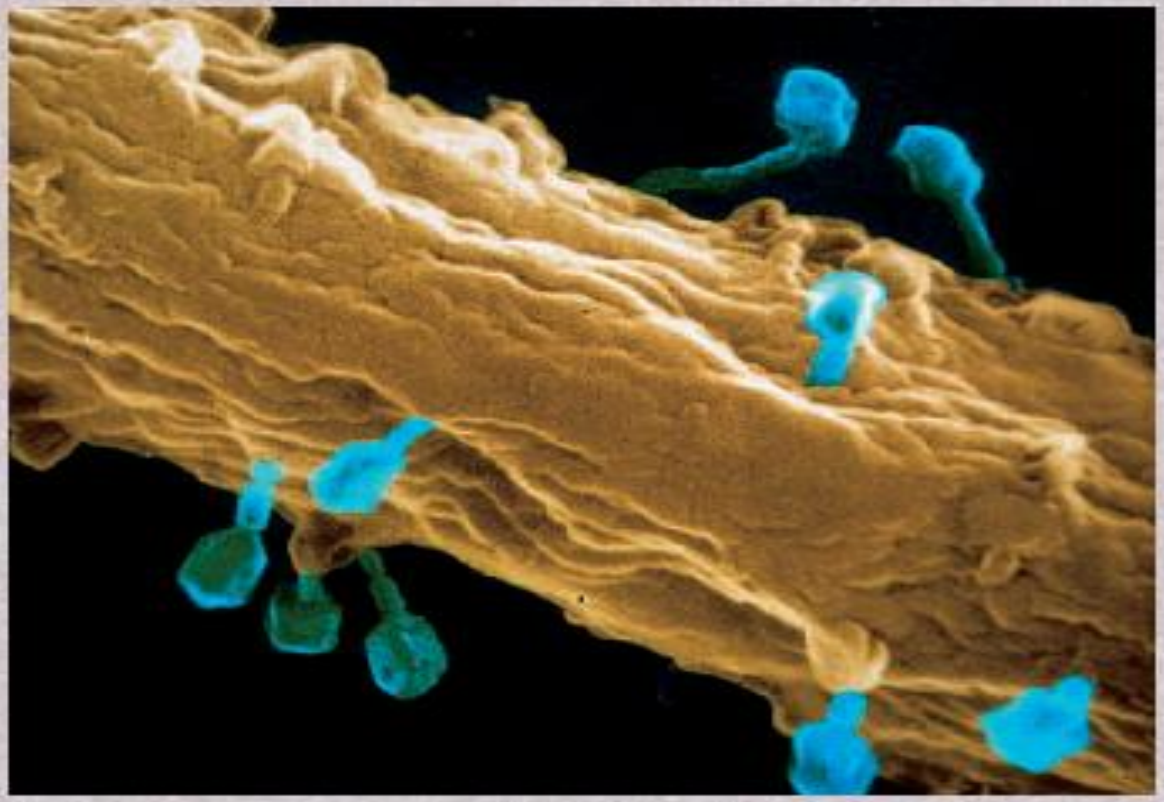
BACTERIOPHAGE: A POTENTIAL BIOCONTROL AGENT FOR MANAGING PATHOGENIC BACTERIA IN WATER/ WASTEWATER

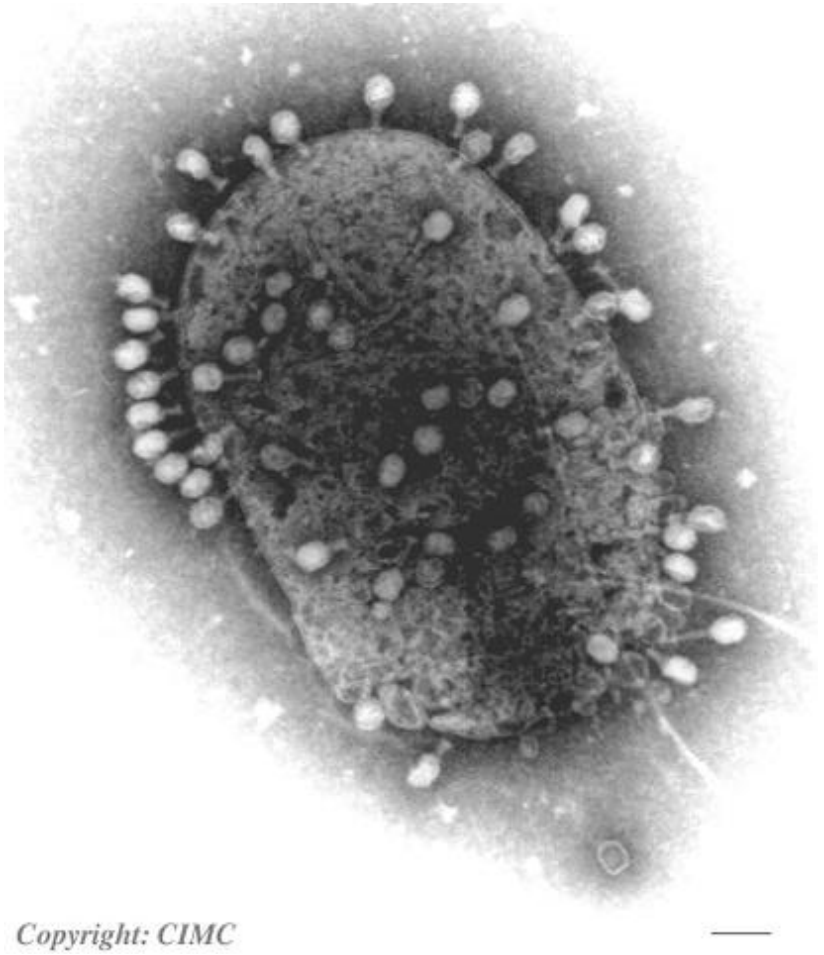
Bacteriophages



Deepesh V., SSA, CPCB, Bangalore.

A scanning electron micrograph of T-even bacteriophages infecting *E. coli*. The phages are colored blue.

Bacteriophage ("eaters of bacteria")



Escherichia coli under mass attack by numerous phage-T4 virions.

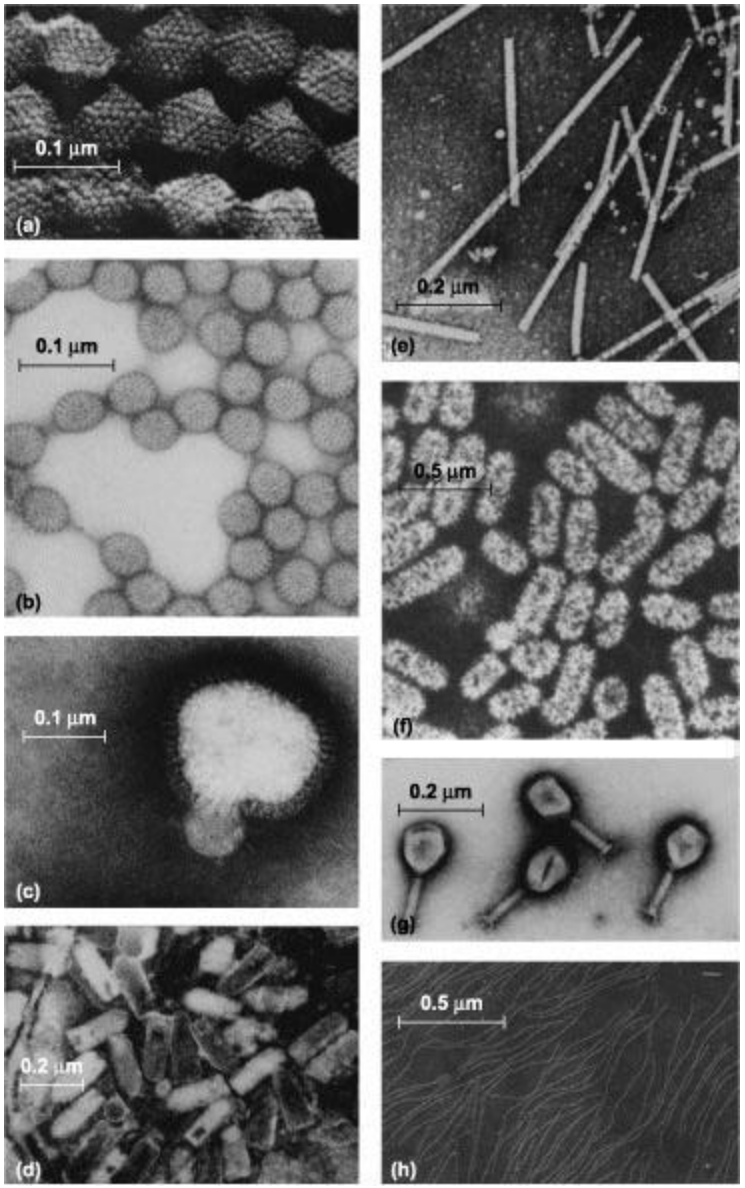
What are viruses?
They lie somewhere between
supra molecular complexes and
very simple biological entities.

Viruses are small infectious particles, typically 20-200 nm consisting of a nucleic acid core (single or double stranded RNA or DNA) enclosed by a protein coat (capsid) and in some cases a lipid envelope.

Viruses exist in two distinct states.
When not in contact with a host cell,
the virus remains entirely dormant.

