BACTERIOPHAGE AND ITS POSSIBLE RELATIONS TO THE CONTROL OF PLANT PARASITES

A RESUME OF RECENT WORK

BY

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1. Introduction.
   (a) What is Bacteriophage.
   (b) Its properties.
   (c) Theories as to its origin.

2. Bacteriophage in relation to bacteria of plants.
   (a) Bacteriophage and the legume bacteria.
   (b) Bacteriophage and the plant pathogenic bacteria.

3. Is such a lytic principle present in the fungi.

4. Future research in relation to control of plant parasites.

Introduction.

What is Bacteriophage: F. W. Twort, an English physician, while attempting to cultivate a filterable virus upon artificial culture media, in 1914, observed that micrococci growing as contaminants on the agar, tended to become "glassy" and transparent. These colonies, it was further observed, would give rise to others, if plated out, which too tended to become ultimately glassy. This led Twort to believe that the glassy material might be some agent causing an acute infectious disease of the micrococci. Filtrates of the glassy substance, diluted 1:1,000,000 in water or saline solution, were just as virulent as the original substance. A similar substance was obtained by filtration from other bacteria of the typhoid-coli group.

Two or three years later, a French-Canadian investigator, d'Herelle, observed that filtrates of faecal matter of convalescents that had suffered from dysentery caused by Bacillus dysentriae, inhibited in vitro the growth of that bacillus, while similar material collected in the early stages of the disease gave rise to profuse growth. As the inhibiting agent did not diminish in its virulence through dilution effected by hundreds of generations of the
B. dysentriae, d'Herelle concluded that he was dealing with a living lytic agent which was capable of reproduction. Under the microscope, he observed that the corpusecular parasite was chemotactically attracted towards the particular bacteria. He further observed how the parasite attacked the bacterium and how the parasitized bacterium ruptured and underwent a sudden dissolution. He named this ultramicroscopic parasite as Bacteriophagum intestinale.

Its Properties: The impetus given by these studies led to a vast amount of research in England, France, Germany and America and, as a result, several of the properties of this substance which, for convenience, has been called as bacteriophage, or simply 'phage, have been found out. It has been observed that the 'phage is strictly specific for certain groups of bacteria from which it originally had been isolated, that the 'phage could not thrive on dead bacteria or in any known artificial culture medium if free from suitable living bacteria, that heating over 60°C for an hour destroyed it, that it could be kept for long periods, that not all strains of the same bacterium were accompanied by this substance, that sometimes it has been observed to arise spontaneously in cultures, that it could be incubated through very great dilutions (one to a million and more) on agar if accompanied by its accompanying bacterium, that it behaves as an antigen causing antibacteriophage serum and when injected into animal's makes them susceptible to the particular bacterial infection.

Theories as to its origin: Having, as the 'phage does, such peculiar properties, naturally it has led scientists to ask, what is this 'phage? Bacteria being too highly organized to represent the start of life, it has been suggested that there must exist forms much more primitive in nature. If that is so, this 'phage and also the viruses which cause disease in plants and animals may be pathogenic representatives of some of these forms. Pathogenicity being the only demonstration of a virus, it is evident non-pathogenic varieties cannot manifest themselves. Moreover, we cannot say that the "pure" bacterial cultures are not contaminated by these non-pathogenic forms. The bacteriophage, then, may belong to such a group which, under certain conditions, becomes pathogenic for the bacterium and is detected only when lysis occurs.

Bacteriophage and Legume bacteria: The vast field that was opened up by these discoveries naturally led agricultural bacterio-
logists to see how this 'phage affected bacteria important in agriculture and Gerretesen et al in 1923 recorded the d’Herelle phenomenon in legume bacteria. They successfully isolated a lytic principle from the nodules of various leguminous plants which were found to be specific for the bacteria of the legume concerned. Roots, stems and the soil around the roots yielded the lytic principle and this signified a particular advance, because of the existence of the 'phage even in the legume-bacteria infested soil.

