Isolation and characterisation of Bacteriophages: A systematic protocol.

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Abstract

Bacteria as well as other organisms are subject to infection by viruses. This was realized when Frederick Twort I 1915 in England an Felix d'Herelle in 1917 in Paris independently observed the phenomenon of the transmissible lysis of bacteria. Twort observed that Micrococcus colonies sometimes underwent lysis and that this lytic effect could be transmitted from colony to colony even at high dilution of material from a lysed effect. However, heating the filtrate destroyed its lytic property from this observation. Twort cautiously suggested that the lytic agent might be a viruses. This was also demonstrated by d'Herelle in the following experiment a few drops of liquid faeces from a case of bacterial dysentery were added to a tube of broth which was incubated overnight; filtration of this culture through a porcelain candle yielded a bacteria – free filtrate which, when added in very small quantities to a young culture of Shigella shigae produced clearing and lysis of the bacterium after incubation for several hours. D'Herelle was able to show that this lysed culture possessed a similar lytic property towards a fresh culture and he was able to carry the effect through more than fifty successive transfers, he thought the effect was caused by "an invisible microbe that is antagonistic to the dysentery bacillus and suggested that this was a minute parasite of the bacteria propagating and multiplying at the expense of the bacterial cells. He called the microbe "Bacteriophage" which means "bacteria eater" a named now frequently abbreviated to phage and this view that the agent was a virus has been fully confirmed.

Keywords: Bacteria, Isolation, Virus.

Isolation and Assay Method

Bacterial viruses and easily isolated and cultivated in young, actively growing cultures of Bacteria in broth or an agar plates. In liquid cultures, lysing of the bacteria may cause a cloudy culture to become clear, where as in agar – plate cultures, clear zones or plagues become visible to the unaided eye.

The principal requirement for isolation and cultivation of phages is that optimal condition for growth of the host organism be provided. The best and most usual source of bacteriophages is the host habitant. For example coliphages or other phages pathogenic for other bacteria found in the intestinal tract can best be isolated form sewage or manure. This is done by centrifugation or filtration of the source material and addition of chloroform to kill the bacteria cells. A small amount (such as 0.0 ml) of this preparation is mixed with the host organism and spread on the agar medium. Growth of phage is indicated by the appearance of plagues in the otherwise opaque growth of the host bacterium.

Morphology and Structure

Bacteria viruses are widely distributed in nature. Phages exist for most, if not all bacteria. With proper techniques, these phages can be isolated quite easily in the laboratory. Bacteriophages, like all viruses, are composed of a nucleic acid core surrounded by a protein coat. Bacteria viruses occur in different shapes, although many have a tail through which they inoculate the host cell with viral nucleic acid. The electron microscope has made it possible to determine the structural characteristics of bacterial viruses. All phages have a nucleic acid core covered by a protein coat, or capsid. The capsid is made up of morphological subunits.

Bacterial viruses may be grouped into ten morphological types. Six are listed below:

Type A: Is the most complex, it has a hexagonal head, a rigid tail with a contractile sheath, and tail fibers. E.g. Myoviridae (T4).

Type B: is similar to type A, it has a hexagonal head. However, it lacks a contractile sheath, its tails is flexible and it may or may not have tail fibers e.g. Styloviridae (^).

Type C: is characterized by a hexagonal head and a tail shorter than the head. The tail has no contractile sheath and may or may not have tail fibers. e.g. Pedoviridae (T3).

Type D: has a head made up of large capsomere but has no tail e.g. Microviridae (S13).

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Type E: has a head made up of small capsomere but has no tail e.g. Leviviridae (MS2).

Type F: members have filamentous shape inorviridae (Fd).

Type A,B, and C show a morphology unique to bacteriophages. The morphological types in groups D and E are found in plant and animals (including insects), viruses as well (Papilloma virus, polio virus and flexuous filamentous virus). The filamentous form of Group F is found in some plant viruses such as potato virus X. Pleomorphic viruses were recently discovered to have a lipid – containing envelope, have no detectable capside and posses double – stranded DNA (ds DNA). The representative phage is Mv-L2 [1].