No internal biological activities

and exist as a **static organic particle**.
In this simple, clearly non-living state
viruses are referred to as **'virions'.**



Bacteriophages are viruses specific to **bacteria**

One of the most abundant "life forms" on earth.

Phages are enormously abundant, making them an absolute majority of all biological entities:

Estimated 1031 particles in the biosphere.

There are about 10 phage particles for one host bacterium.

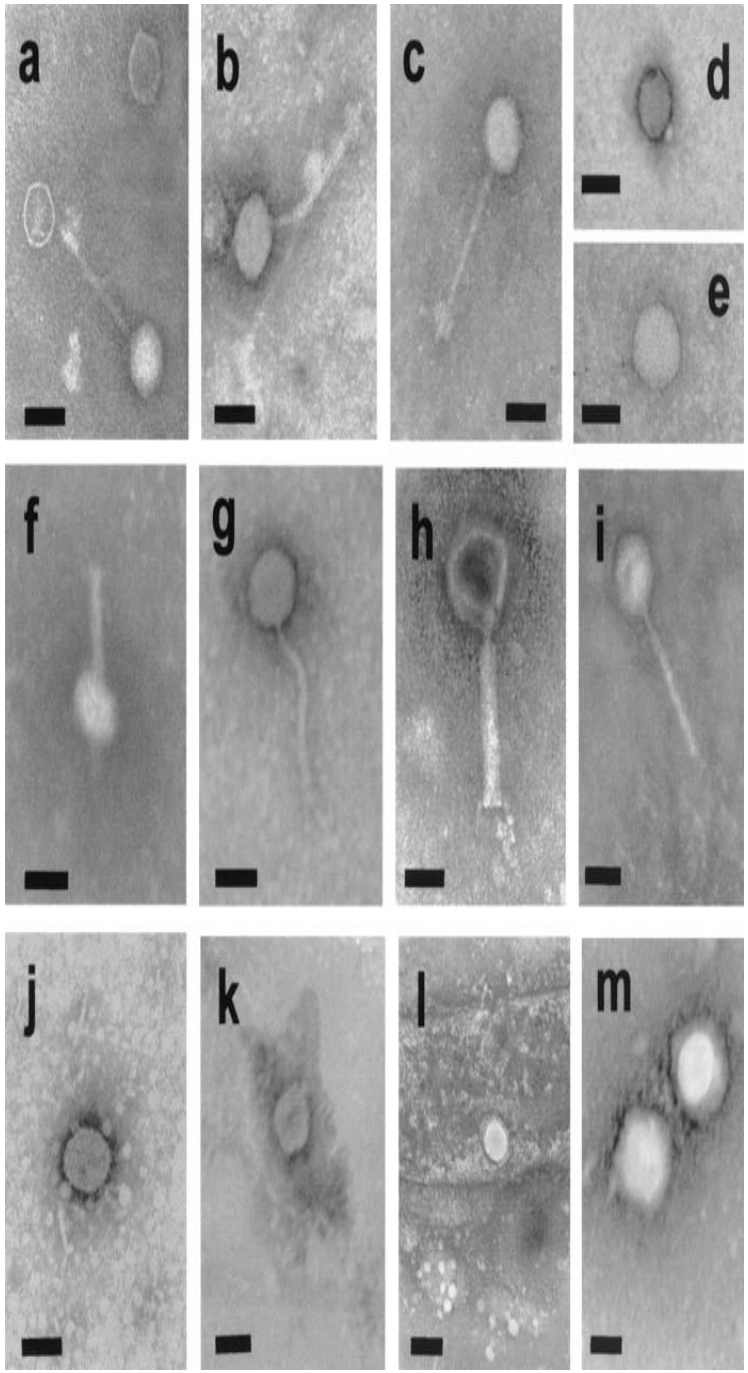
1023infections per second across the planet.

They encompass enormous genetic diversity:

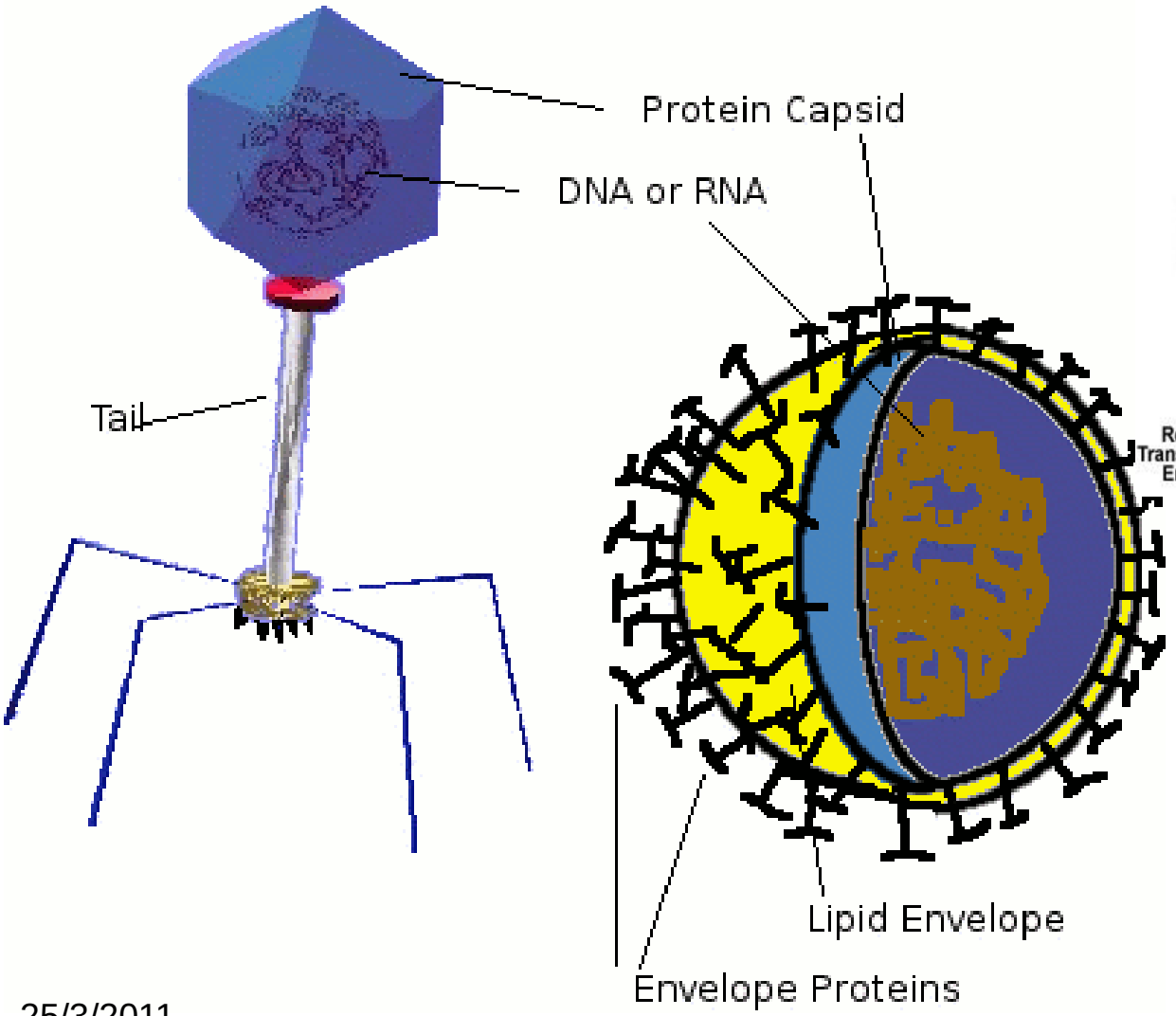
Phages can also be grouped according to host genera.

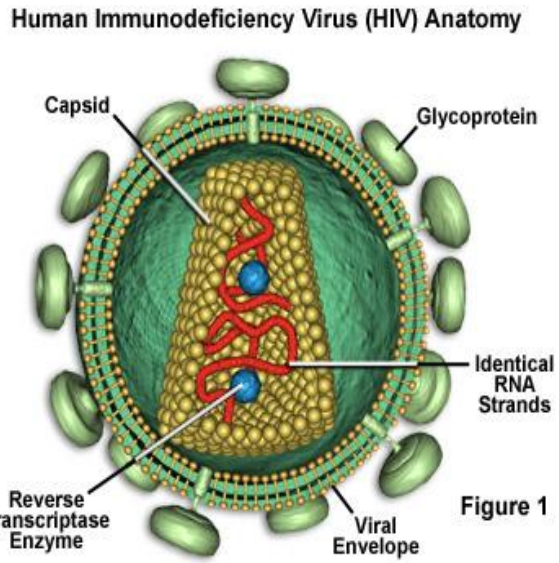
Enterobacteria (>900),
Lactococcus (700),
Bacillus (380) and
Streptococcus (290).

The top one millimeter of the world's oceans could contain a total of over $3x10^{30}$ virus particles!

