to each dilution tube, including tube 7 with no phage added.

5. Label your Tryptone agar plates 1-7 (include your initials and date)

6. Add 4.0 ml of top agar to each of the seven prepared sample tubes, and then immediately pour the mixture onto an agar plate and swirl the plate gently. Do this one tube at a time to reduce cooling of the top agar. To reduce contamination problems, start with the most dilute phage samples, then proceed to the less dilute ones (i.e., start at tube 7 and go to tube 6)

7. After the plates solidify, invert and place them in a 37˚C incubator. Incubate them overnight.

Protocol-Day 2

Count the plaques on the plates. If there are too many plaques, the plate will be "clear". Pick the plates that have a countable number of plaques and count them. A countable # is between 30-300 plaques per plate. By back calculating, you should be able to calculate the titer of the phage stock.

Worksheet

Tube Number	Dilution	# of plaques	Stock Titer
1			
2			
3			
4			
5			
6			
7			

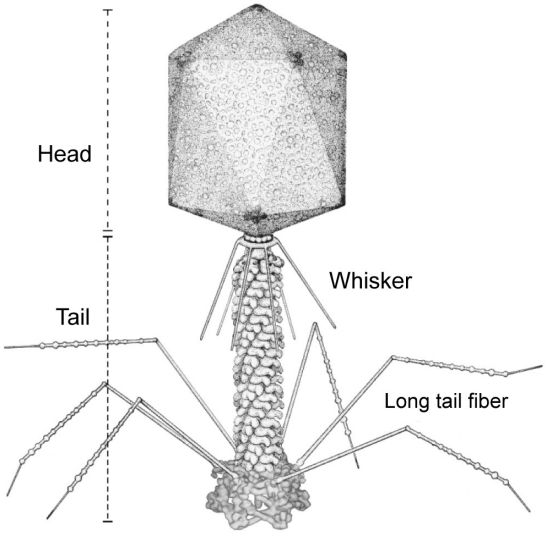


Fig.1-1 Structure of T4 phage
(Eiserling, 1983)