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Characteristics of Bacteriophages. Methods of Working With Bacteriophages

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ABSTRACT

In this article, we will study the characteristics of bacteria and bacteriophages, their distribution in nature and the requirements for working with them in microbiological laboratories. shows the necessary conditions for self-recovery. If a bacteriophage-infected culture is examined under a microscope, instead of bacteria, fragments of bacteria with no specific shape will be seen. Some bacteriophage can affect only one type of bacteria without affecting all types. For example, dysentery, cholera, staphylococcus and other bacteriophages affect only a specific pathogen. So, the bacteriophage has a specific feature, this feature can be related to one type of microbe, even some types of one type of bacteria. We have carried out microbiological examinations of these bacteriophage bacteria with our own eyes..

Bacteriophages :(bacteria and Greek phegos - decomposing) are viruses that swallow, destroy and destroy bacteria; bacteria group. In 1917, the French scientist F. D'Erell gave the name "bacteriophage" to organisms that can pass through bacterial filters and have the ability to dissolve.

N. F. Gamaleya was the first to observe this process (1898). Where there are microorganisms, there are definitely bacteriophages. They have a more rounded or hexagonal head with a diameter of 45–140 nm and a tail that is 100–600 nm in length. The shell of the head and tail is made of protein. Bacteriophages stick to the bacterial cell with their tumor

(adsorption), then the substance of the head penetrates into the cell and forms new phage particles. As a result, the cell is destroyed (lysis), and new bacteriophages are released into the environment. Virulent and moderate (symbiotic) bacteriophages are distinguished. The first produces new particles by breaking down cells (lysis). Others enter the cell but do not dissolve it (prophage). Bacteria that contain moderate phage are called lysogenic bacteria. Some bacteriophages are highly specific, that is, they affect only one type of microbe (monophage). Other Bacteriophages lyse different types of cells (polyphages). Bacteriophages are important in the variation and evolution of microbes. All bacteriophages are composed of proteins and nucleic acids, and depending on the type of these acids, they are divided into bacteriophages that capture DNA and RNA. Bacteriophages are also used as therapeutic and preventive agents. It is also used to diagnose infectious diseases (dysentery, diarrhoea, etc.), and to determine the type of bacteria isolated from the patient's body

Bacteriophages, which destroy various disease-causing (phytopathogenic) bacteria in plants, are also known. Bacteriophages of some pathogenic microbes have been found in the soil and drains of cultivated fields. The experience of treating seeds against various diseases before planting has shown that bacteriophages can be used to disinfect planted seeds. When examined by electronic photography, it was determined how bacteriophage affects the body of bacteria and the reproduction of phage, and it consists of 4 phases. 1. It is adsorbed. 2. Enters the bacterial cell. 3. It develops in a bacterial cell. 4. Dissolves the bacterial cell and releases it into the environment. Phase 1. In this case, the phage particle comes to the body of the bacterium and sticks to its tail. Phase 2. The phage penetrates the bacterial cell membrane, or without entering, directs the DNA of its head into the cell like a syringe. Phase 3. In this case, the phage feeds on various necessary substances of the bacterial cell, lives in the body of the bacterium, and begins to multiply, that is, it creates a special dissolving enzyme, dissolves and digests the protein substances of the cell, and as a result, it multiplies. Phase 4. As the bacteriophage particles multiply, the pressure inside the bacterium increases, the bacterium's body swells, the shape of the bacterium changes, and finally the bacterium cell bursts. Young bacteriophage particles begin to emerge from the ruptured bacterium.

If a bacteriophage-infected culture is examined under a microscope, instead of bacteria, fragments of bacteria with no specific shape will be seen. Bacteriophage particles reproduce only by moving to the side of young bacteria. It cannot multiply in an old culture or in the body of a dead bacterium. If the bacteriophage affects the culture in liquid food (broth), the broth becomes clear. After 1-2 days (sometimes even more) in the young culture in the infused broth under the influence of these substances, it is possible to see the growth of bacteria again. As a result, under the action of bacteriophage, the bacteria lyse and the broth becomes cloudy again, which is a sign of the increase of bacteria. In this way, a new culture is called a secondary culture. Now the reasons for the emergence of this culture have been determined; in each bacterial culture, there may be variants of bacteriophage-resistant and bacteriophage-resistant bacteria. When a culture of bacteria resistant to a bacteriophage is affected by a bacteriophage, the microbes in it are preserved unchanged, and then they multiply and form a secondary culture. Microbes forming secondary cultures are called lysogenic cultures. The variant of phage-resistant bacteria differs from the variant of bacteria resistant to bacteriophage, although it does not differ morphologically, culturally and biochemically, but it is very different in terms of antigenic properties.

