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TESTS WITH
PASTEURIELLA PSEUDOTUBERCULOSIS
AND
PASTEURIELLA PESTIS BACTERIOPHAGE

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TESTS WITH PASTEURELLA PSEUDOTUBERCULOSIS AND PASTEURELLA PESTIS BACTERIOPHAGE

Following is the translation of an article by Werner Knapp of the Hygiene Institute at the University of Tubingen (Director: Prof. Dr. R. E. Bader) in Zeitschrift für Hygiene (the German-language publication (Journal of Hygiene), Vol 148, 1962, pages 375-383.

Through tests done by Flu (1927), Girard (1942, 1943), and Flu & Flu (1946), bacteriophage strains were discovered which have a lytic effect on individual strains of Escherichia coli, Shigella dysenteriae, and Pasteurella pseudotuberculosis.

Among other authors who have dealt with the lytic action of bacteriophage strains on Past. pseudotuberculosis (Past. pstbc.) and other types of bacteria, Sugino (1932), Advier (1933), and Bezenova & Co. (1938), were unable to determine this property in their bacteriophage strains, while Lazarus and Gunnison (1947) and Gunnison and Lazárs (1948), in transferring one of the bacteriophage strains which had been tested by Advier (1933) on Past. pstbc. found that 31 out of 40 underwent lysis prior to the adaptation, and all of the strains tested lysed after the adaptation. Out of 42 strains of Salmonella which represented 25 different species, 3 strains (one each of Salm. schottmueller, Salm. hirschfeldii, and Salm. rubislaw) and out of 37 strains of Shigella, 6 strains were sensitive to concentrated suspension of bacteriophage. The attempts of Gunnison and Co. (1951) to differentiate between Past. pestis and Past. pstbc. with the aid of the same strain of bacteriophage and applying a critical testing dose at incubator temperatures of 22°C, resulted in a lysis of pestis tuberculosis strains; however, it did not affect any strains of pseudo-tuberculosis.

Apart from the above transfers of bacteriophage on Past. pstbc., there are no extensive tests with Past. pstbc. bacteriophage. Girardi's report (1942) that some Past. pstbc. strains show the same sensitivity to Past. pestis bacteriophages as to Past. pstbc. bacteriophages fails to give more detailed data. Recently, during a small epidemic of pseudo-tuberculosis among marmots, Flankina and Ogneva (1961) succeeded in isolating the irritant and in finding bacteriophage strains in four Past. pstbc. strains. These phages, however, showed a reaction only with Past. pstbc. strains and a pestilence vaccine strain, but none with strains of Salmonella, Listeria, and Coli. The Past. pstbc. strains, cultured during the epidemic, in addition were lysed by a Past. pstbc. bacteriophage strain which was described by Kotitarova (1956).

Following our unsuccessful attempts to isolate phages in strains of human and animal origin which were cultured at the Institute of Hygiene, or received from other institutes for successive testing, we tried
to obtain a Paste. pseudotuberculosis bacteriophage strain from elsewhere. Dr. Girard (Pasteur Institute, Paris) was kind enough to supply a bacteriophage strain (Y phage) and a Paste. pseudotuberculosis bacteriophage strain which, according to reports (Girard 1960), had never been in contact with Paste. pestis.

[[Note] Unfortunately, until now we have failed to obtain the bacteriophage strains described by Kotliarova (1956) or Plankina and Co. (1961) for the purpose of extending our tests.]

This article reports on our tests in answering the following questions:

I. Does the PST-bacteriophage strain have an uniform effect on Paste. pseudotuberculosis strains of serological types I - V, or can we prove clear differences in the sensitivity of individual strains of the same or different serological types to this phage?

II. Are there differences in the lytic action of the PST-phage strain on strains of Paste. pseudotuberculosis and Paste. pestis, following separated concentration of the original phage suspension over Paste. pseudotuberculosis or Paste. pestis?

III. What is the lytic action of the bacteriophage Y on strains of Paste. pseudotuberculosis, following separated concentration of the original phage suspension over Paste. pestis and Paste. pseudotuberculosis?