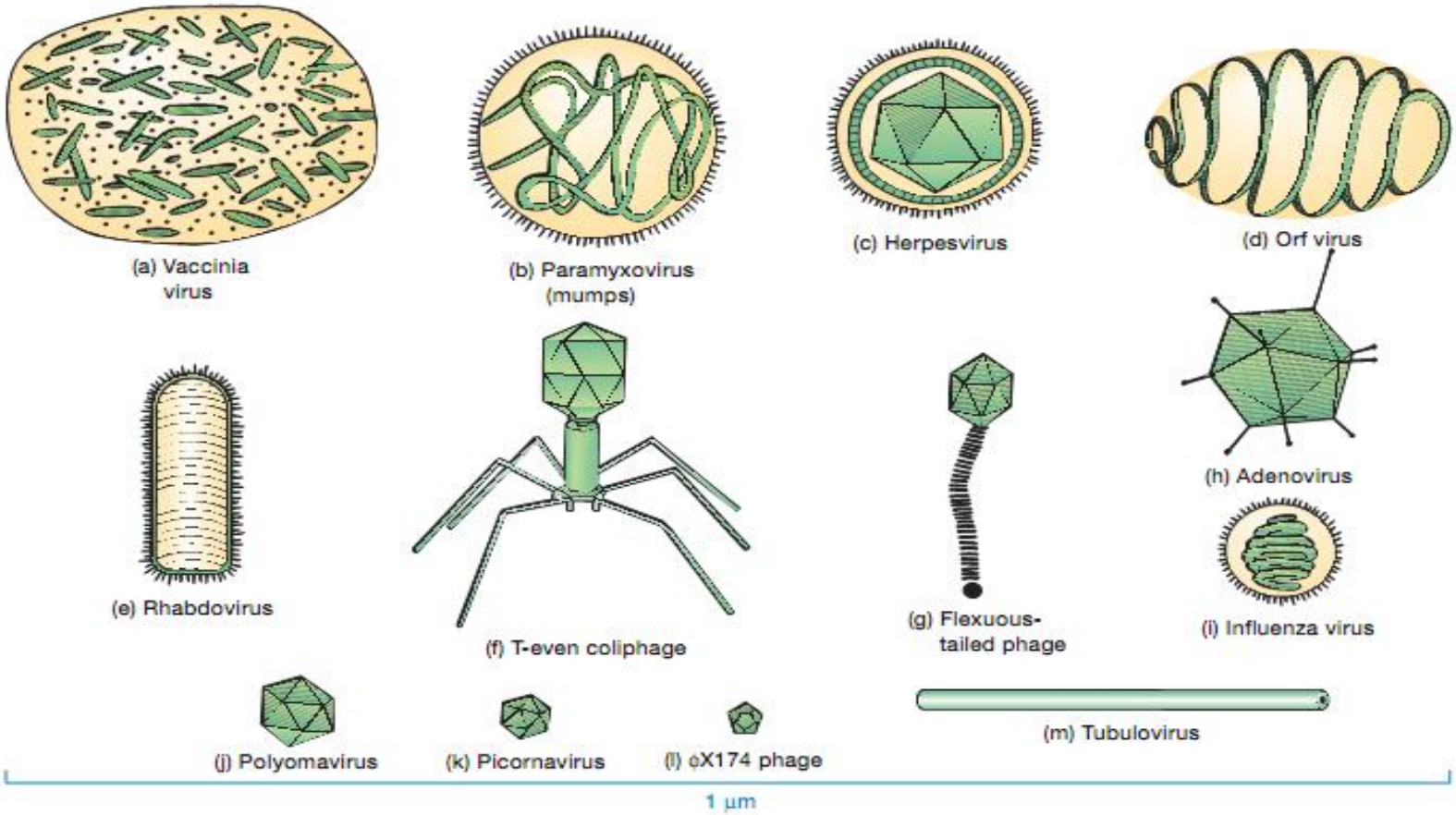


Virus structure





Size and morphology of selected viruses



Bacteriophage therapy

Use of phage in place of antibiotics and other control measures

Felix d'Herelle: Demonstrated the efficacy of phage for the control of *Shigella* infection in the World War I

King Edward VII Pasteur Institute in Assam tried phage therapy in villages in Assam that had cholera epidemics every year.

Nowgong, the village that used phage treatment had fewer than 10 deaths due to cholera,

whereas Habibganj that did not use phage had over 300 deaths.

GangaGen Biotechnologies, Bangalore

Is close to getting the **world's first phage-based product** ready to tame deadly bacteria strains.

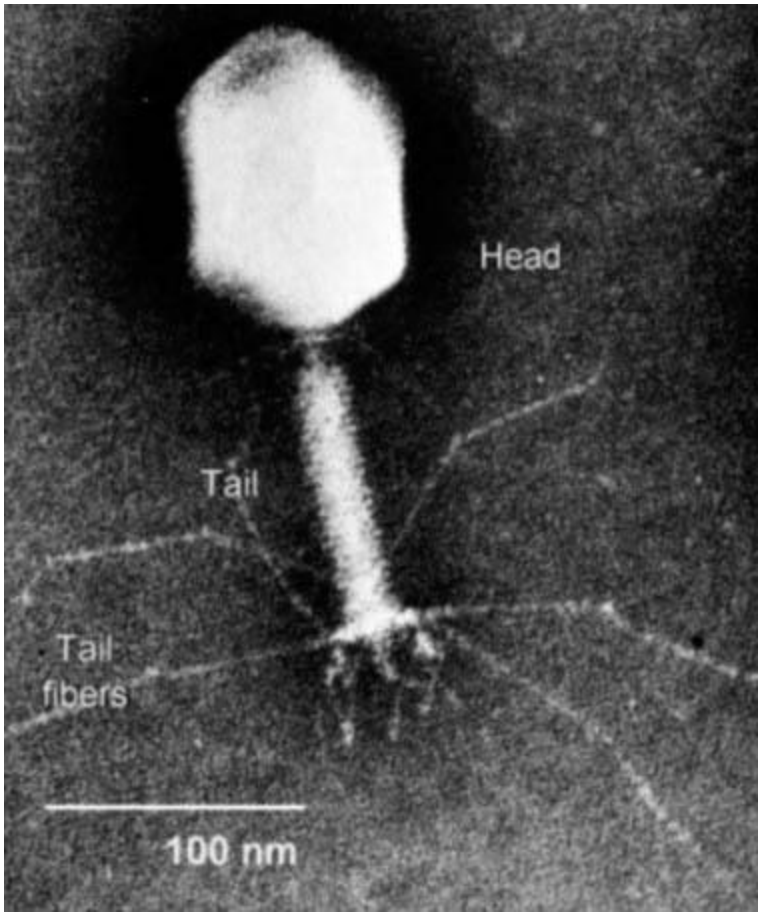
Human trials of the world's first "superbug" killer,

StaphTAME, a genetically-modified protein developed from phages.

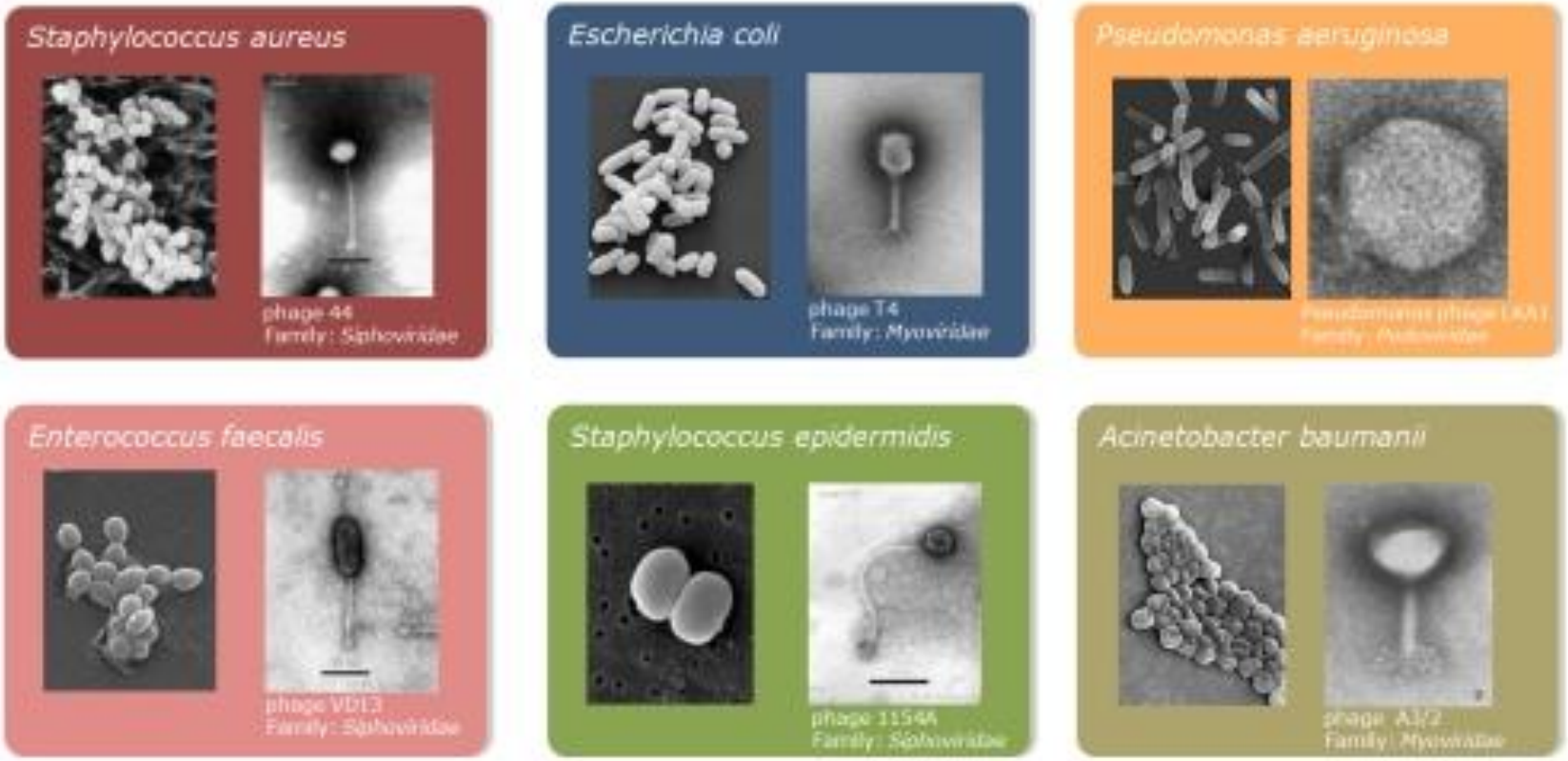
Host specificity of bacteriophage

A particular phage can usually infect only one or a few related species of bacteria.