Although phage-resistant bacteria are resistant to one strain (copy) of a bacteriophage,

they can also be destroyed under the influence of another strain of the same bacteriophage. Therefore, the secondary culture can dissolve and lyse only if it has a specific bacteriophage. Specific properties of bacteriophage. One of the main features of bacteriophage is that some of them can lyse only the same bacteria. This is its unique feature. As a result of careful examination of this feature of bacteriophage, it was found that it is a very intuitive feature. Some bacteriophage can affect only one type of bacteria without affecting all types. For example, dysentery, cholera, staphylococcus and other bacteriophages affect only a specific pathogen. So, the bacteriophage has a specific feature, this feature can be related to one type of microbe, or even to some types of one type of bacteria. On the contrary, the specificity of some bacteriophage is quite wide and can lyse antigenically close descendants of one type of bacteria. A bacteriophage that affects one species or one type of bacteria is called a monovalent phage. On the other hand, if a phage affects and lyses a type of bacteria itself and its close descendants, this is called a polyvalent phage. Antigenic property of bacteriophage Another property of bacteriophage is that it also has antigenic property. For example, if the same phage is administered parenterally to a rabbit, neutralizing substances (antilysins) against this phage are formed in its body. If a rabbit is immunized with this phage for a long time, its blood serum will have the power to completely neutralize this bacteriophage. For example, phage-neutralizing rabbit serum is added to a dense nutrient medium in a petri dish, and the phage injected into the rabbit is mixed into it.

Then, if a bacterium that undergoes lysis under the influence of this phage is planted in the cup, these planted bacteria grow normally and form colonies without lysis, because the special substances (antilysins) in rabbit serum do not neutralize the phage. When a rabbit is immunized with this phage, the formation of a neutralizing substance against it indicates that the phage has antigenic properties. Bacteriophage resistance Bacteriophage resistance to chemical and physical factors is different. Heat resistance of bacteriophage is the same as that of viruses, and most phages are weakened in broth at a temperature of 65-80°. At a temperature of 50-55°, the activity of some phage may decrease. Different phages have different temperature tolerances. For example, the staphylococcal phage can withstand heat of -60-62°, and the Escherichia coli phage +70-75°. The phage's resistance to heat depends on the pH and composition of the nutrient medium.

Sometimes it is possible to restore the power of a phage that has lost its activity. For this, if the weakened phage is mixed (massaged) with its bacterium several times, the phage can become strong again. Bacteriophages are quite resistant to cold - they may not lose their activity even at a temperature of 185°C. Bacteriophage is very resistant to drought. Using this, phage, which is used against colic, is currently being used dry.

Thus, ultraviolet light kills bacteriophage in 10-15 minutes, and direct sunlight in 2-3 hours. Compared to vegetative forms of bacteria, phages are more resistant to most chemical disinfectants. They quickly die in an acidic environment, and they are more resistant to a weak working environment. A 0.5% solution of antimony, 1% phenol can destroy phage activity in a few days, sometimes a week. Alcohol, ether, and chloroform change very little the potency of phage. Under the influence of 1% formalin, the phage loses its activity in a few minutes. Trypsin cleaves phage within 24 hours. Phage is resistant to the effects of hydrochloric acid in the stomach. Highly concentrated glycerin also kills phage. Another feature of the bacteriophage is that it can adapt to the lysis of other microbes as well as having a specific effect on the bacterium. This feature is called adaptation. To check the activity of the bacteriophage, it is

