

JoVE: Science Education

Detection of Bacteriophages

--Manuscript Draft--

Manuscript Number:	10190
Full Title:	Detection of Bacteriophages
Article Type:	Manuscript
Section/Category:	Manuscript Submission
Corresponding Author:	Ian Pepper UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Ian Pepper
First Author Secondary Information:	
Order of Authors:	Ian Pepper
	Bradley Schmitz
	Luisa Antoinette Ikner
	Chuck Gerba
Order of Authors Secondary Information:	

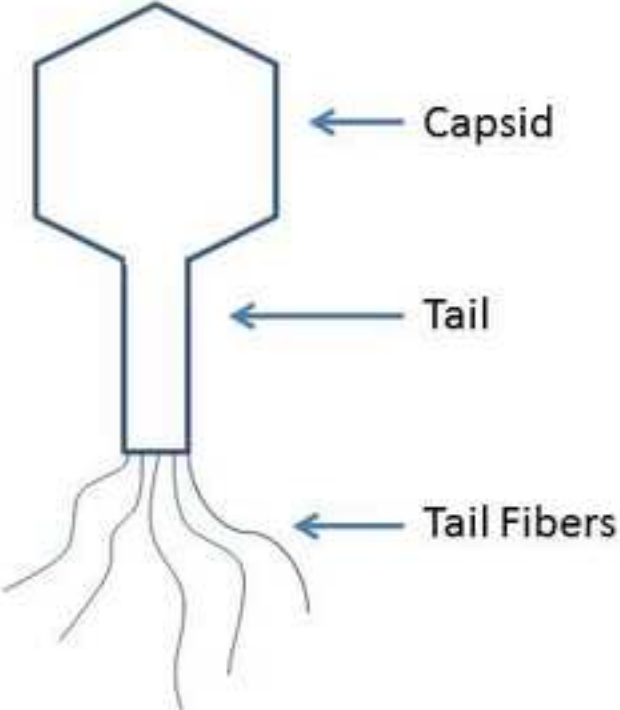
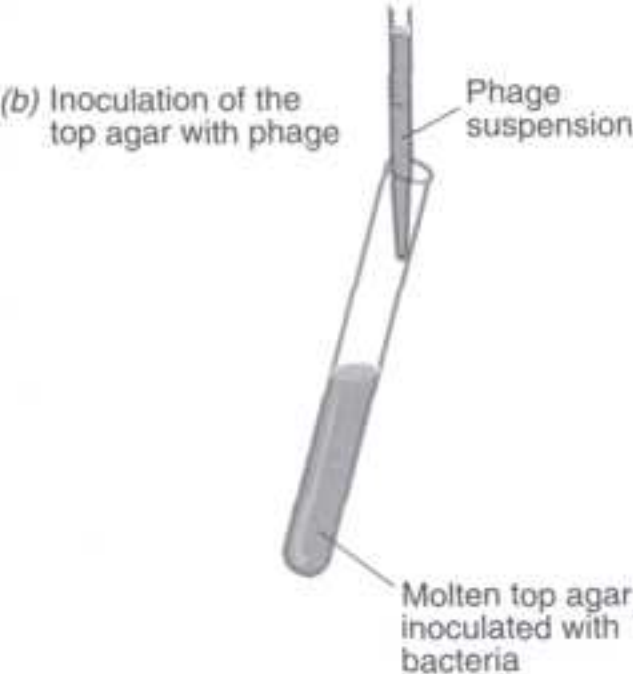
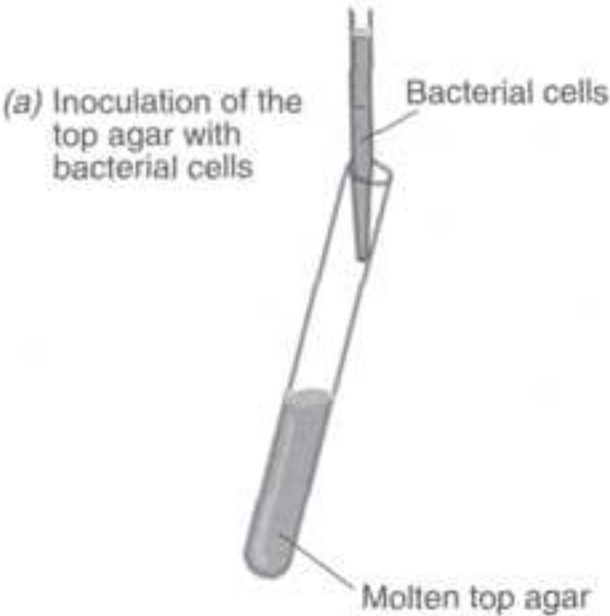
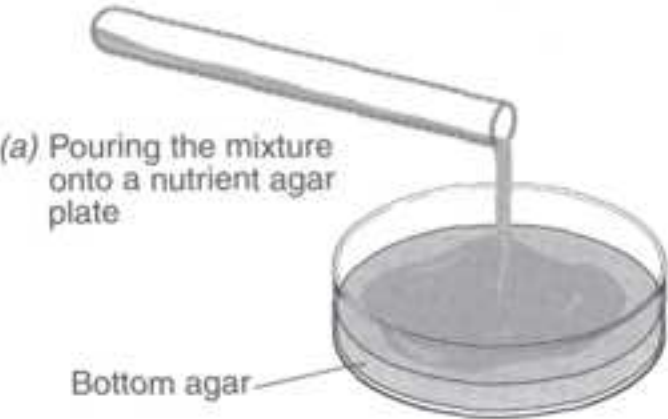


Figure 1 . Coliphage T2.