IV. Can the PST-phage strain, as the bacteriophage Y, be transferred to strains of Salmonella; is this transfer particularly possible to Salmonella strains which have antigenic relations to Paste. pseudotuberculosis?

V. Is it possible to adapt Salmonella phages to Paste. pseudotuberculosis, and how does a Salmonella phage strain which has been transferred on Paste. pseudotuberculosis compare with strains of Salmonella, Paste. pseudotuberculosis, and Paste. pestis?

Materials Used in Testing.

A. Pasteurella Phages. PST-phage: Paste. pseudotuberculosis bacteriophage strain; supplied by Dr. Girard, Pasteur Institute, Paris.

Y-phage: Bacteriophage strain Y; supplied by Dr. Girard, Paris.

B. Salmonella Phages. Salmonella Typhimurium phage 1: supplied by Prof. Dr. Brandis, Institute of Hygiene, Gottingen.

Salmonella O-1 phage: supplied by Prof. Brandis, Institute of Hygiene, Gottingen.

C. Bacteria Strains. Paste. pseudotuberculosis: testing strains of serological types I to V (No 21, 16II, 43III, 2200III, 32IV, IkegakiIV, 25V, and 74RI), as well as other Paste. pseudotuberculosis strains mentioned in this text are from the collection of the Institute of Hygiene, Tubingen.

Paste. pestis: strains of avirulent pestis described as A122, EV 76, and TWJ; supplied by Prof. Dr. K. F. Meyer, San Francisco.

Salmonella strains: Strains from the collection of the Institutes of Hygiene in Tubingen and Gottingen (Prof. Dr. Brandis), see test for particulars.

D. Culture Medium. Bacto-proteose-agar or solution, Difko (Difko-Mamal 1953) for the purpose of conducting bacteriophage experiments on strains of Paste. pseudotuberculosis and Salmonella.
Bacto-tryptose-agar, Difko (Difko-Manual); Tryptose-bouillon (Pepton 20 g (Merck), cooking salt 5 g, dextrose 1 g, broth 1000 ml) for the purpose of conducting bacteriophage experiments on Past. pestis.

([Note]: Our sincere thanks to Prof. Dr. K. F. Meyer, Prof. Dr. Brandis, and Dr. Girard, for their kind cooperation in supplying the strains, and to Prof. Dr. Brandis for his valuable advice during the experiments dealing with question IV.)

Method.

The transfer of phages to the various strains of bacteria and the successive concentration, unless otherwise mentioned, was done by means of the usual liquid or solid culture media (bibl. by Brandis 1957, Adams 1959, a. o.). As a rule, the Oese method was used to test the phage suspension. The Overlay method was used only for individual control tests.

Tests to Question I.

Following isolated concentration of the PST-phage over 7 different test strains of Past. pstbc. type I - V (strain No 2I, 16II, 43III, 2200III, 32IV, Ikegaki IV, and 25V), the 7 bacteriophage concentrations were tested against 42 Past. pstbc. strains, that is, 14 strains of type I, 10 strains of type II, 5 strains of type III, 4 strains of type IV, and 9 strains of type V. All bacteriophage concentrations which were transferred with an oese to cultures pre-hatched for ½ to 1 hour, lead at a minimum dilution of $10^{-2}$ to a confluent lysis of the strain used for the concentration, and at a dilution up to $10^{-6}$ or $10^{-9}$, to numerous or sporadic holes (philques) in the liquid area. All bacteriophage suspensions were concentrated, and tested at a dilution from 10-1 to 10-3. A critical testing dose, that is, a concentration which produces a confluent lysis on the homologous strain during the application was not used in these tests.