Phage structure

Most phages occur in one or two structural form having either cubic or helical symmetry. In overall appearance, cubic phages are regular solids or more specifically, polyhera (singular polyhedron); helical phages are rod shaped. Polyhedral phages are icosahedral in shapes. The icsahedron is a regular polyhedron with 20 triangular facets and 12 vertices). This means that the capside has 20 facets, each of which is an equilateral triangle; these facets come together to form 12 corners. In the simplest capside, there is a capsomere at each of the 12 vertices, this capsoemre, which is surrounded by fire other capsomere, is termed a PENTON. For example, the phage 0 × 174 exhibits the simplest capsid. In large and more complex capsids, the triangular facets are subdivided into to progressively larger unit number of equilateral triangles. Thus a capside may be composed of hundreds of capsomeres but it is still based on the simple icosahedrons model [2].

The elongated heads of some tailed phages are derivatives of icosahedrons. For example, the head of the T2 and T4 phages is an icosahedrons elongated by one or two extra hands of hexons. The road shaped viruses have their capsomeres arranged helically and not in stacked rings. An example, of the bacteriophages M13.

Some bacteriophages, such as the T-even coliphages (T2, T4 and T6) have very complex structures including a head and a tail. They are said to have binal symmetry because each virion has both an icosahedral head and a hollow helical tail [3].

List of bacteria cells affected by bacteriophages

The following examples of bacteria ells are affected by Bateriophages; Alkaligenes affected by A6 phage, Brucella by F1, F2 Rhzobium by F9 Anabaena by A-1 (L) Norcardia by B1 Escherichia coli affected by T2, T4, T7 etc. C1.

Classification and nomenclature of bacterionphages

It may be apparent by now that the common names of bacteriophages do not follow particular guidelines. They have simple designation or code symbols assigned in investigators. Although, serving the practical needs of the laboratories, this is a haphazard way of naming a group of micro-organisms Table 1.

Consequently, the international committee on Taxonomy of viruses (ICTV) has a bacterial virus subcommittee working on the classification and nomenclature of bacteriophages. However, taxonomic development within the bacterial viruses remains slow and difficult for two mains reasons. First, some 1,900 descriptions of bacterial virus isolates of known morphology have been published. About 150 new descriptions are published each year. Many of this description give a characterization of the virus that is quite inadequate for establishing its relationships with other phages. Secondly, a substantial proportion of the scientists who work with bacterial viruses are molecular biologist rather than virologists. They work with a relatively small selection of extremely well characterized viruses and are satisfied with simple code designations for these viruses because these biologists have little need for viral classification nomenclature, or natural relationships.

The bacterial virus subcommittee has now recommended names for families of phages, names ending in-viridae [4].

The viral multiplication cycle

The sequence of events initiated by the injection of the phage nucleic acid and culminating in the release of newly synthesized virions is termed the Viral Multiplication Cycle. It can be plotted as a one step multiplication cycle, which describes the production of progeny virions by cells as a function of time after infection under lytic phage one-step condition (that is the cells are infected simultaneously and secondary infection by progeny virus is eliminated by dilution).

Specifically, during the first ten minutes or so after injection of phage DNA, no phage can be recovered by disrupting the infected bacterium. This is termed the ECLIPSE Period. At the end of this period, mature phages beings to accumulates intracellularly until they are released by cell lysis. No newly released extracellular phages can be seen until lysis begins;

Table 1. *Characteristics of Commonly Studies.*

PHAGE	HOST	DNATYPE	RNATYPE	MW*
0 X174	*Escherichia coli*	Single stranded (ss) circular (Circ)		1.7
Fd	*E. coli*	Ss, circ.		1.7 – 2.0
T1	*E. coli*	Double stranded (ds)		30
P22	*Salmonella*	Ds		26
SPO1	*Bacillus substilis*	Ds		100
P2	*Pseudomonas*	Ds, Circ		6
N1	*Cyanobacteria*	Ds		43
F2	*E. coli*		Ss	
R17	*E. coli*		Ss	
QB	E. coli		Ss	

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the time from infection until lysis is the LATENT Period. The extracellular phage number increases until it reaches a constant titer at the end of the multiplication cycle this time interval is termed the Rise Period. The yield of phage per bacterium is called Burst Size. This procedure affords observation of a single cycle of bacteriophages growth.