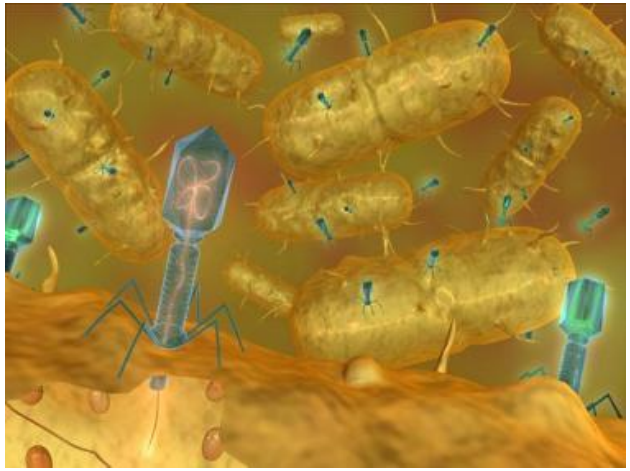
T4 Coliphage infect only the bacterium ***Escherichia coli.***

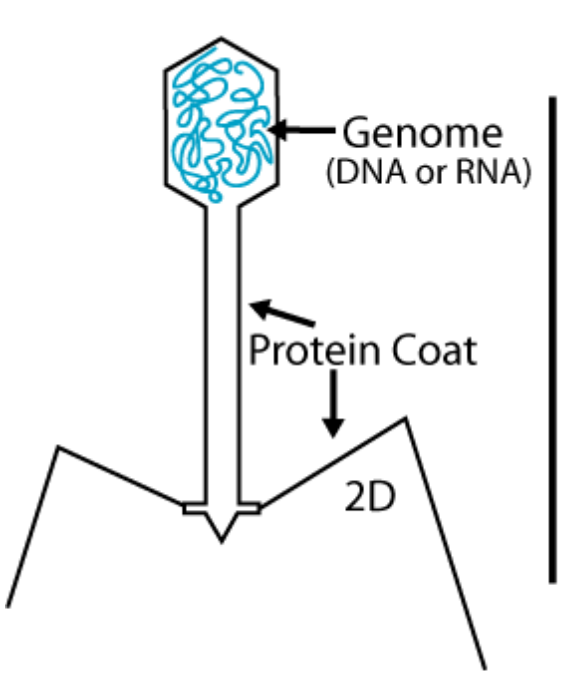


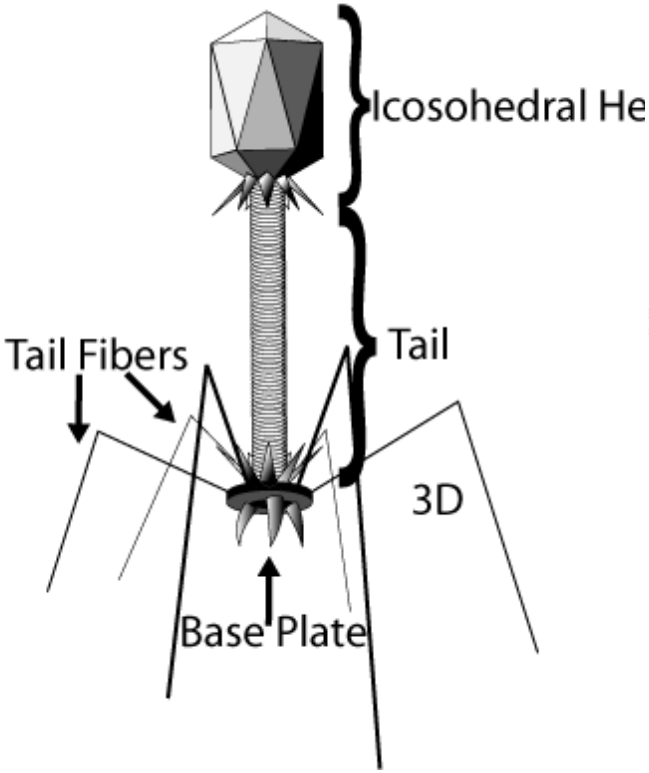
Host specific bacteriophages

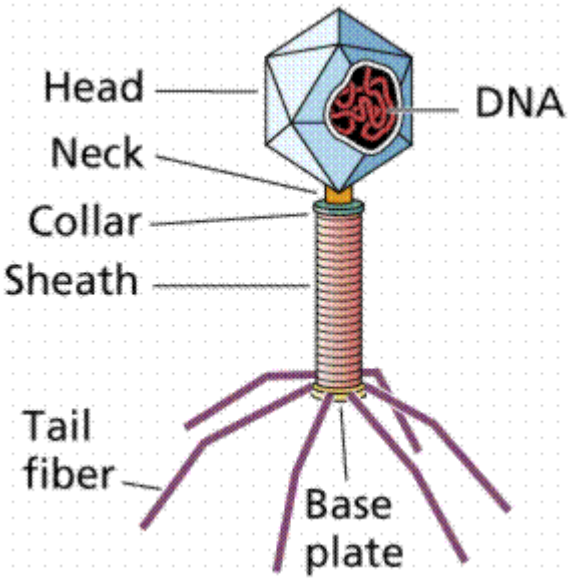


Coliphage:

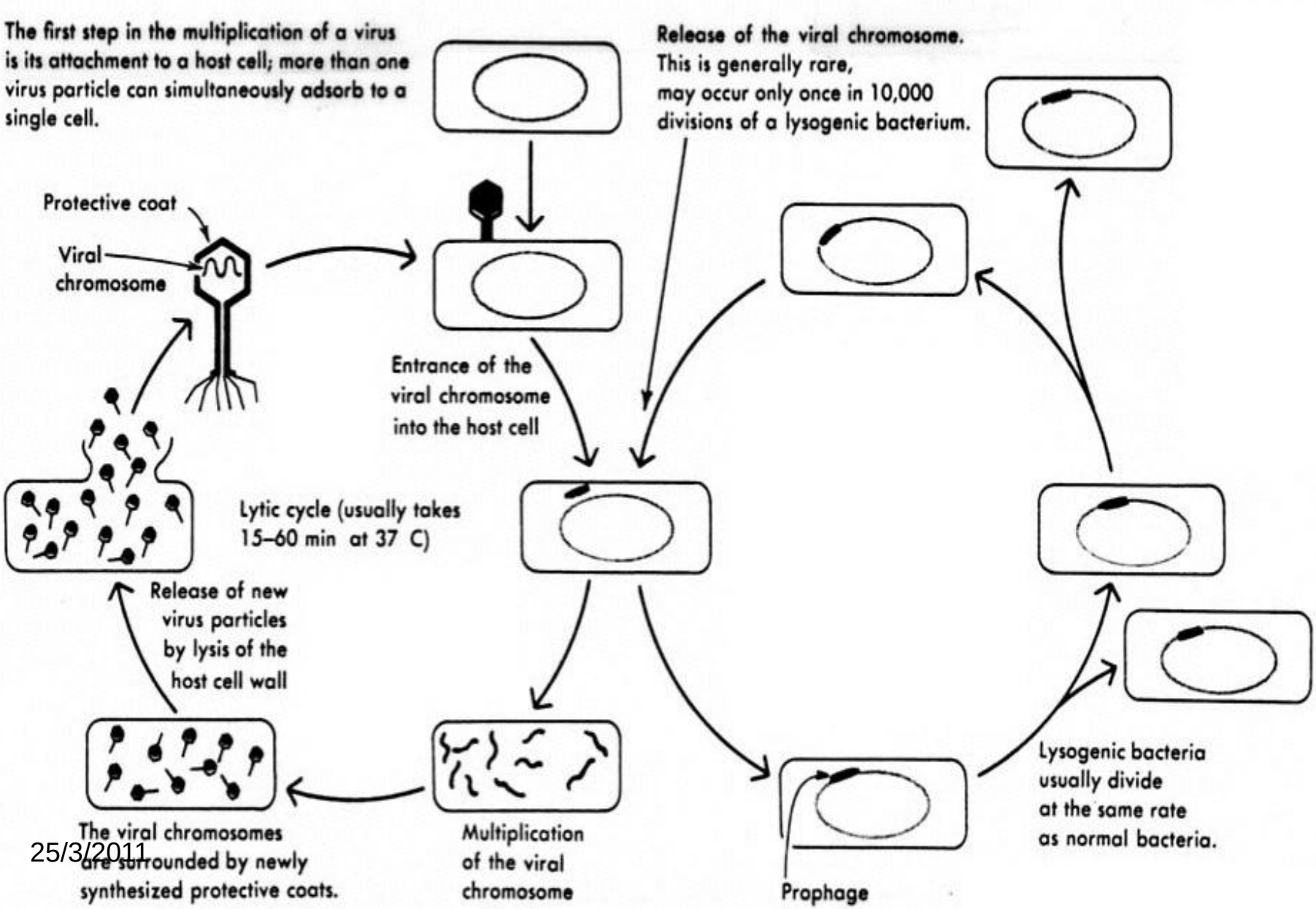



Genome
(DNA or RNA)
Protein Coat
2D


Icosohedral He
Tail
Tail Fibers
3D
Base Plate


Head
DNA
Neck
Collar
Sheath
Tail
fiber
Base
plate

Bacteriophage multiplication

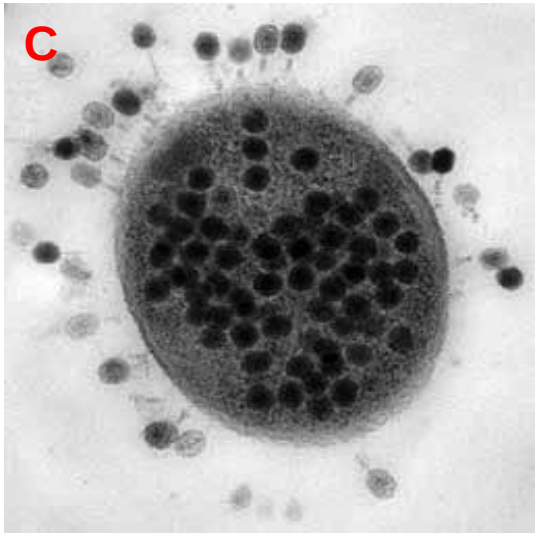


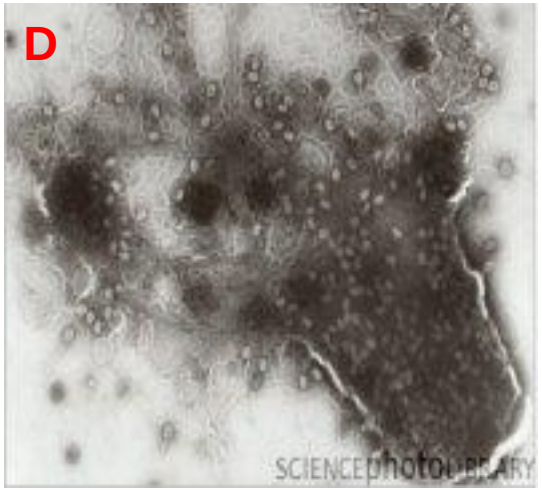
Lytic bacteriophage life cycle

- Adsorption of the phage on the bacterial cell by binding to a specific receptor.
- Injection of the nucleic acid into the bacterium.
- Expression of the phage early genes, synthesis of early proteins, most involved in the shutting down of the host bacterium systems and phage genome replication.
- Replication of the phage genome.
- Expression of the phage late proteins involved in the formation of new phage particles and lysis of the host bacterium.
- Assembly of the phage heads and tails and packaging of the genome.
- Lysis of the host bacterium and release of the new phage progeny.

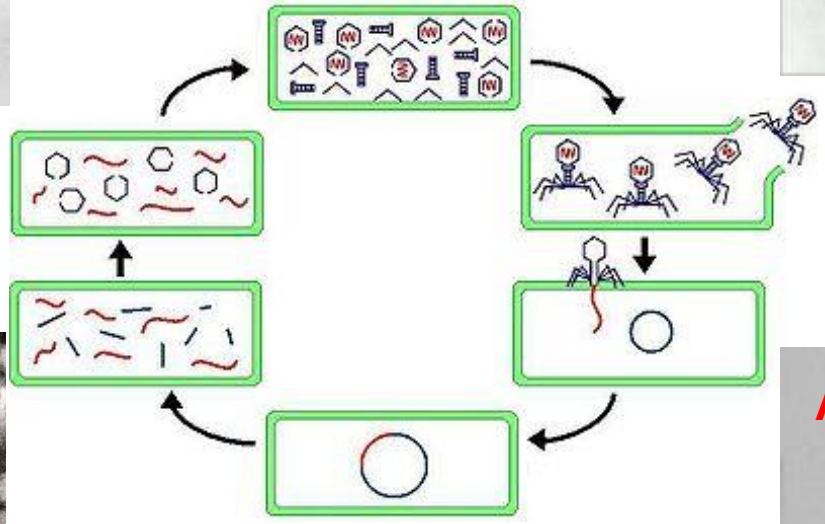
Lytic bacteriophage lifecycle

Lysis and rupture of host bacterial cell, releasing phage progeny to the environment.

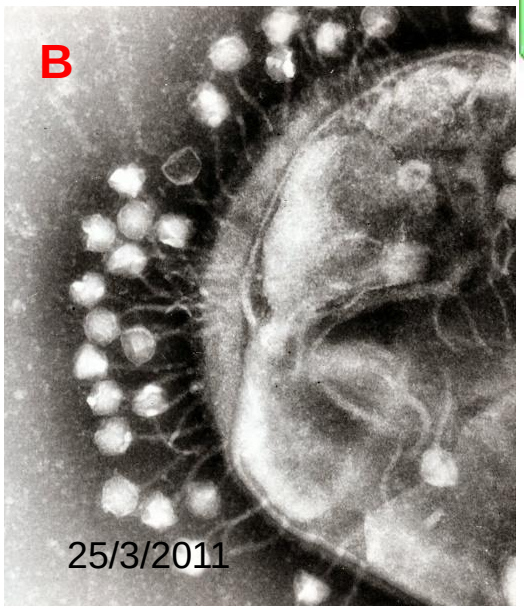




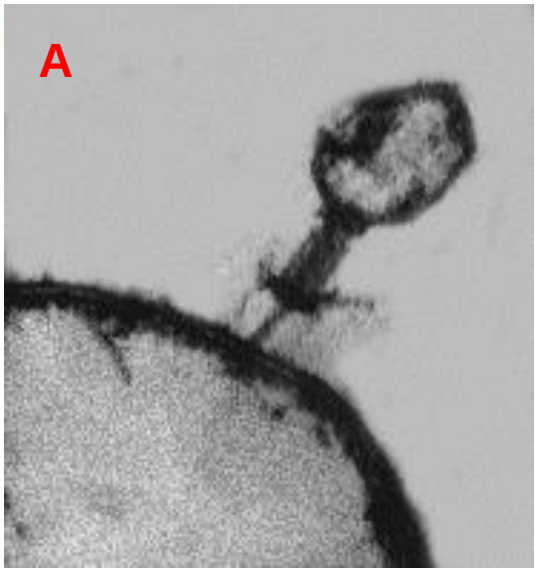
Assembly of phage particles inside the host bacterial cell.

