B.Sc (Botany Hons.) Part-1, Paper-II

Bacteriophages

1.Discovery of Bacteriophages:

Viruses which attack or parasitize the bacterial cells are known as bacteriophages. These were first discovered by **Edward Twort** in **1915** in **England** and later by **d'Herelle (1917)** in **France**. They observed that broth cultures of certain bacteria of the intestinal group (dysentery organisms) could be dissolved by the addition of a bacteria free filtrate of certain sewage specimens.

The clear broth filter again added to a fresh culture of the susceptible bacteria, which cleared bacteria. This is known as the **Twort-d'Herelle phenomenon**. **d'Herelle (1917)** termed the virus which destroys bacteria as **bacteriophage**, which literally means **'bacteria eaters' (Greek phage-to eat)**. They are also known as **phages.**

Bacteriophage are **obligate parasites** and are dependent on living bacteria for their growth. They may be found in nature where bacteria are growing. They are abundant in soil, sewage, intestine of men, animals, insects, vegetables, milk etc. Some viruses parasitize Actinomycetes (actinophages), yeast cells (zymophages) and blue green algae (cyanophages).

2. Types of Bacteriophages:

The phages have specific host. E. coli has been studied most extensively from this point of view. The bacteriophage capable of destroying E. coli is called coli-phage. The types of coli-phages have been called as T-phages.

They have been classified into many arbitrary groups such as:

(i) T-Even Phages (T_2, T_4, T_6):

These phages have an angular head and contractile tail. The DNA contain a unique base 5-hydroxyl methyl cytosine in place of cytosine. These viruses are most thoroughly studied viruses. These are also called virulent as they cause death of the host cells.

(ii) T-Odd Phages (T_1, T_3, T_7):

These viruses have an angular head and a short non-contractile tail. The DNA contain cytosine. These are temperate viruses as their genetic material becomes integrated with bacterial chromosomes and the host remains unaffected.

(iii) T_5 Phages:

These viruses have an angular head and non-contractile tail. The DNA of these viruses also contains cytosine.

3. Structure of Bacteriophage:

With the help of electron microscopy, the morphology of the bacteriophage has been studied. The T even phages show complex symmetry. These viruses are generally tadpole shaped i.e., a 'head' followed by a 'tail'.

The head is hexagonal and like a prism in outline (Fig 1A, B). This shape is also known as elongated **icosahedron**. It is 950 A° in length and 650 A° in width. The head has a 2-layered protein wall that encloses the double stranded DNA. The wall is 35 A° thick and is composed of about 2000 similar **capsomeres.** DNA is tightly packed in the head and is about 50 μ long.

Attached to one of the points of the head, through a neck and collar is the tail (Fig. 1C). The tail has a complex structure and is proteinaceous in nature. It is made up of a cubical, hollow, cylindrical core. This core is 800 A° long, 70 A°in diameter and has 25 A° wide central canal. This core is surrounded by a contractile sheath. The sheath is 165 A° in diameter.

