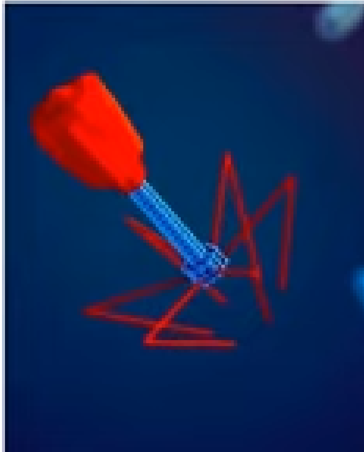
Lab# 9 Isolation of bacteriophage

bacteriophage or phage =**Bacteriophages** are viruses that infect only bacteria. In addition to the nucleocapsid or head, some have a rather complex tail structure used in adsorption to the cell wall of the host bacterium



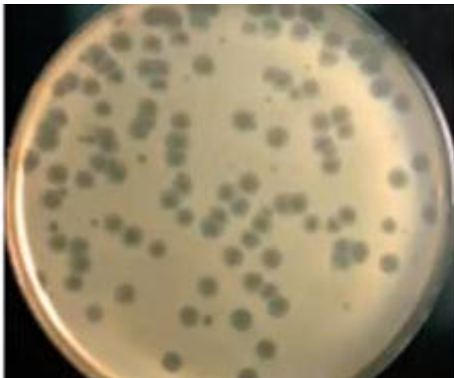
each phage is specific to 1 bacterial species . phages are important in maintaining ecological balances of bacteria(i.e. in the ocean).

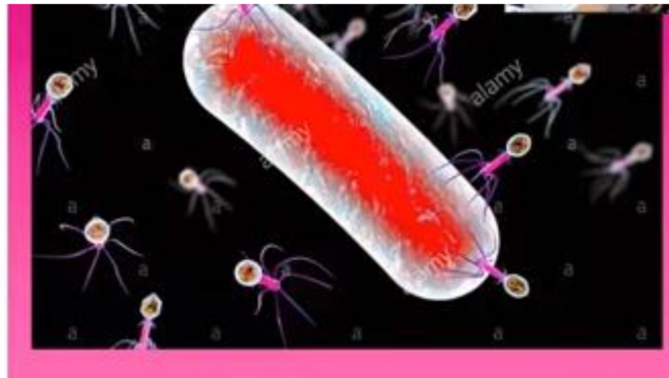
Also being explored for phage therapy to treat antibiotic-resistant bacteria

Coilophage= phage that attacks coliform bacteria such as E.coli

2 types of phages: lytic and temperate

Lytic phages: infect bacteria , replicate,lyse the cells to release of new phages; lytic phage





produce plaques. A plaque is a small, clear area on an agar plate where the host bacteria have been lysed as a result of the lytic life cycle of the infecting bacteriophages. As the bacteria replicate on the plate they form a "lawn" of confluent growth. Meanwhile, each bacteriophage that adsorbs to a bacterium will reproduce and cause lysis of that bacterium. The released bacteriophages then infect neighboring bacteria, causing their lysis. Eventually a visible self-limiting area of lysis, a plaque, is observed on the plate.

While temperate phage : have a lytic &lysogenic cycle(ability to integrate into the host genome as prophage, remain dormant until trigger the lytic cycle.

Vibrio cholerae is a bacterium that causes cholera through a toxin that it gained from a prophage.

There are many many different type; mostly dsNA tailed phage

In this Lab. you will isolate Coliphage that infect the bacterium *Escherichia coli*

Protocol: Phage enrichment

The goal of this method is to enrich your sample for phages capable of infecting your desired host. This is done by removing the endogenous bacteria from your sample and adding it to bacterial culture media and a growing culture of your host, and then incubating it. If even a single phage capable of infecting the inoculated bacterial strain is present in the sample, it will replicate to levels which should be detectable by normal plating techniques.

I. Fluid samples (water, sewage influent, etc.)

1. Centrifuge your sample at 8000 x g, 10 min.

2. Filter the clarified supernatant through a 0.45 or 0.22 µm syringe filter to remove any

endogenous bacteria. Your sample is now sterile and must be handled aseptically from now on.

(Note : that filtered sewage may still contain human pathogenic viruses such as Hepatitis A or Norwalk virus; handle these samples accordingly)

3. Add 10 ml of filtered sample to 10 ml of sterile 2X broth medium in a 50 ml conical tube, inoculate with 100 µl of a fresh overnight host culture. Incubate this enrichment culture overnight at 37˚C

II. Solid samples (sewage sludge)

1. Add your solid sample at a 1:4 (w/v) ratio to sterile broth medium (e.g., 10 g soil + 40 ml broth) in a beaker or centrifuge bottle. Shake or stir the resulting slurry for 1-2 hrs at RT.