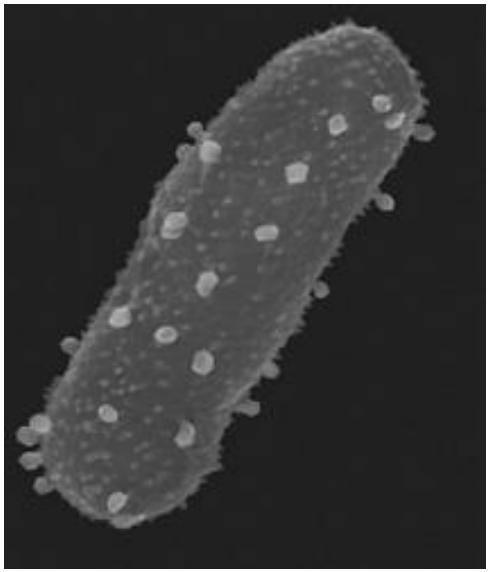


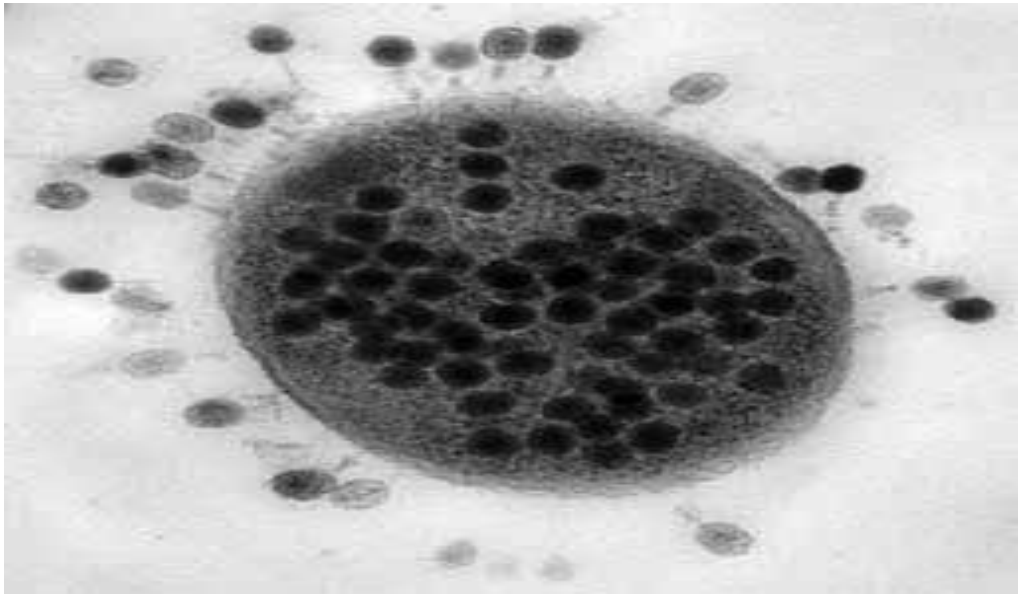
Phage attachment and DNA insertion

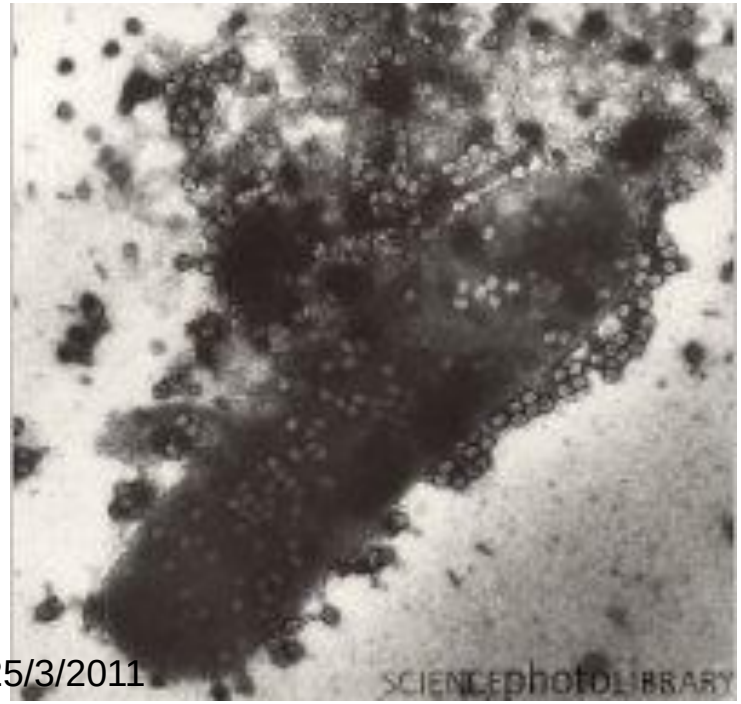


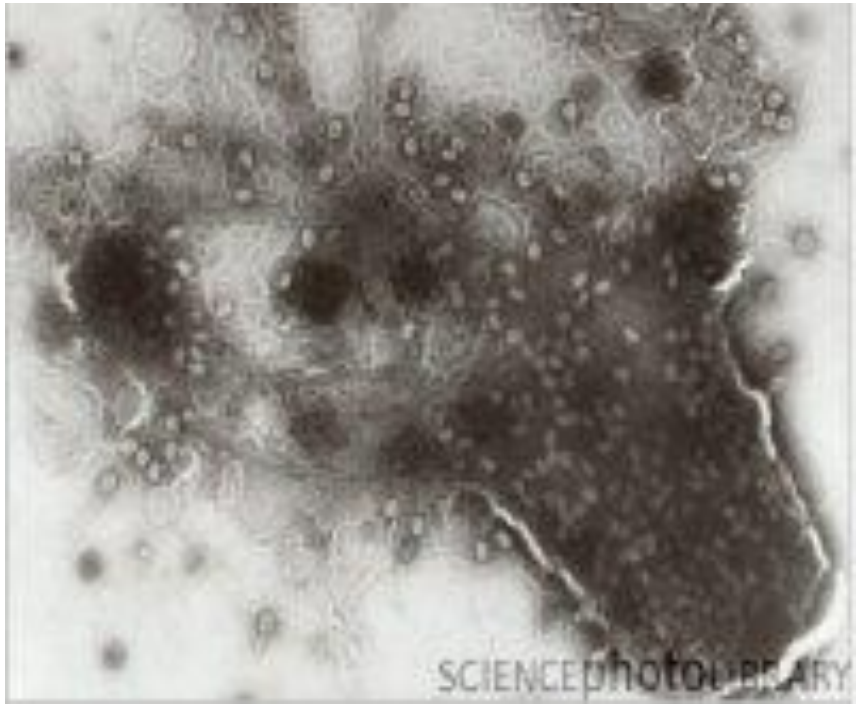
Phage DNA take control of the bacterial cell, replicate itself, directing the synthesis of phage particles like capsid.








SCIENCEphotoLIBRARY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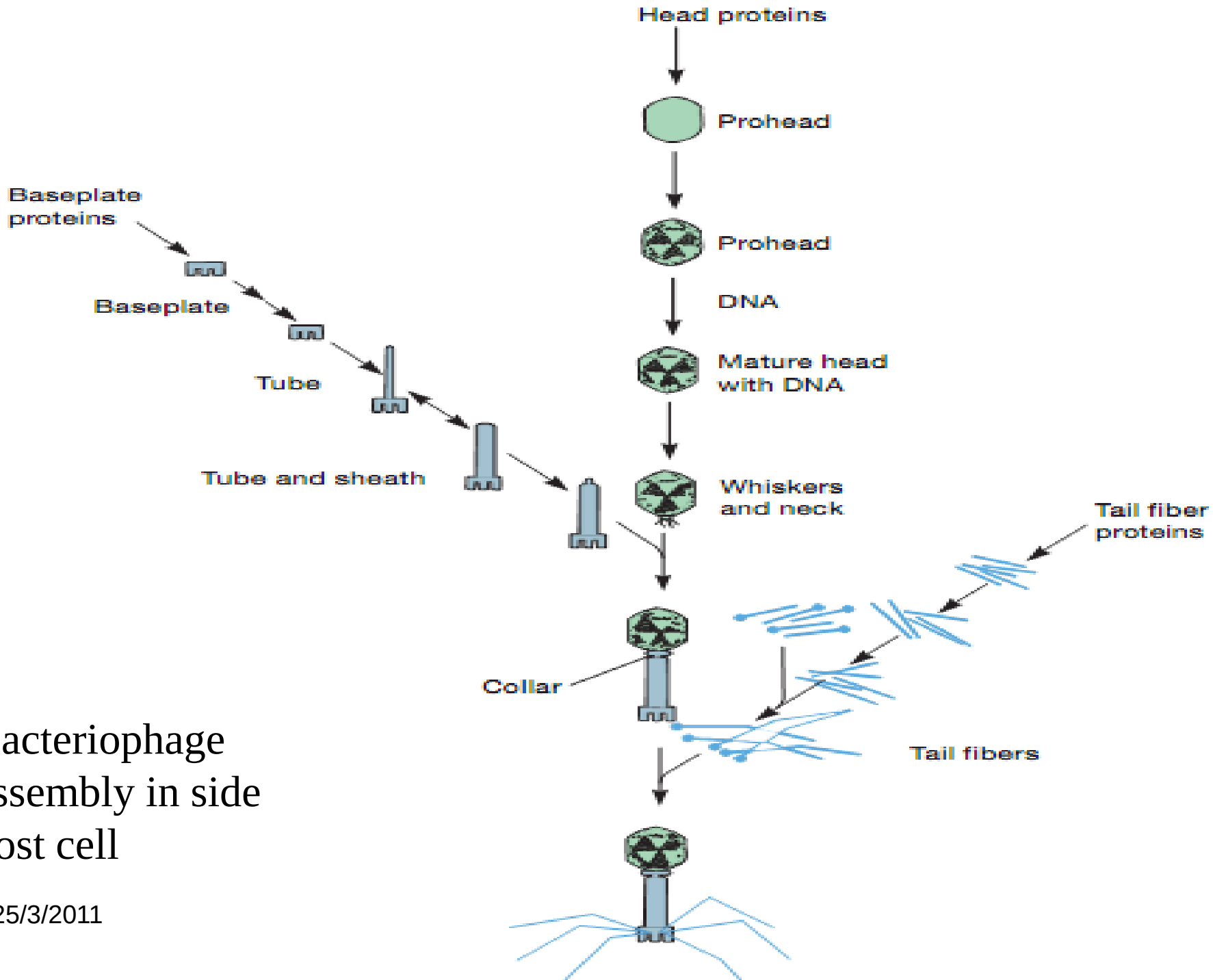