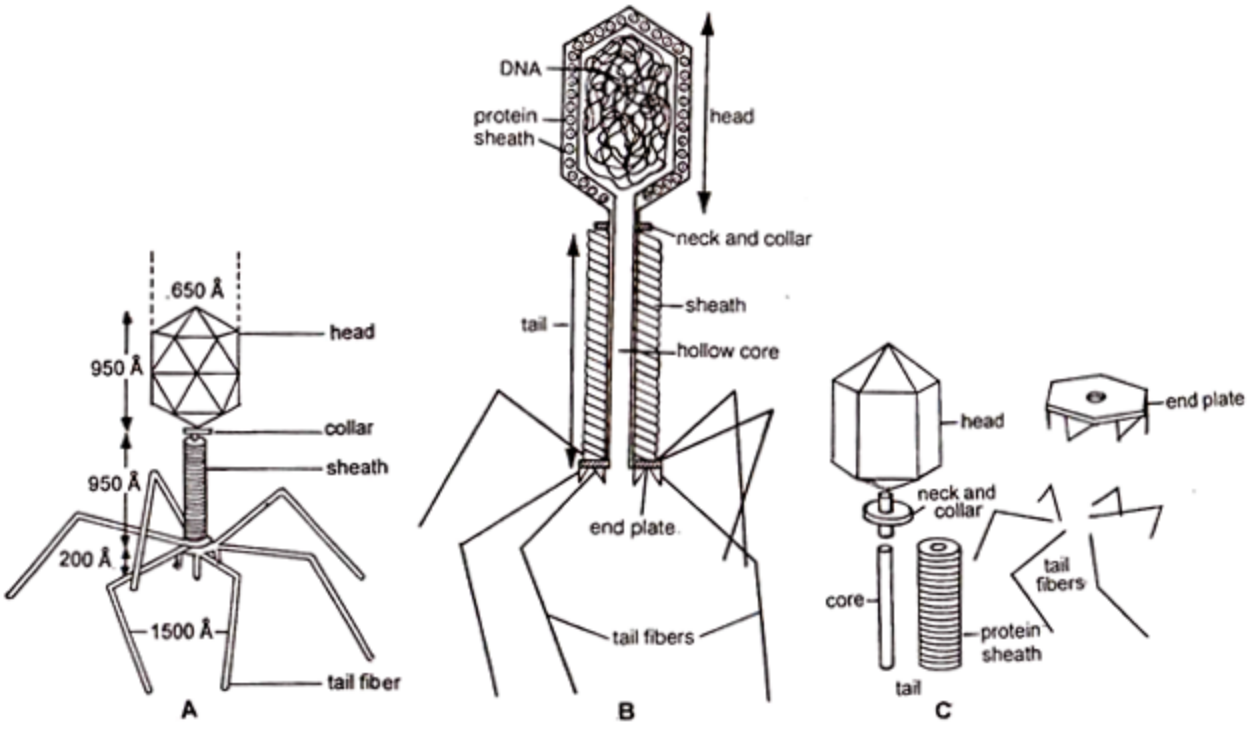

Fig. 5. (A-C) Bacteriophage : A. External morphology, B.L.S. of bacteriophage, C. Various components of bacteriophage

The internal diameter of the tube formed by it is equal to the core diameter of 70A°. The core is terminated into a hexagonal plate which has six small tail fibres (tail 'pins') at every corner and 6 tail fibres. Each tail fiber is 1500 A° long and is composed of fibrillar protein.

The main function of the short tail fibres is to hold the phage fast to the host during sheath contraction and DNA injection while long tail fibres helps in adsorption of the phage on the bacterial wall.

4. Chemistry of Bacteriophages:

Bacteriophages are made up of nucleoproteins. The proteins are about 50-60% and nucleic acid 40-50%. The nucleic acid is either double stranded DNA or single stranded DNA or single stranded RNA (Both never occur together).

Important Characteristics of Some Bacteriophages:

Phage	Nucleic acid type	Head	Tail
T_1, T_3, T_5, T_7	Double stranded DNA	Hexagonal	short, non-contractile
T-even	Double stranded DNA	Polygonal	contractile tail
$\phi \times 174$	Single stranded DNA	Hexagonal	tail absent
f_2, R_{17}, f_r	Single stranded RNA	Hexagonal	tail absent

5. Life Cycle or Multiplication of Bacteriophages:

Bacteriophages show two types of life cycles:
(i) Lytic cycle, and

(ii) Lysogenic cycle.

(i) Lytic Cycle:

In this type the sensitive bacterium lysis and large number of newly formed virus particles are liberated. These viruses are also known as virulent phages (e.g., T_2, T_4 phages).
The whole process can be summarized in six stages:
(a) Attachment (adsorption) of Virus Particle to Sensitive Cells:
Random collision brings the phage particles in contact with the susceptible bacterial cell. The phage particle attaches itself by adsorption on specific 'receptor sites' on the wall of the sensitive bacterium with the help of tail fibres (Fig. 2A).

(b) Penetration into Cell of Virus Nucleic Acid:
The host cell wall is dissolved by phage enzyme secreted by the plate and an opening is formed. This enzyme is of lysozyme type which hydrolyses muramic acid peptide complex of the bacterial cell wall. The elastic protein sheath of the tail contracts, the core forces its way through the cell wall and the DNA is injected into the cell (Fig. 2 B-C). The empty protein head left out-side is called as the 'ghost' or doughnut.

