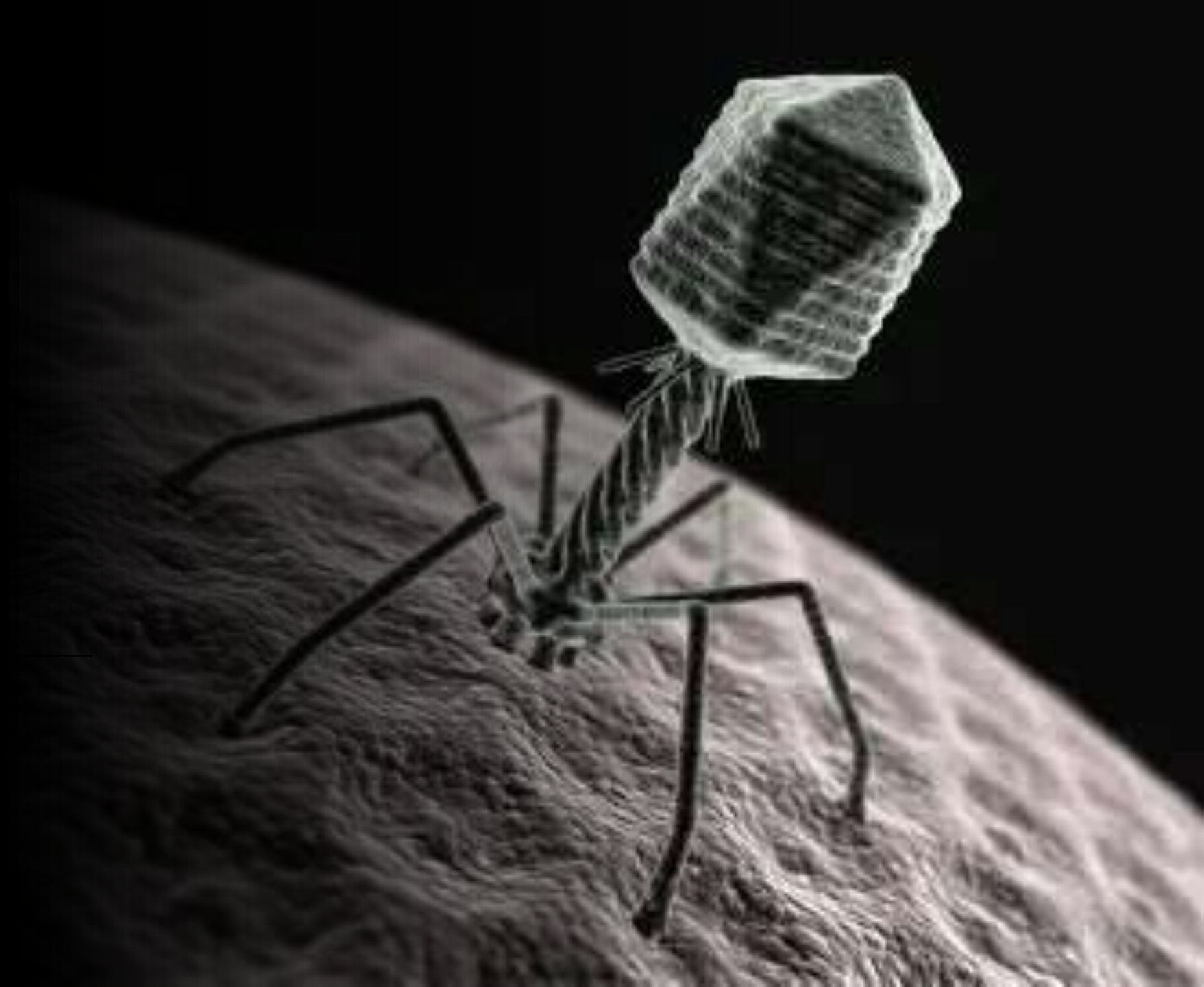
Bacteriophage



- Bacteriophages, also known as phages, are viruses that infect and replicate only in bacterial cells. They are ubiquitous in the environment and recognized as the earth's most abundant biological agent. They are extremely diverse in size, morphology, and genomic organization.
- However, all consist of a nucleic acid genome encased in a shell of phage-encoded capsid proteins, which protect the genetic material and mediate its delivery into the next host cell.

- Electron microscopy has allowed the detailed visualization of hundreds of phage types, some of which appear to have "heads," "legs," and "tails." Despite this appearance, phages are non-motile and depend upon Brownian motion to reach their targets.
- Like all viruses, bacteriophages are very species-specific about their hosts and usually only infect a single bacterial species or even specific strains within a species. Once a bacteriophage attaches to a susceptible host, it pursues 1 of 2 replication strategies: lytic or lysogenic

The Discovery of Bacteriophage

- The Twort-d'Hérelle phenomenon, discovered independently by Frederick Twort (1915) and Félix d'Hérelle (1917), refers to the transmissible, glassy transformation and lysis (destruction) of bacteria, which led to the discovery of bacteriophages—viruses that infect bacteria. It established the existence of agents smaller than bacteria that require bacterial hosts to replicate