It may be mentioned that there are other phages which can reproduce without drastic interruption of host cell physiology. For example, the filamentous single stranded DNA phage Fd can replicate within a host cell and is released from it as mature virions without accompanying cell lysis or death. That is, the infected cells continue to reproduce themselves as well as the virus, and the mature virions are excluded from the cell surface continuously over a long period of time. This type of release mechanism is called productive infection.

Lytic cycle

Much of what is known about bacteriophages replication has come from studies of the virulent even numbered T-phages (T2, T4, T6) of Escherichia coli. However, it is apparent that the basic sequence of events during phage replication is similar for most phages. Variations occur with respect to the number of phage proteins made, degree of usurping of host functions. T-even phage would be used as a model for discussing phage replication [5,6].

Adsorption

The first step in infections of a host bacterial cell by a phage is adsorption. The tip of the virus tail becomes attached to the cell via specific receptor sits on the cell surface [7]. Attachment is specific in that certain viruses and susceptible bacteria have complementary molecular configurations at their opposing receptor sites. In some cases, the specific receptor of the bacterium is part of the bacteria lipopolysaccharide although many surface structures can function as a specific phage receptor. Including flagella, pili and carbohydrates and proteins in the membrane or cell wall.

It should be noted that infection of a host bacteria cell cannot occur without adsorption. Some bacterial mutant have lost the ability to synthesis specific receptors; they also become resistant to infection by the specific phage.

Initial absorption of the phage to the receptor is reversible (that is the phage can be washed away), when only the tips of the tail fibers attached first to the cell surface. But this soon becomes irreversible when the tail pins attach [8].

Penetration

If too many phages are attached to the bacterium and penetrate it, there may be premature lysis (lysis (lysis from without) which is not accompanied by the production of new virus. The actual penetration of phage into the host cell is mechanical. But it may be facilitated by localized digestion of certain cell surface structure either by phage enzymes (e.g. Lysozyme) carried on the tail of the phage or by viral activation of host degradative enzyme [9].

In this T-even phages, penetration is achieved when:

- The tail fibers of the virus attach to the cell and hold the tail firmly against the cell wall.
- The sheath contracts driving the tail core into the all through the cell wall and membrane; and
- The virus injects its DNA the way a syringe injects a vaccine.

The protein coat, which forms the phage head and the tail structure of the virus remain outside the cell.

Phage such as T1 ad T5 that do not have a contractile sheath also inject their nucleic acid through the cell envelope possibly at adhesion sites between the inner and outer membrane. That is, sheath contraction is not a prerequisite for phage infection. The filamentous, rod shaped DNA phages (such as fd and M13), like animal viruses enter the bacteria cell as discrete virions prior to the liberation of DNA from the phage capside [10].

Transcription

In the case of phage T4, transcription occurs in several stages leading to the formation of immediately early, delayed early and late gene products so named on the basis of their time of appearance. The sequence of transcription events of the phage DNA in other bacteriophages may vary from that of the T4 model discussed here. In brief bacterial mRNA and bacterial proteins stop being synthesized within a few minutes after entry of phage DNA. Bacterial DNA is quickly degraded to small fragments and the nucleoid region of the bacterium becomes dispersed. Some phage mRNA is made immediately after infection [11]. The amount of phage DNA increase after a brief delay virion. Specific protein appear somewhat later, followed by appearance of organized capside precursors and resulting in the formation of mature infections capsids. Immediately early phage genes are transcribed using the existing bacterial RNA polymerase. For the most part, these genes code for nucleases that breakdown host DNA (rendering its nucleotides available for phage DNA synthesis) and for enzymes that alter the bacteria RNA polymerase so that it will preferentially transcribe delayed early phage genes.