Bacteriophage assembly in side host cell


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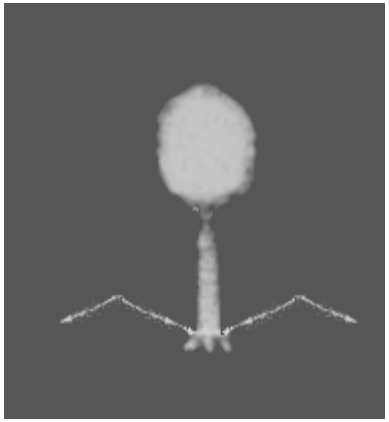
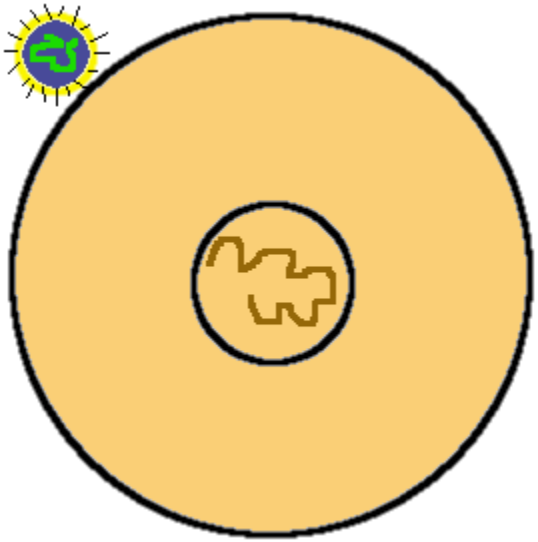
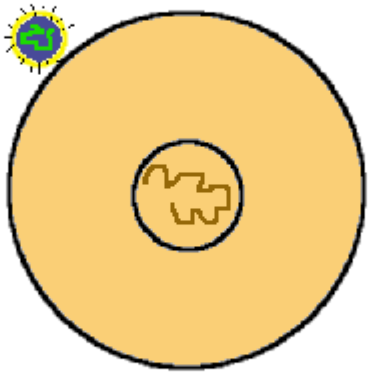
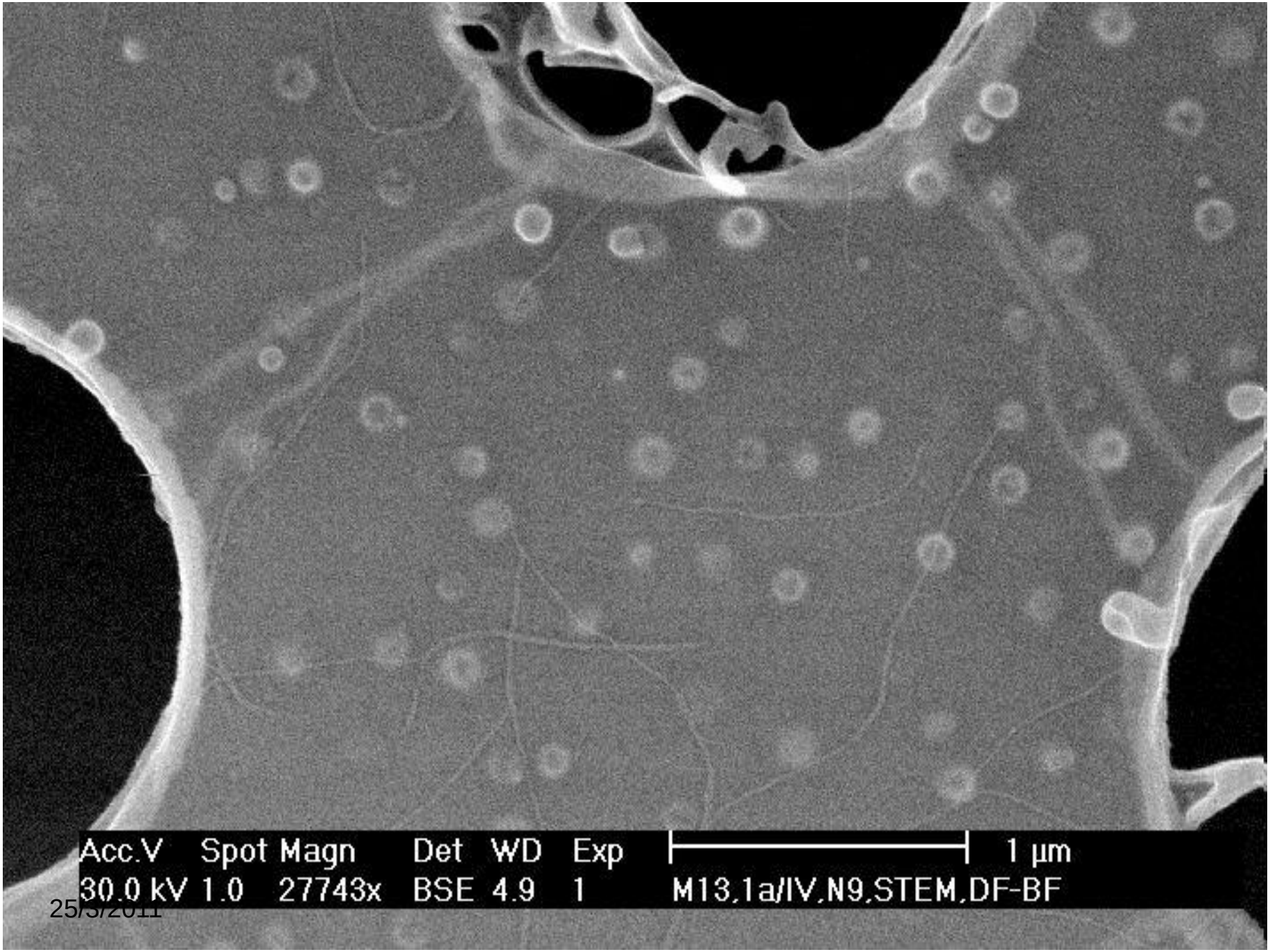
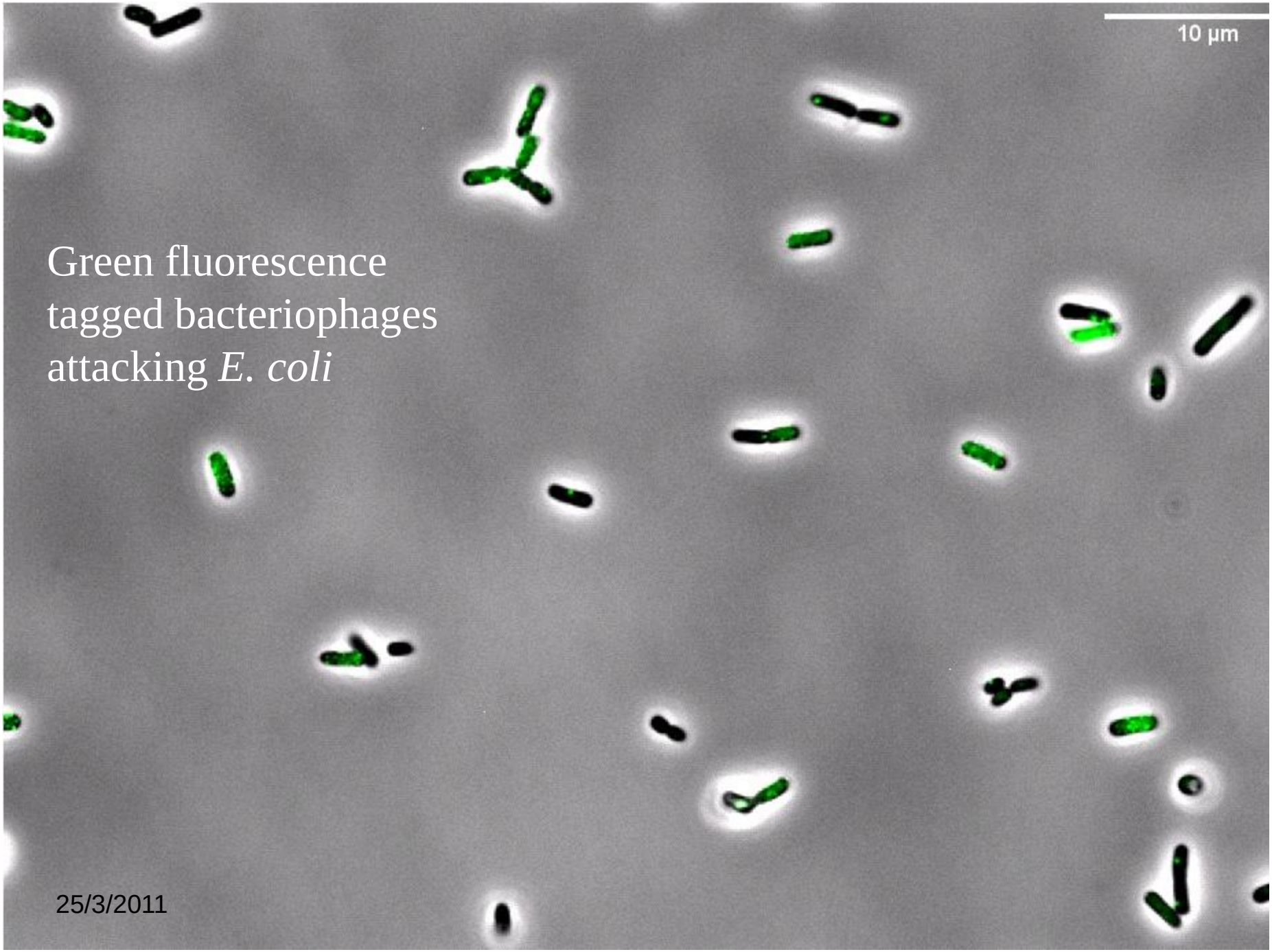







Acc.V Spot Magn Det WD Exp 1 µm
30.0 kV 1.0 27743x BSE 4.9 1 M13,1a/IV,N9,STEM,DF-BF


10 µm
Green fluorescence tagged bacteriophages attacking *E. coli*
25/3/2011

Isolation and enrichment of host specific bacteriophage from sewage

Bacteriophages will be present where ever its host bacterium thrives!