Composition of bacteriophages

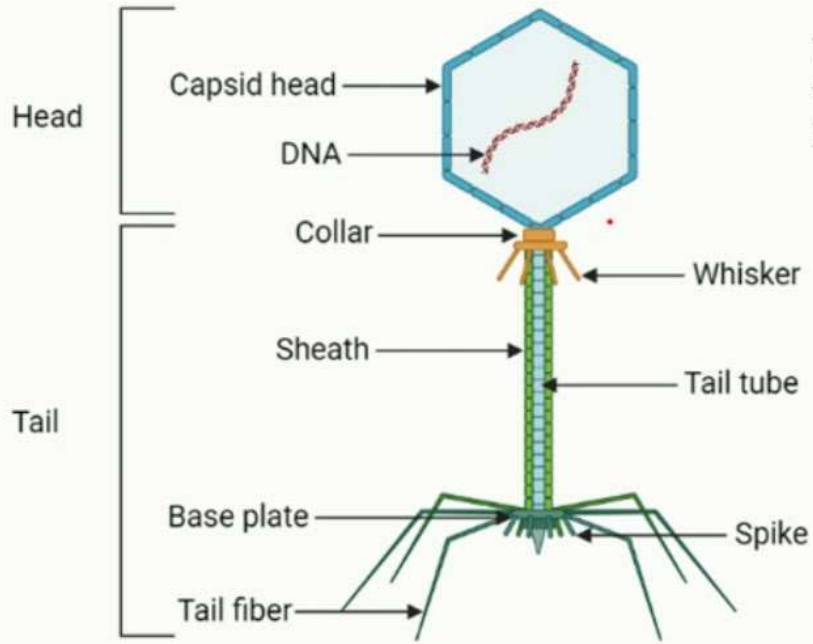
- Different bacteriophages may contain different materials they all contain nucleic acid and protein. Depending upon the phage, the nucleic acid can be either DNA or RNA but not both .
- Typical phages have hollow heads (where the phage DNA or RNA is stored) and tunnel tails, the tips of which have the ability to bind to specific molecules on the surface of their target bacteria.
- The viral DNA is then injected through the tail into the host cell, where it directs the production of progeny phages often over a hundred in half an hour. These "young" phages burst from the host cell (killing it) and infect more bacteria.

Structure of bacteriophages

Bacteriophage comes in many different sizes and shapes. The basic structural features of bacteriophages are (which depicts the phage called T4)

1. Size - T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide. Other phages are smaller. Most phages range in size from 24-200 nm in length.
2. 2. Head or Capsid - All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.
3. 3. Tail - Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even have a tail structure. In the more complex phages like T4 the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail the more complex phages like
4. 4. have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In these instances other structures are involved in binding of the phage particle to the bacterium

Structure of T4 Bacteriophage



Under an electron microscope, the T4 phage resembles a tadpole having a head and tail.



types of phages

- 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 selflimiting area of lysis, a plaque, is observed on the plate.
- Temperate phage : have a lytic &lysogenic cycle,ability to integrate into the host genome as prophage, remain dormant until trigger the lytic cycle.





plaques

Isolation of bacteriophage

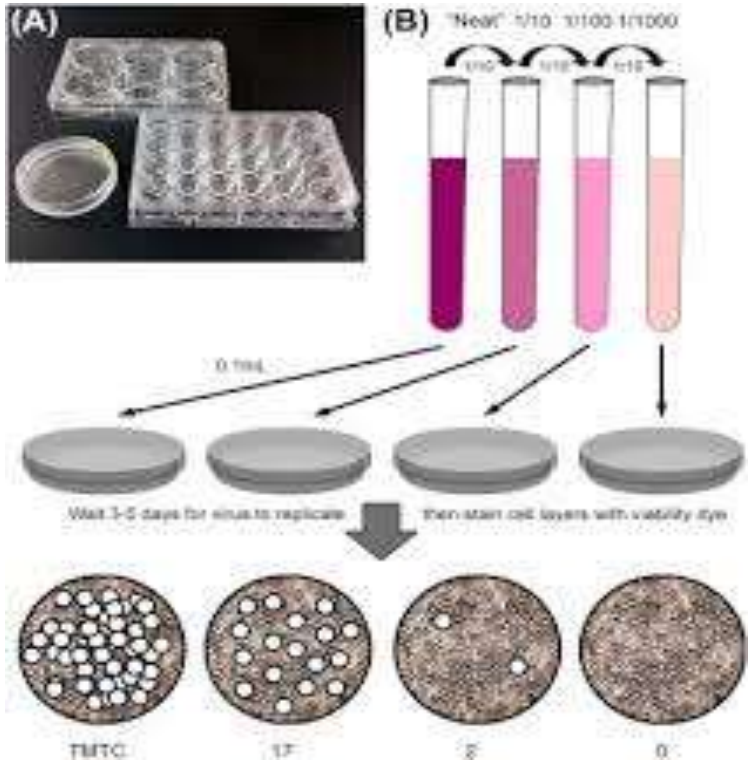
- Bacteriophages are isolated from diverse, bacteria-rich environments.
- **Common Sources of Bacteria**
- **Sewage/Wastewater:** High concentration source for targeting fecal bacteria like *E. coli*, *Salmonella*, and *Shigella*.
- **Soil:** Rich in diverse bacteria; used for finding phages against soil-borne pathogens like *Bacillus subtilis*.
- **Food Samples (Raw Milk/Whey):** Used for isolating dairy-associated phages.