Delayed early genes code for phage enzymes which produce unique phage DNA constituents such as 5-hydroxymethyl-cytosines (which replaces cytosine in the bacterial DNA) which glycosylate these nucleotides; or which destroy precursors of cytosine deoxynucleotides so that no bacterial cytosine will be incorporated into phage DNA. These alteration enable the phage to survive because bacterial restriction enzymes (nucleases) are unable to degrade phage DNA modified by substitution of 5-hydroxy methylcytosine. For cytosine and by glycosylation of this substituted base. Further a phage nuclease will destroy and DNA that has unsubstituted cytosine. Delayed early genes also code for polymerase and ligases that play specific roles in phage DNA replication and recombination and for a second altered RNA polymerase that will transcribe the late gene.

Late genes products include the structure components of new phage lysozyme (an edolysin) which will lyse the bacterial cell, releasing the matue virions.

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Assembly and release

Only after the synthesis of both structural proteins and nucleic acid is well under way do the phage components begin to assemble into mature phages. About 25 min. after initial infection some 200 new bacteriophage would have been assembled and the bacterial cell bursts releasing the new phage to infect bacteria and being the cycle over again.

Lysogeny

It is not all infections of bacterial cells by phages that proceed as described above to produce more viral particles and terminate in Lysis. An entirely different relationship, known as Lysogeny, may develop between the virus and its bacterial host. In lysogency the viral DNA of the temperate phage, instead of taking over the functions of the cell's genes, is incorporated into the host DNA and becomes a prophage in the bacterial chromosome, acting as a gene. In this situation the bacterium metabolizes and reproduces normally, the viral DAN being transmitted to each daughter cell through all successive generations. Thus, prophages behave as plasmids and are in fact considered as such by molecular biologists. Sometime however, due to unknown reasons, the viral DNA is removed from the host's chromosome and the lytic cycle occurs. This process is called Spontaneous Induction.

Infections of a bacterium with a temperate phage can be detected by the observations that the bacterium is resistant to infection by the same or related phage and that it can be induced to produce phage particles. A change from lysogeny to lysis can sometimes be induced by irradiation with ultraviolet light.

Mechanism of Lysogeny

A good part of our knowledge on lysogeny comes from studies on the coliphages lambda (^). It is generally considered to the typical of the temperate phages.

When a sensitive bacterium is infected by temperate phage, two things may happen. In some of the infected cells, multiplication of the phage occurs and a lytic cycle takes place. In the other infected cells (ranging from a few to 100 percent, depending on both the host and the phage), the multiplication of the phage is repressed (because late genes required for phage multiplication and host lysis are switched off) and lysogenisation occurs. Specifically, the temperate phage possesses a gene that codes for a repressor protein which makes the cell resistant to lysis initiated either by the prophage or by lytic infection by other viruses.

The repressor protein (also called Immunity Repressor, since the cell is resistant to lysis from externally infecting phage) from ^ phage has been isolated and purified. It is an acidic protein with a molecular weight of 26,000. It reacts with two different operator sites on the ^ phage genome to prevent the expression of phage lytic functions and the formation of mature phage particles. Thus, the repression of phage genes is very much like the repression of bacterial operons.

More specifically, the lysogenic state is governed by the activity of the regulatory region of the ^ phage genome, which both bestows immunity to externally infecting phages and causes integration of the phage genome into cellular DNA. This region is termed the Immunity Operon. Upon infection by ^ phage, the phage cro genes is transcribed, resulting in the synthesis of a protein repressor that inhibits the synthesis of the immunity repressor. Thus the basic mechanism in the production and maintenance of the lysogenic state is the antagonism of two repressor – the immunity repessor and the cro-repressor, which prevents immunity.

As previously mentioned, the lytic cycle of bacteriophages ^ can be induced by radiation e.g. ultraviolet light. At the molecular level, this induces the synthesis of a host cell protein encoded in the rec. A gene of Escherichia coli. This protein has protelytic activity; once induced to accumulate, it cleaves the immunity repressor, preventing the later from binding to the, ^ prophage. It is suggested that spontaneous induction of lysis may involve the same mechanism.

No RNA phages have yet been shown to be temperate. It is possible that temperate RNA phage exist; the phage could form a DNA copy of the RNA genome, which can then be integrated into the bacterial chromosome.

Importance of Phages

The effects of bacteriophages on bacteria has merits and demerits which are evident within the industry, medical field and as a research tool in studying host-parasite relationship, viral multiplication molecular genetics.