2. Centrifuge the slurry at 8000 x g, 10 min. Filter-sterilize the supernatant through a 0.45 or 0.22 µm syringe filter

NOTE: These supernatants are often difficult to filter-sterilize; samples may be spun harder (e.g., 12,000 x g, 10 min) or pre-filtered using a sterile paper filter (e.g., Whatman filter paper).

3. Because the sample was processed using culture medium, the sample can be aliquoted into a culture flask and inoculated with host culture directly. Add 10 ml of filter-sterilized sample to 10 ml of 2X broth medium in a 50 ml conical tube,, inoculate with 100 µl host culture and incubate overnight.

III. Processing enrichment cultures

Remove 10 ml of the enrichment culture and place into a sterile 15 ml Falcon tube, centrifuge at 8,000 x g, 10 min. Filter sterilize the resulting supernatant through a 0.22 or 0.45 µm syringe filter, store at 4 ºC until use.

note: Even if an enrichment culture appears turbid, it may still contain phage.

Titering phages (Plaque count)

Titering phages is count phages by using a double agar layer method. There are two common methods for phage tittering:

1) Spot titer

2) Whole plate titer

Materials required:

• Host culture

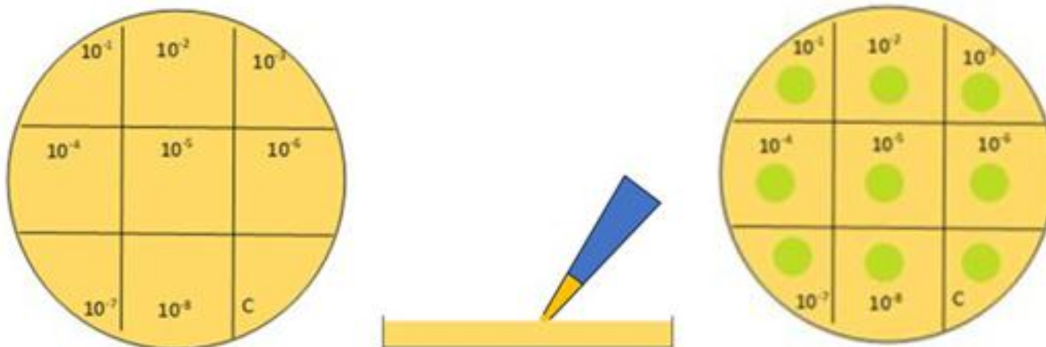
• serially diluting from phage lysate stock that prepared in the step of (Processing enrichment cultures) from 10^{0} (undiluted) to 10^{-8}

• Petri plates containing solid culture medium "bottom agar" appropriate for the host bacterium

• Aliquots of molten soft agar "top agar" for overlays (3 – 4 ml each, enough to cover one plate), held at ~50 ℃ in a heat block or water bath
Molten soft agar (Top agar) is low concentration agar medium (typically 0.5-0.7% agar). Plaques form when bacteria are immobilized in a lawn of top agar while phages can slowly diffuse, leading to the formation of a plaque from a single phage-infected cell
• Tube racks, pipettors, sterile tips
• Aseptic working area (near a flame or in an aseptic cabinet)

Spot titer

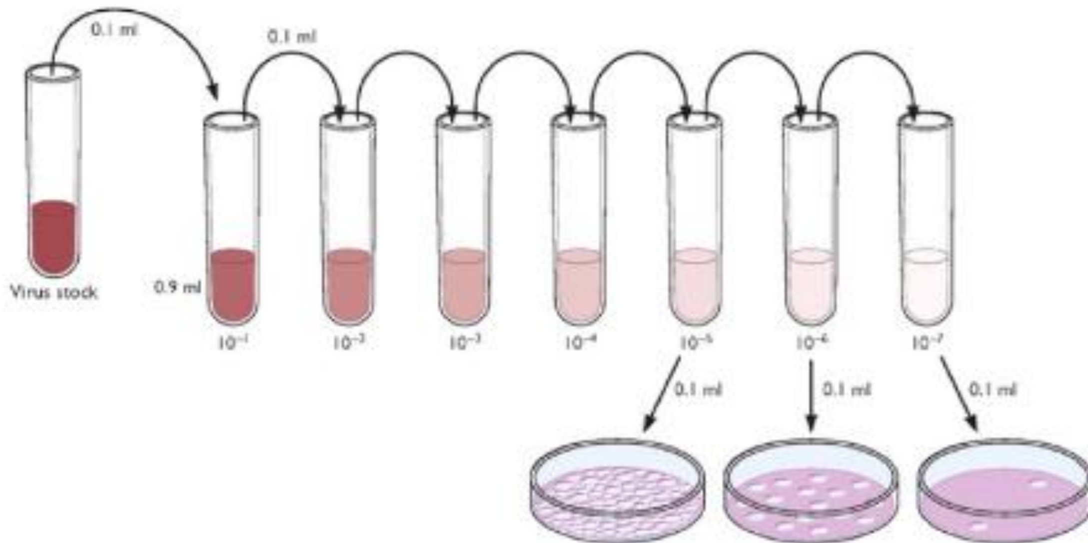
Spot titers involve pouring a lawn of bacteria then placing aliquots of phage dilution onto the lawn. Typically, a small volume of dilution (5-20 μL) is spotted onto the lawn, so a single lawn can hold up to ~15 spots. We have found that 5 μL spots tend to not run into each other on 0.5% top agar, so we most commonly use this volume. However, a larger volume aliquot will give more accuracy as long as the spots remain separate and do not spread into each other. Label your plate(s) and mark your plate(s) with the location that each dilution factor (or control/ negative control) spot will be placed. Pour your lawn of bacteria and then leave it to set. Starting with your control tube (and working from 10 -8 to 10 -1 , not the other way around) aspirate 5 μL of sample with apipettor then carefully place that aliquot onto the lawn. Leave the plate open for no more than 5 minutes, if the top agar dries out it will affect plaque formation. Once the spots are sufficiently absorbed the lid can be closed and the plate inverted and incubated.