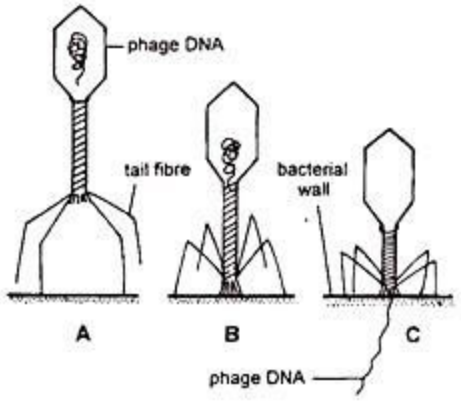

Fig. 2. (A-C). Process of bacteriophage infection : A. Infection in which the phage attaches to the host baterium, (B-C). The nucleic acid core is emptied into the bacterial cell.

(c) Replication of the Virus Nucleic Acid:
After penetration, the viral DNA takes control of all metabolic processes of the host cell (Fig. 3 A-C). For about 12-22 minutes no changes are found to take place in the bacterial cell. This stage is known as latent period phase or period of eclipse.

The chromatin body of the host, which also contains DNA is broken down and its contents are dispersed within the host cell. Viral DNA replicates and gives rise to several copies of the viral genome for the production of new virus particles.

(d) Production of Protein Capsomeres and Other Essential Viral Constituents:
The viral DNA forms messenger RNA and synthesizes the protein of its own type. All the viral components are synthesized separately (Fig. 3 C).

(e) Assembly of Nucleic Acid and Protein Capsomeres into New Virus Particles:
This process is also called maturation. In this process the components already made are grouped together forming new phage particles (Fig. 3 D).

(f) Release of Mature Virus Particles from the Bacterial Cell:
In the final stage the bacterial cell wall bursts and releases new phage particles. This process is also known as lysis. The viral genome synthesizes the enzyme lysozyme (Fig. 3 E).

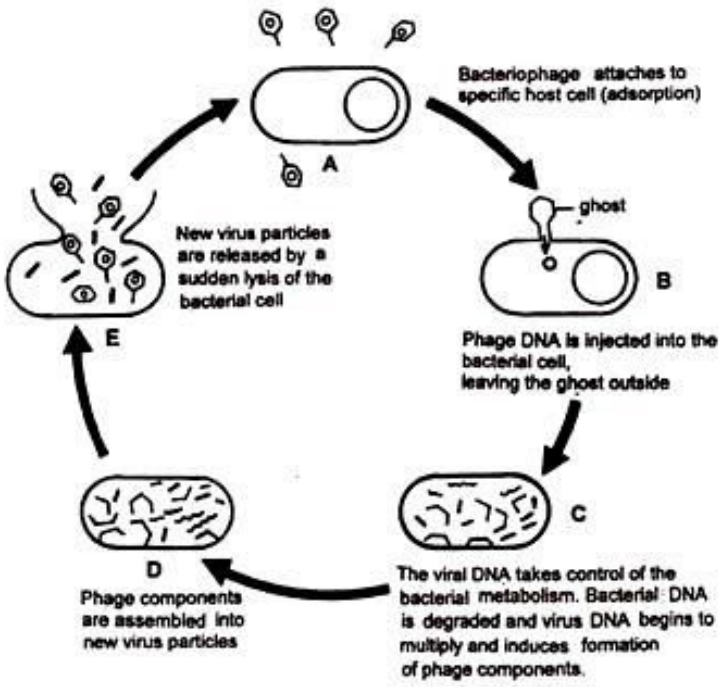

Fig. 3. (A-E). The lytic cycle of bacteriophage.

(ii) Lysogenic Cycle:

In this type of life cycle the virus does not multiply and there is no death of host cells. No virus particles are formed. These viruses are known as avirulent or lysogenic temperate phage. In it, the viral genome gets integrated with the bacterial genome and then replicates along with bacterial DNA. The viral genome in the integrated state is called 'prophase' (e.g. F_2, M_{12} (Fig. 3A-H)).