Industrial use of bacteriophage

Certain manufacturing processes such as antibiotic production; cheese-making which depend on the activity of particular specie or strains of bacteria can be inhibited by phage active against those bacteria can be inhibited by phage active against those bacteria like the phage attacking Streptomyces griseus which is involved in streptomycin production.

Also, twenty-two (22) bacteriophages were isolated from cheese-vat when sample over a priod of four years were found to be active against one or more of four different strains of lactococcus, Lactis subsequent Cremoris used in a defined strain starte-system in an Irish Cheddar Cheese Factory.

Biological use of bacteriophage

Due to the ease with which bacteria can be grown and infected with phage, a lot of basic information on the biology of the virus-host cell interaction has been developed with the phage bacterial call system. The knowledge of the mechanisms of phage infection and reproduction is far more advanced than knowledge of the corresponding mechanisms is animal viruses. There is therefore, a tendency to regard phages as model viruses and to adapt the methods used successfully in their study to the more complex relationship between the animal virus and it's host cell.

Use of bacteriophages in molecular genetics

Phage particles exhibit the same two fundamental genetical properties that are characteristic of organized cells; general stability of type and a low rate of heritable variation. All phage properties are controlled by phages gene and are subject

to change through gene mutation. Most of our knowledge concerning the chemical basis of mutation comes from studies on phages genetics.

Cytological, biochemical and genetic studies show that bacteria have a nucleus containing deoxyribonucleic acid (DNA) and tha this DNA contains genetic information. This is because bacteria oridinarily grow by vegetative reproduction, change in the genetic material is usually the result of gene mutation. Markers that may be used in genetic studies with bacteria include antibiotic resistance; nutritional requirements and sugar fermentation. Gene recombination between bacteria can occur by conjugation in which there are recognized donor and recipient cells. Transformation may take place when DNA from one cell is taken in by other cells, which then become genotypically (genetically) altered to express phenotypically (physically) a characteristic of the first cell type. Furthermore, genetic material from one bacterium may be introduced into another bacterial cell by temperate bacteriophages, a bacteria-destroying agent, this process being known as transduction. Finally, temperate bacteriophages may invade the genetic material of a bacterium and become a prophage and this may be expressed phenotypically in the altered bacterium.

Medical use of virulent phages

Since virulent of lytic phages can destroy their host bacterial cells, it is logical to think that inoculation of such phages into a bacterial infected individual would result in the elimination of the pathogens. However, after numerous studies, there is no evidence to show that phages and be used therapeutically to destroy bacterial pathogens in the human body principally because phages do not persist in the body. Consequently, the primary uses of bacteirriophages are in the identification of bacterial strains and as genetic models in molecular biology.

Thus, lytic phages have been used in the detection and identification of pathogenic bacteria. Strains of bacteria may be characterized by their resistance or susceptibility to lysis by specific virulent phages. The resulting pattern of lysis (from visible phages on a lawn of bacteria growth) of a bacterial strain by different phages types gives an induction of the identity of the bacterium. This laboratory procedure is termed bacteriophages typing and is used routinely for the identification of certain strains of bacterial pathogens such as the Staphylococcus and the typhoid bacilli.

In this way phages serve as a tool for medical diagnosis and of tracing the sources of a disease spreading a community.

Medical aspects of Lysogeny

Diphtheria is caused by the bacterial pathogen Corvnebacterium diphtheria. Its capacity to cause disease is directly related to its ability to produce to a toxin. It can only produce toxin when it carries a temperate phage. In the same way, only those streptococci which carry temperate phages can produce the Ertyrogenic (rash producing toxin of scarlet fever). In other known instances some types of botulism toxin are produced Clostridium botunilium as a result of lysogeny.

Conclusion

This phenomenon in which a prophage is able to make changes in the properties of host bacterium in lysogeny is termed lysogenic conversions. Its occurrence is based on the fact that not all phages genes in lysogeny are blocked by the immunity repressor. Part of the phage DNA can be transcribed to form new proteins.

Bacterial lysogeny is a good conceptual model for study of oncogenic or cancer-producing viruses. Since these viruses also have the capacity of perpetuating their genomes in infected cells.

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