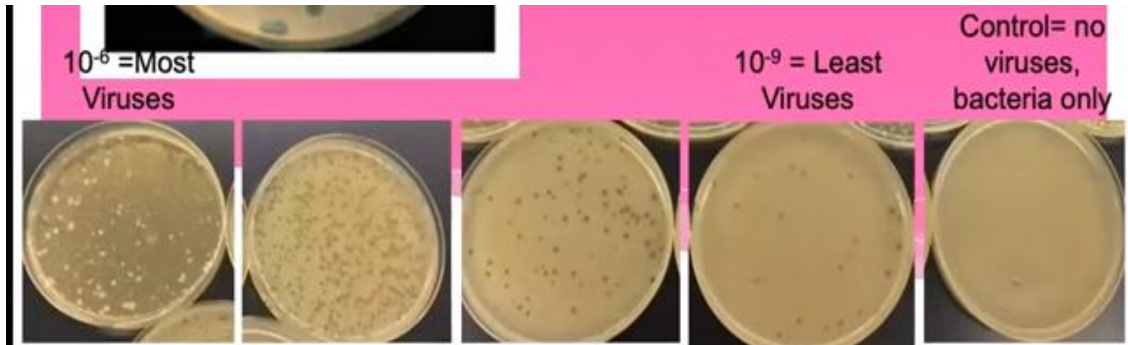


Whole plate titer

Whole plate titers are more accurate for a number of reasons. A larger aliquot of phage is used (typically 50-100 μL), which is itself more accurate than pipetting smaller volumes. The plaques from a single aliquot are spread over the surface of the entire Petri dish, so they are far less likely to overlap, meaning that more plaques can be counted more accurately. Finally, the phage and the cells are premixed, allowing the phage to adsorb to

their host cells prior to lawn formation. This tends to lead to more plaques, and more uniform plaques than spot titers because all plaques are initiated at the start of lawn formation, whereas for spot titers the phage must diffuse through the top agar and adsorb to their host, which will take different amounts. If a phage takes too long to find a host then the lawn will already be growing, possibly approaching stationary phase, and the resulting plaque will be smaller or invisible as a result. To carry out a whole plate titer, first label your plate(s), including the dilution factor to be used for each plate. In sterile microcentrifuge tube, mix your cell culture aliquot (usually 10-100 µL of overnight log phase cells) and your phage sample aliquot (usually 100 µL) , then leave for 5-10 min for the phages to fully adsorb. Some phages can produce progeny in as little as 15-20 minutes, so it is important not to let the mixture sit for too long. Aspirate the entire mixture into a fresh pipette tip and pour a lawn with this entire reaction. Allow the lawn to set before inverting and incubating the plate.





Results and calculations

After incubation, take a look at your plate(s). **For spot titers**, check to see if your control spots are devoid of plaques. If they are not then you may have to repeat the titer with fresh buffer, check with your instructor. Check to see if your serial dilutions are behaving as expected. As in, do you see the spots going from complete lysis to individual plaques. Then choose a spot to count, this will be the least most concentrated dilution to show entirely separate plaques. Note the concentration and count the plaques. For **whole plate titers**, you also want to check your control plate (no plaques) and look to see your dilutions performed roughly as expected. Identify the dilution that lead to the largest number of entirely separate plaques and count the plaques.
To calculate the titer in PFU/mL you need to know your plaque count (or mean count if n>1), the

dilution factor and the aliquot volume of your phage sample. The calculation is as follows:

Phage titer (PFU/mL) = plaque count / (dilution factor x aliquot volume (mL))

So, if you counted 37 plaques (PFU) from a 100 µL aliquot of 10 -6 dilution, the titer will be:

37 PFU/(1E-06 x 0.1mL) = 3.7E+08 PFU/mL