necessary to titrate it (that is, to determine its strength). Phage titer is determined by two methods. 1. Appelman's method. In this case, the phage is diluted and checked to determine the quantity of phage particles. For this, bacteriophage liquid 1:10; It is diluted from 1:100 to 1:10-10-10 and more. Then take 0.1 ml of each diluted liquid and mix it with the microbial culture in liquid food. After that, test tubes are placed in a thermostat for 12-13 hours, and then it is taken into account in which test tube the cultures were lysed. It is necessary to find the tube with the most diluted phage and lysis. The titer of the bacteriophage will be the number of times the phage was diluted in the liquid in the last test tube. One milliliter of this culture should contain 250 million microbial cells. 2. Determination of phage titer in dense food. The filtrate of the test material is diluted from 10-1 to 10-10. A dense nutrient medium is placed in a Petri dish, and after it has cooled, it is divided into 10-12 parts by drawing with a pencil from the bottom of the dish. Then a young (12-18 hours old) culture is planted on the surface of the food in the Petri dish. After that, from the examined filtrate, the filtrate diluted in the ratio of 10:1 to the first part of the agar in the cup, to the second part diluted to 10-2 ratio, to the third part to 10-3, and to the remaining parts, one drop of the diluted filtrate is dripped until it is diluted to 10-10 ratio. For about 18 hours, "sterile" areas are formed on the food touched by these drops, and as a result of the action of the phage, it is possible to see that no microbes have grown there. Microbes can grow without the strength of the phage in the parts where the filtrate is too diluted. The titer of the phage is determined depending on which section the microbe begins to grow. In general, the titer of most bacteriophages reaches 10-8. Bacteriophage keeps its titer up to 2.5 years. Sometimes, if the titer of the bacteriophage is low and weak, it can be transplanted several times into a suitable bacterium to increase its strength. GENETIC METHODS OF STUDYING MICROORGANISMS

Genetic research has determined the basic laws of heredity by classical methods: the ability of the gene to self-renew, its variability, the ability to maintain the relative stability of the structure, the ability of recombination and discrete operation in the genotype system and the phenotype system. Genetic analysis is an experimental study of the relationships between bacteria (mutants). Two main methods are used to determine these relationships: 1) Recombination determines the spatial location of genes (or mutations) on the genetic map, that is, mapping the genome; 2) Complementation - determines the functional relationship of genes (or mutants). Bacteria that can multiply quickly allow you to spend a relatively short time on the experiment.

Moreover, methods for selecting mutants or recombinants are simple and convenient. The main method of selection is to use a minimal set of media (for example, with single amino acids) along with inoculation of the Replicator stamp. This method makes it possible to determine the nature of the mutation (or recombination) and the location of the corresponding gene on the genetic map. The following mapping methods are the most common: 1) Using canceled conjugation (with sowing of microbes in a minimal medium at certain time intervals during the conjugation process); 2) Using transduction (that is, using phage); 3) By conversion (after the artificial destruction of the donor bacteria, the nature of the transformation of the recipient bacteria is studied). Genetic analysis can be performed using DNA (RNA) probes in a hybridization reaction. This method is important not only theoretically, but also practically - as the most specific method for identifying microbes and viruses. DNA-(RNA) is a radioactive (32R, etc.) fragment of DNA (RNA) to which a probe is attached. These parts of nucleic acids are obtained: a) by limiting the DNA (RNA) part of certain microorganisms (dividing the

molecule into two chains), b) by chemical synthesis. The hybridization reaction consists of the following steps: 1) Destruction of the studied microbes, denaturation and cutting of DNA (unidirectional sites are required) and attachment to the polymer film; 2) It is done by inserting a DNA probe. In short, if the probe is filled with the DNA of the microbes under investigation, then the binding, i.e. hybridization, unrelated probes are removed by washing; 3) taking into account the results - using autoradiography. Another important reaction based on genetic engineering methods is polymerase chain reaction (CPD), which is used in medical microbiology, virology, and immunology. The essence of the method is to identify not the patient's microorganism, but the interconnected parts of the genome (DNA), its biosynthesis, and then identification of species-specific parts by molecular-genetic methods. Biosynthesis is carried out under special physicochemical conditions. The reaction mixture contains the matrix (a part of the single-stranded DNA of the pathogen under study), nucleotides, primers (that is, DNA regions where biosynthesis begins), polymerase enzyme. As a result, copies of the DNA of microorganisms are formed, which can be detected in the CPR hybridization reaction. This indicates that its properties are changing. We young researchers saw all types of bacteriophage and made different reactions to them. we are conducting.

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