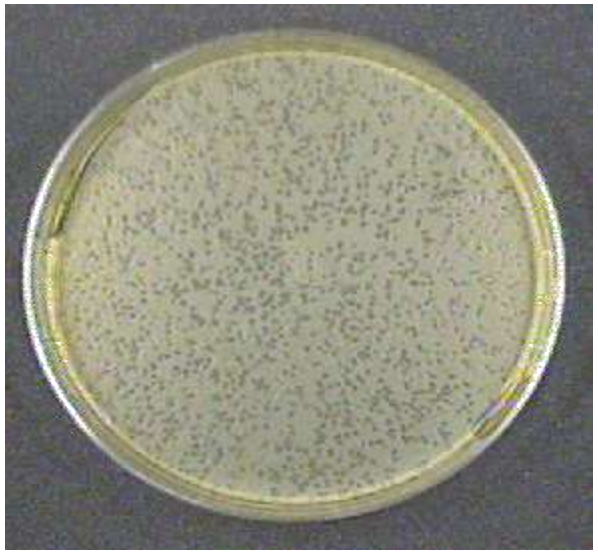
- Materials
- • NA plates
- Semi solid(soft) NA-agar
- • Bacterial culture
- • Phage dilution series
- Spot Assay Protocol
- (Spot tests involve dropping phage lysate on a lawn of bacteria)
- 1. . Add 100µL of bacterial culture to 3mL of pre-melted soft agar and mix by vortexing.
- 2. Pour the soft agar mix on the top of a plain NA plate and let it solidify.
- 3. Spot small volume (1-3µL) of the diluted phage solution on the top of soft agar and incubate at 37°C overnight.
- 4. Determine an appropriate concentration of phage for plague assay, usually the highest dilution with which plaques appear..

- Plaque Assay Protocol (Double Agar Layer)
- 1. Melt soft NA-agar. Wait for it to cool .
- 2. Prewarm the NA plates at 37°C.
- 3. Put 100µL bacterial culture, 1µL of phage dilution in 3mL soft NA agar tube and vortex.
- 4. Spread the mixture quickly on the NA-plate and wait for it to solidify.
- 5. Incubate plates at 37°C for 12 h

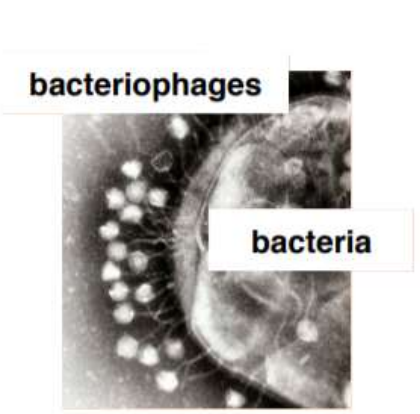
- **Calculation:** The concentration (PFU/mL) is calculated by multiplying the numberof plaques by the dilution factor.

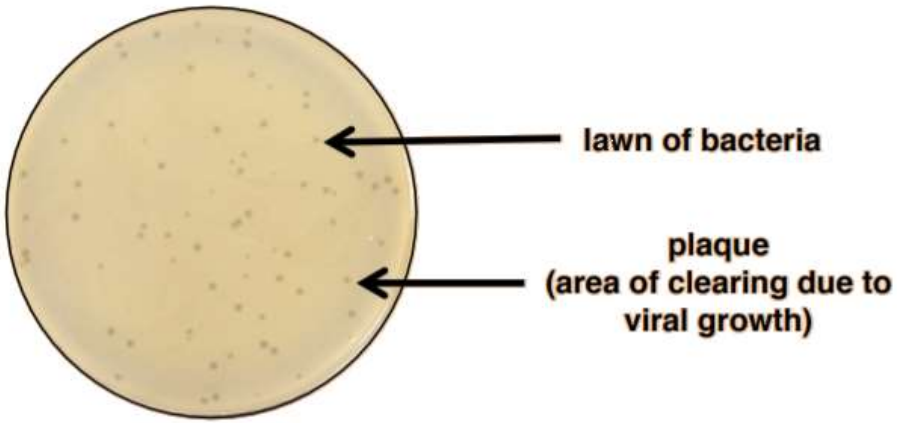
No. of Plaques / (D x V) = pfu/ml
D = Dilution factor
V = Volume of diluted virus
Sample calculation:
- An average of 50 plaques formed in the 1:10,000 dilution
- Volume of diluted virus added: 0.2ml
= 50/ (0.0001 x 0.2) = 2.5 x 10^6 pfu/ml











Plaques on a 1/100,000 (10-5) Dilution of Coliphage T-4