
LAB #3
BACTERIOPHAGE DETECTION *infect bacteria*

Introduction

Viruses are particulate, non-cellular, ultramicroscopic microorganisms composed primarily of a genome, either DNA or RNA, and protein. Basically, the viral particle, or *virion*, is a nucleic acid core surrounded by a protein coat, or *capsid*, composed of protein subunits called *capsomers*. Viruses are obligate, intracellular parasites whose nucleic acid genome controls and utilizes the synthetic capabilities of their host cells for replication.

Viruses that infect the intestinal tract of humans and animals are known as *enteric viruses*. They are excreted in feces and can be isolated from domestic wastewater. Alternatively, viruses that infect bacteria are known as *bacteriophages*, and those that infect coliform bacteria are called *coliphages*.

There are many potential applications of bacteriophages as environmental indicators or surrogates, including: use as indicators of sewage contamination, measurement of the efficiency of water and wastewater treatment processes, and to model survival of enteric viruses and bacteria in the environment. The use of bacteriophages as indicators of the presence and behavior of enteric animal viruses is attractive because of the ease of detection and low cost associated with the assays. Additionally, bacteriophages can be quantified within 24 hours, as compared to the days or weeks necessary to quantify human enteric viruses.

Coliphages in water are assayed by the addition of a sample to soft overlay agar along with a culture of host bacteria (e.g., *E. coli*) in the log phase of growth. The bacteria produce a confluent lawn of growth, except for areas where the phage has grown and lysed the bacteria. The resulting clear areas are known as *plaques*. A soft agar (0.7% instead of 1.5% agar) overlay is used to allow limited physical spread of viruses between bacterial cells. *10 min*

Procedure

First Period

Materials:

- Water sample containing bacteriophages
- 3 – 4 hour nutrient broth culture of log phase *E. coli* or *Salmonella* bacteria
- 8 dilution tubes: 9 ml of 1X tri-buffered saline (TRIS)
- 8 tubes of 5 ml of soft (top) agar (0.7% agar)
- 8 petri dishes with bottom agar (10-12 ml, 1.5% agar)
- 1 ml pipettes
- Water bath at 45 – 48°C
- Incubator at 37°C

Methods:

1. A sample containing bacteriophages is provided.
2. Perform a 10-fold serial dilution, as described in the Lab #1 handout.
3. Autoclave the tubes of soft agar (5 ml of 0.7% agar). Place the top agar tubes in a water bath at 45 – 48°C, and allow 15 min. for the temperature of the agar to adjust to 47°C.

4. To the first tube, add 1 ml of a log phase broth culture of *E. coli* or *Salmonella* and 1 ml of water sample. Remove the tube from the water bath and gently roll it between your hands to mix the suspension. Wipe the water from the tube with a paper towel and pour the agar over the petri dish containing the bottom agar. Quickly rotate the plate to spread the top agar. Be sure the agar covers the entire surface.
5. Repeat Step #4 using 1 ml of bacteria and 1 ml of each diluted sample.
6. Prepare a negative plate using only 1 ml of bacteria and no water sample.
7. After the agar has solidified, invert the agar plates and incubate at 37°C for 24 hours. Knock any moisture off the lid of the petri dish. If a drop of moisture falls on the plaque, it will cause it to spread across the agar surface.

Second Period

1. Count the number of plaques for each plate/dilution and calculate the concentration of bacteriophage in the original sample.
2. Record any differences in the size or appearance of the plaques.

Questions

1. List and describe the steps of virus infection and replication. 5 pts
2. What are some advantages of using bacteriophages as indicators for enteric viruses? 4 pts
3. What are some sources of coliphages in the environment? 4 pts
4. Why do we use bacteria in the log phase of growth for this assay? 4 pts
5. What are some factors that might determine the plaque size? 3 pts