Plaque assay/ test

To confirm the presence of bacteriophage, cell free culture supernatant was dropped on a freshly prepared lawn of host bacterium. Clearing zone on dropped area indicates the presence of bacteriophage

Water/ waste water (Sample which may contain bacteriophage)

Incubate sample with freshly prepared culture containing targeted host bacterium at dark with mild agitation

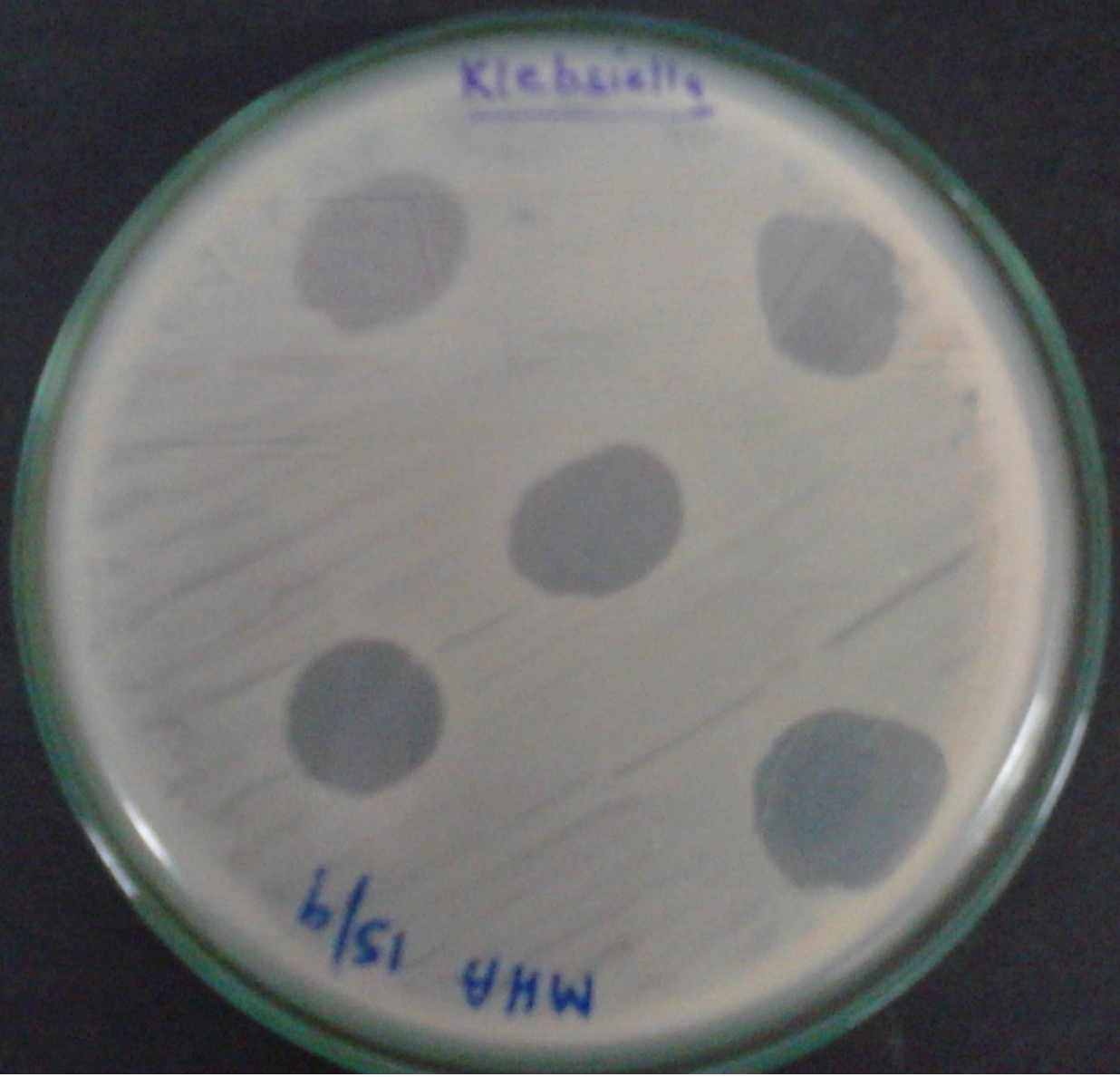
After incubation remove cells by centrifugation and membrane filtration

If plaque test is positive inoculate the cell free culture extract to a freshly prepared liquid culture and repeat this process until the complete clearing of liquid culture

25/3/2011

Plaque assay: drop test method

Plaque assay: Clearing zones due to host cell destruction by bacteriophage

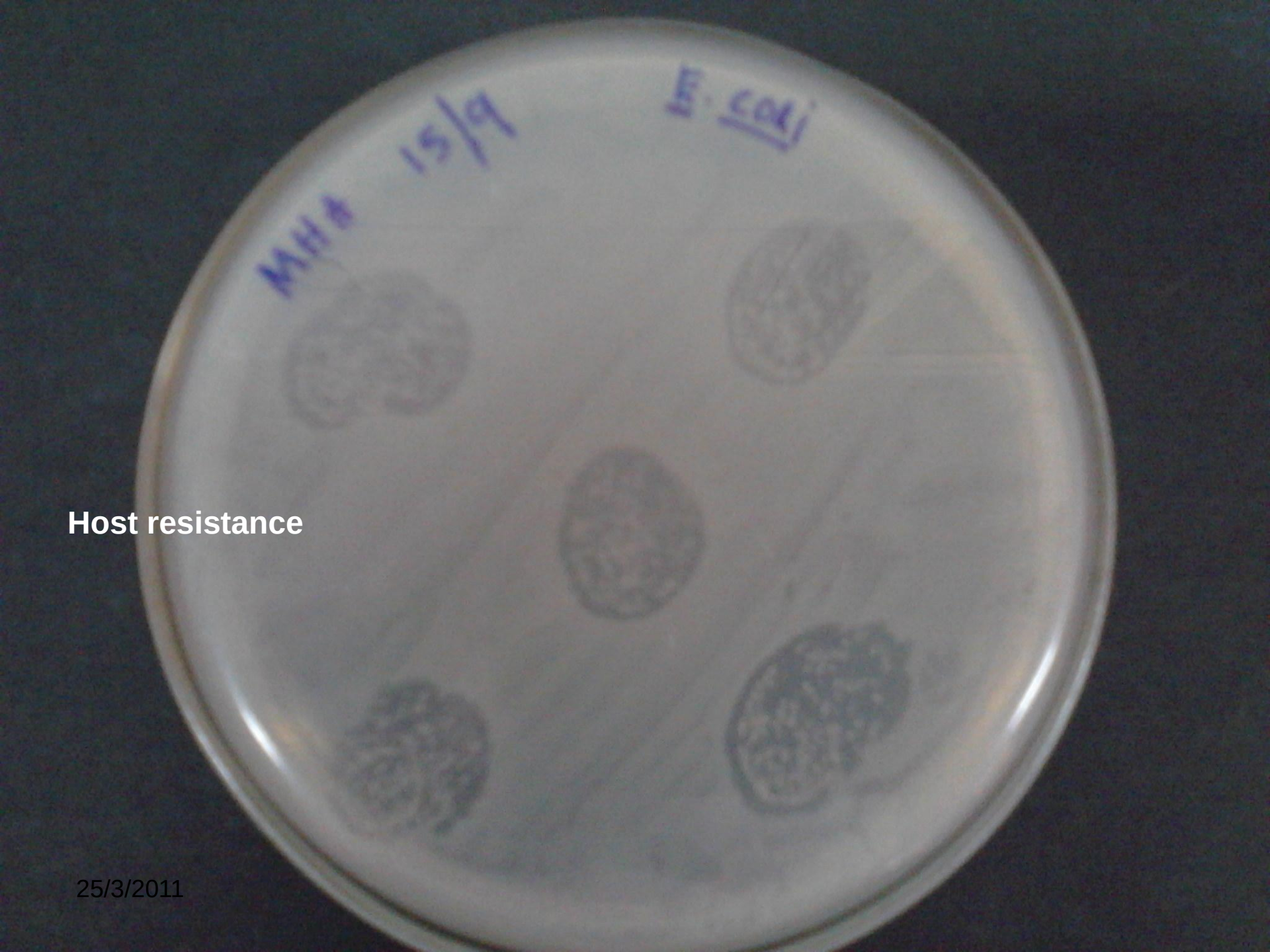


25/3/2011


SAL
KLEB

Phage obtained from *Salmonella* & *Klebsiella* enrichment effective against *Klebsiella sp.*

25/3/2011



Host resistance


SRom
SHK
E. Chi
HOST - SALMONELLA
25/3/2011

Presence of *E. coli* specific bacteriophage: Clearing of the culture broth

Presence of *E. coli* specific bacteriophage: Clearing of the culture broth

E. coli

Shigella sp.

Salmonella sp.

Klebsiella sp.

25/3/2011

Host augmented sewage experiment

Raw sewage	Autoclaved sewage
Number of already present host bacterium enumerated	Since, it is sterilized no host bacterium present
Host bacterium was added to sewage to get a final cell count of $1x10^8$ cells/ mL	Host bacterium was added to sewage to get a final cell count of $1x10^8$ cells/ mL
Phage extract was added (4mL/ 500mL)	Phage extract was added (4mL/ 500mL)
Very good removal (98%)	Good removal (80%)

Progress:

Isolated *E. coli*, *Klebsiella sp.*, *Salmonella sp.* and *Shigella sp.* from sewage.

Isolated and enriched host specific bacteriophages against *E. coli*, *Klebsiella sp.*, *Salmonella sp.* and *Shigella sp.* from sewage.

Standardized the protocol for phage isolation and enrichment of host specific bacteriophage.

Standardized the protocol for plaque assay and phage titre determination.

Potency of host specific bacteriophage was assessed in lab scale simulated microcosms.

Thank you!


cpcb
CENTRAL POLLUTION CONTROL BOARD
Ministry of Environment & Forests
(Govt. of India)