Bacteriophage and the plant pathogenic bacteria: Very shortly afterwards, Mallaman and Hemstreet isolated an “inhibitory substance” from a plant source. Using the fluorescent bacteria that cause a rot of cabbages, they demonstrated how an extremely dilute (1:100,000,000,000) filtrate inhibited the growth of these particular bacteria though they were unable to show lysis. This work was very soon followed by that of Coons and Kotila who obtained the lytic principle (‘phage) from Bacillus carotovorus, B. atrosepticus and Pseudomonas tumefaciens, the principle retaining its lytic characteristic in even as high dilutions as 1:100,000,000. The actual effect of the principle on the bacteria was a loss of motility, malformation, and agglutination and ultimate lysis. When spread on susceptible plants, slices of carrots and potatoes, the ’phage seems to have reduced or prevented the infection by B. carotovoros and B. atrosepticus, respectively. In Russia, Israilsky has demonstrated the ’phage in the galls produced by Pseudomonas tumefaciens on beets. The ‘phage dissolved the particular strains of the parasite, and in his later work he studied the physico-chemical nature of the normal plant and tumor juice along with that of P. tumefaciens in culture. He concluded that the disappearance of the bacterium from the galls should be attributed to the action of a ‘phage and not to the formation of acids in the galls. He also treated the roots, stems, and seeds with the ‘phage before inoculation by the organism and found that the ‘phage reduced appreciably the amount of infection. Feeling that his work was not sufficient enough to draw definite conclusions, he stated that in order to prove the prophylactic action of the ‘phage, it was necessary to accomplish a great deal more than what he had done. The work on the galls produced by the tumefaciens was done also by d’Herelle and Peyre. They state that they isolated two kinds of the tumefaciens from the tumor, one of which was ultrapure and non-parasitic and
the other was affected by the 'phage and was parasitic. They then advance a curious theory that the bacteria when affected by this 'phage became invisible which they call protobacterial forms.

To support this contention, they state that microscopical examination of the galls does not show any bacteria, because they are then in a protobacterial form and that when the filtrate of these galls is injected into healthy plants, galls are produced which is because of these protobacterial forms which then become visible. They, therefore, believe that these protobacterial forms can act as virus filtrates. Muncie and Patel also obtained a lytic principle from beet galls as well as a pure culture of the gall organism. The 'phage was lytic in its action but showed a definite specificity and cultures of the parasite treated with the 'phage failed to produce galls in tomatoes.

Brown and Quirk, however, did not find any complete lysis in the 'phage which they obtained from Ricinus tumors and their work did not confirm the results obtained by d'Herelle and Peyre regarding protobacterial forms of organism.

These authors obtained very vigorous and extremely larger galls than the normal galls, when their cultures of the tumefaciens were treated with a highly diluted juice from the galls or even from normal juice and they believe that the closer union is established between the 'phage and the bacterium by the juices, temporarily establishing a higher degree of virulence in the pathogen. They were able also to retard the formation of galls if the bacterium was treated with the 'phage. Aside from these studies, brief reports of the presence of alytic principle have been made by Kauffmann whose results seem to be entirely negative to those of d'Herelle and Peyre. Introduction of the 'phage exerted no action, according to this investigator, in tumor formation or its prevention.

A lytic principle has also been reported from Bacterium tabaci, causing the Wildfire of Tobacco, by Moore and from B. pruni by Anderson.

*Is such a principle present in fungi?:* Investigators doing work on the physiology of fungi do not yet seem to have taken up this matter seriously though Gratia and Dath state that the filtrates of the cultures of Penicillum glaucum were able to cause a lysis in some bacteria.
Future Research and its Relation to Control of Plant Parasites.

Can the bacteriophage be used as a protective agent in the control of plant diseases due at least to bacteria? This is the big question that arises in one's mind when the different manifestations of the 'phage are taken into consideration. The work that has been done, however, is too meagre and very much contradictory to come to any definite conclusions. The work of Coons and Kotila, Israilsky, Muncie and Patel has shown that the 'phage may be used as a prophylactic agent. Fulton observed that *Pseudomonas citri* in the soil degenerated after some time and the diminution in numbers of legume bacteria in the soil has been shown by Gerretsen et al. Whether it would be possible to control the soil inhabiting *B. cartovorous, B. atrosepticus, P. insidiosum*, etc., it is difficult to prophesy. The work of Israilsky in connection with crown gall, while showing hope, is contradicted by that of Brown and Quirk and that of Kauffmann. We are, however, only at the beginning of the development of an interesting line of science which, at the most, is still a theory, at least as far as agriculture is concerned. Yet, we can expect that with the development of bacteriophagy as a workable hypothesis, much light can be brought in to the still lasting darkness of phytopathological problems.

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BACTERIOPHAGE AND ITS POSSIBLE RELATIONS. 217