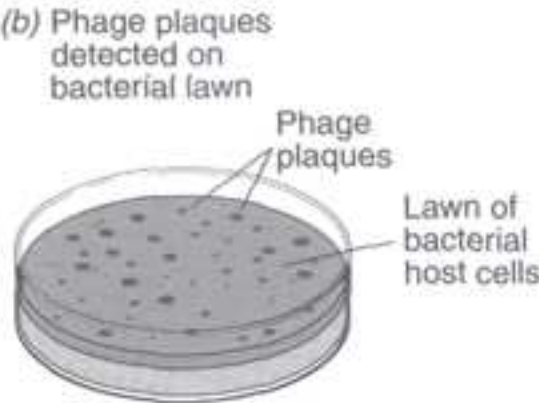
1) Preparation of the top agar

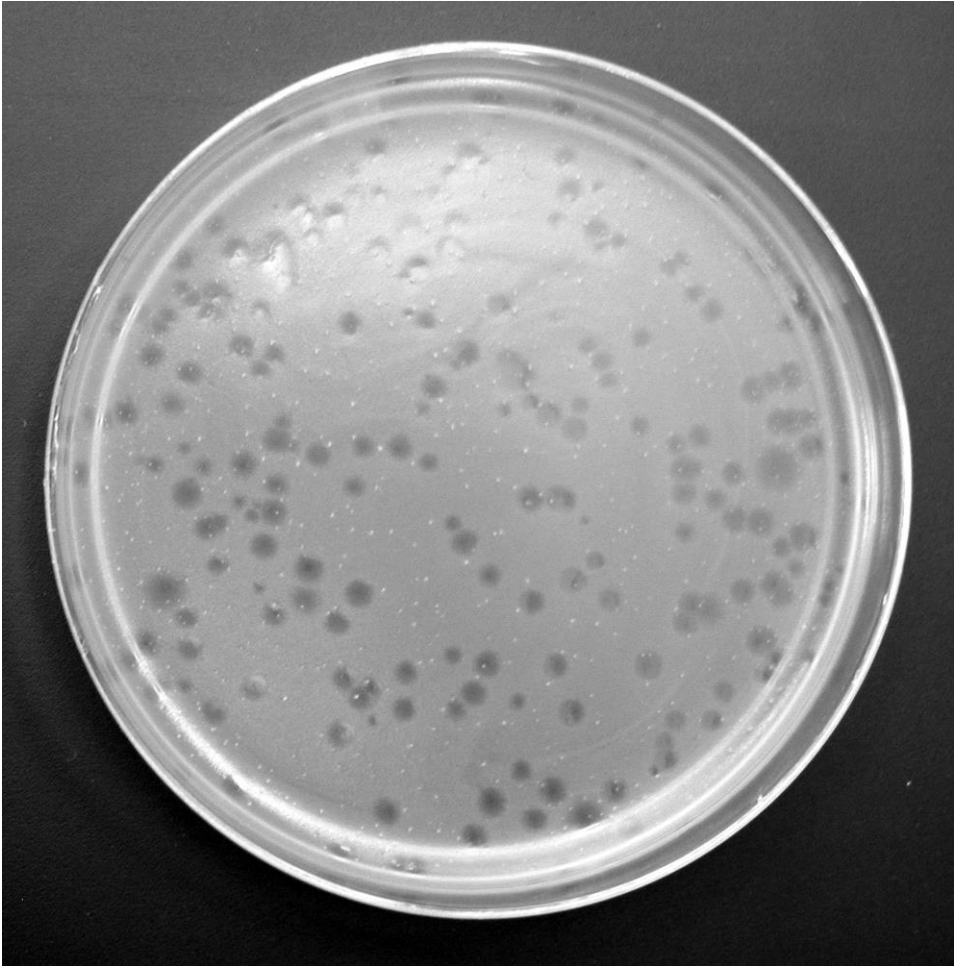


2) Plating and detection



Incubation





PI Name: Bradley W. Schmitz, Dr. Luisa A. Ikner, Dr. Charles P. Gerba, and Dr. Ian L. Pepper
Environmental Science Education Title: Detection of Bacteriophages

OVERVIEW

Viruses are a unique group of biological entities that can infect eukaryotic or prokaryotic organisms. Viruses are also unique in that they are obligate parasites that have no metabolic capacity and rely on host metabolism to produce viral parts that self-assemble during replication.