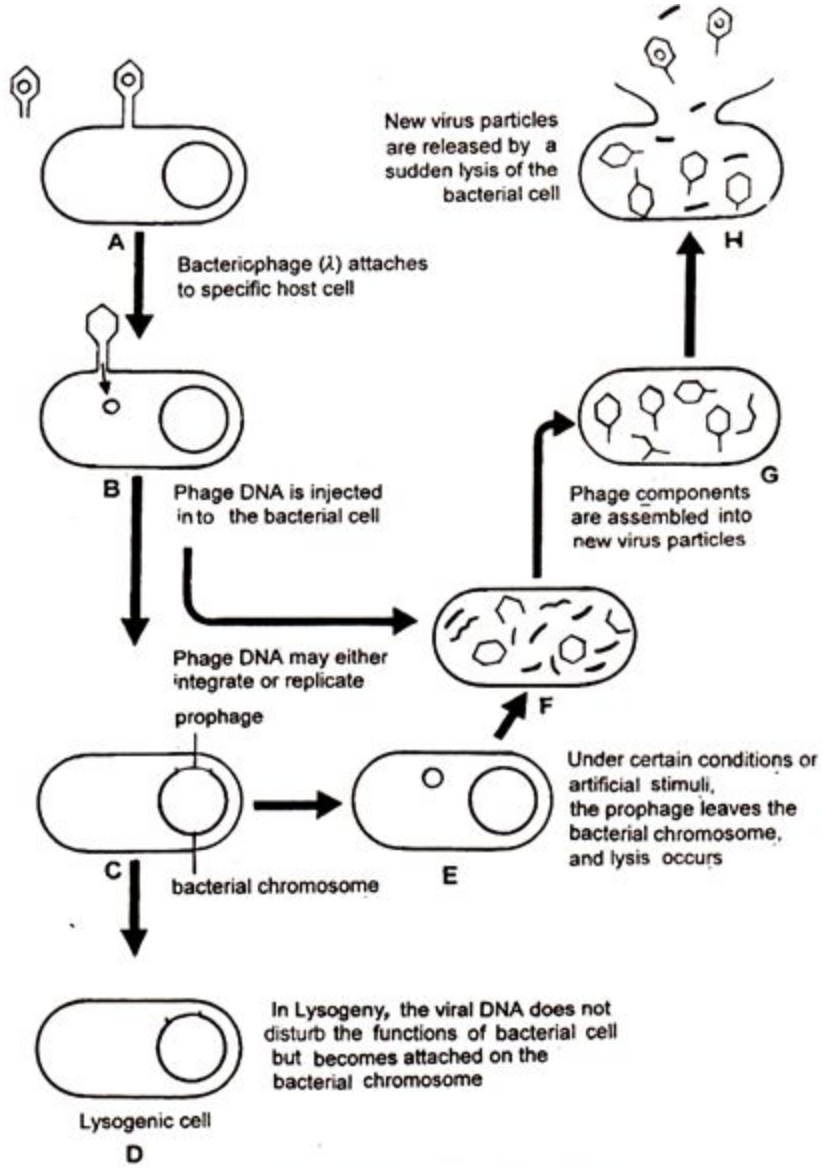

Fig. 4 (A–H). Lysogenic cycle of bacteriophage

6. Biological Importance of Bacteriophages:

Bacteriophages have been used in prophylaxis and medical treatment against several pathogenic bacterial diseases e.g., cholera, plague, dysentery, enteric fever etc. They are also used in the diagnosis of certain infections like plague, cholera etc. Bacteriophages feed on pathogenic bacteria present in polluted water.

So, they can also be used as scavengers. In many cases bacteriophages determine the micro-flora of the soil. Thus, they play an important role in agriculture. In space microbiology, lysogenic cultures are used as radiation detectors and are used in USSR spaceship Vostok 2.

Temperate phages serve as ‘vector in transferring the genetic material from one bacterial cell to another (transduction). Bacteriophages are very harmful during the process of manufacturing of antibiotic and milk products because they kill beneficial bacteria by their lysogenic activity.

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