Viruses are ultramicroscopic—too small to be viewed with the light microscope, visible only with the greater resolution of the electron microscope. They are composed primarily of a nucleic acid genome, either DNA or RNA, and protein.

Viruses which 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. Viruses which infect bacteria are known as bacteriophages, and those which infect coliform bacteria are called coliphages (Figure 1). The phages of coliform bacteria are found anywhere coliform bacteria are found.

Basically, a viral particle is a nucleic acid core surrounded by a protein coat, or capsid, composed of protein subunits or capsomers. In some more complex viruses, the nucleocapsid is surrounded by an additional envelope and some have spike-like surface appendages or tails.

Principles

Bacteriophages are studied in environmental science because they are a critical component of biological systems. They are the most abundant biological entity on earth and are important because they help control bacterial populations, food web processes, biogeochemical cycles, as well as enhance prokaryotic diversity via horizontal gene transfer. Finally they are now being considered by EPA as possible viral indicators of fecal contamination in ambient water.

Coliphages in water are assayed by addition of a sample to soft or overlay agar along with a culture of *E. coli* in the log phase of growth. The phage attach to the bacterial cell and lyse the bacteria. The bacteria produce a confluent lawn of growth except for areas where the phage has grown and lysed the bacteria. These resulting clear areas are known as plaques. A soft agar overlay is used to enhance the physical spread of the viruses between bacterial cells.

To obtain optimal plaque formation it is important that the host bacteria is in the log stage of growth. This ensures that all the phage attach to live bacteria and produce progeny. This requires that a culture of host bacteria be prepared each day that an assay is performed. Usually, a culture is incubated the day before the assay to obtain a culture in the stationary phase. This is then used to inoculate a broth which is incubated to obtain enough host bacteria

Commented [JJ1]: The Application section contains information relating bacteriophages and Environmental Science as a whole. Include similar discussion here.

It also includes more of the “why” bacteriophages are studied in Environmental science. Add similar content to the Principles.

Commented [DM2]: Do we want to qualify this sentence so it isn’t dated in a year or 2? Or perhaps cut it altogether? If the former, perhaps:
“As of 2015 the EPA finally began considering the possibility of using bacteriophages as potential viral indicators of fecal contamination in ambient water.”

in the log phase for the assay (this usually requires 2–3 hours of incubation in a shaking water bath at 35 to 37 °C).

PROCEDURE

First Period

1. Obtain a sample of sewage or water containing coliphage.
2. Dilute the sample 1:10 and 1:100 by making 10-fold dilutions in Tris buffer by transferring 1.0 mL to 9 mL of Tris buffer.
3. Melt four tubes of soft agar (0.7% agar/3 mL tube) by placing in a steam bath or autoclaving.
4. Place the agar in a water bath at 45–48 °C and allow 15 min for the temperature of the agar to adjust to 45 °C.
5. To the first tube, add 1 mL of a log phase broth culture of *E. coli*¹ and 1 mL of undiluted sample.
6. Remove the tube from the water bath and gently rock between hands to mix the suspension for 2–3 seconds.
7. Wipe the water from the tube with a paper towel and pour the agar over the Petri dish containing bottom agar.
8. Quickly rotate the plate to spread the top agar. Be sure the agar covers the entire surface.
9. Repeat Steps 5–8 using 1 mL of bacteria and 1 mL of each sample dilution (Figure 2).
10. After the agar has solidified, invert the Petri dishes and incubate at 37 °C for 48 h. 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 on each dilution (Figure 3) and calculate the concentration of phage in the original sample.
2. Record any major differences in the size or appearance of the plaques.

CALCULATIONS AND REPRESENTATIVE RESULTS

Dilution of sewage sample = 10^{-1}

Number of plaques obtained = 9

Therefore, number of phage per mL of sewage:
= 10×9
= 90

Raw sewage typically contains $10^3 - 10^4$ coliphage per mL with a range of $10^2 - 10^8$ per mL.

Applications

There are many potential applications of coliphages as environmental indicators. These include their use as indicators of sewage contamination, efficiency of water and wastewater treatment, and survival of enteric viruses and bacteria in the environment. The use of bacteriophages as indicators of the presence and behavior of enteric bacteria and animal viruses has always been attractive because of the ease of detection and low cost associated with phage assays. In addition, they can be quantified in environmental samples within 24 hours as compared to days or weeks for enteric viruses.

Legend:

Figure 1: Coliphage T2

Figure 2. Procedure for the preparation of a bacterial lawn in the top layer of agar in which the detection of coliphage takes place.

Figure 3. Phage plaques originating on a bacterial lawn.

References:

¹*E. coli* strain ATCC 15597 usually will produce the greatest number of plaques from sewage samples. It should be grown overnight in a 250-mL Erlenmeyer flask containing 100 mL of nutrient or trypticase soy broth and incubated under shaking conditions at 35 °C. Three hours before the phage assay inoculate one mL of this culture into a fresh flask containing 100 mL of nutrient or trypticase soy broth and place in a shaking waterbath at 35 °C to 37 °C. This will ensure that the bacteria are in the log phase of growth.