Bacteriophage suspensions, concentrated on the Past. pstbc. strain 2I, 16II, 32IV, Ikegaki IV, and 25V, lead to a confluent lysis in most Past. pstbc. strains of type I, II, III, and V. The 4 Past. pstbc. strains of type IV showed only numerous or sporadic plaques, without leading to a confluent lysis. The sensitivity of the Past. pstbc. strains type I, II, IV, and V, was noticeably smaller than that of the bacteriophage suspensions concentrated on Past. pstbc. strain No 43III and 2200III, which in most strains lead to a confluent lysis or plaques when concentrated or at a dilution of $10^{-1}$, while the strains of type III used for concentration attained a confluent lysis even at a bacteriophage dilution of $10^{-4}$.

Within the Pasteurella genre, only 3 avirulent pestis strains reacted to the PST-phages concentrated on the Past. pstbc. strain No 2I. There was no reaction in 18 Past. multocida and 4 Past. tularensis strains. No effect of the PST-phages could be proved in 20 Coll, 12 Shigella, 5 Pseudomonas or 5 Klebsiella, and 6 Hemophilus strains.
In answering the first question, we can state that the PST-phage possesses a great specific effect on Past. pstbc. strains, and represents an additional aid in type diagnosis. In countries with the occurrence of pestilence, however, a differential diagnosis of Past. pestis (for example, according to the method of Gunnison and Co. 1951) should be taken into consideration. There are differences in the sensitivity of the individual strains of Past. pstbc. to the various concentrations of PST bacteriophage; however, all strains of serological types I-V were grasped by the PST-phage.

The questions still remain open as to whether our observations of the PST-phage apply to other Past. pstbc. bacteriophage strains as well, and whether the transfer of PST-phage to suitable Past. pstbc. strains and the discovery of further phage strains make a lytic typification of Past. pstbc. strains possible.

**Tests to Questions II and III.**

Concentrations of the PST-phage over Past. pstbc. strain 21 (titer: confluent lysis up to $10^{-5}$, numerous or sporadic plaques up to $10^{-7}$ or $10^{-9}$ of bacteriophage dilution) and over Past. pestis strain A 1122 (titer: confluent lysis up to $10^{-9}$, numerous or sporadic plaques up to $10^{-11}$ of bacteriophage dilution) or concentrations of the Y-phage over Past. pestis strain A 1122 (titer: confluent lysis up to $10^{-5}$, numerous or sporadic plaques up to $10^{-8}$ or $10^{-10}$), Past. pstbc. strain 21 (titer: confluent lysis up to $10^{-7}$, numerous or sporadic plaques up to $10^{-11}$) were tested (as in I) against 42 Past. pstbc. strains and 3 Past. pestis strains.

We received the results as briefly summarized below:

Concentrated over Past. pstbc. strain No 21, the PST-phage lead to a confluent lysis in the liquid area in 39 strains of Past. pstbc. types I -- V, at a dilution of $10^{-3}$ (highest dilution) or in 3 strains at a dilution of $10^{-2}$, or $10^{-1}$. Following the concentration over Past. pestis strain A 1122, a confluent lysis was observed in 7 strains (1 strain type I, 4 strains type II, and 2 strains type IV) at a PST-phage suspension up to $10^{-3}$, and in 31 strains of types I and V at a concentrated or diluted 1:10 phage suspension. All pstbc. strains of type II with no peculiarities from the cultural or biochemical points of view either showed no reaction to the PST-phage (strain No 784 and 255), or else only the concentrated phase suspension effected numerous or individual plaques in the area of the phase liquid (strain No 792 and 798).

Both PST-phage concentrations, diluted up to $10^{-3}$, lead to a confluent lysis in the 3 pestis strains.

Following the transfer of the bacteriophage strain Y to Past. pestis strain A 1122 or Past. pstbc. strain No 21 and the testing of the two phase suspensions on Past. pstbc., the results shown in the chart below were received.
CHART. Reaction of Past. pstbc. with bacteriophage Y concentrated over 
Past. pestis and Past. pstbc.

<table>
<thead>
<tr>
<th>Serological type</th>
<th>Number of strains</th>
<th>Past. pestis strain A 1122</th>
<th>Past. pstbc. strain No 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13 (74R)</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>1 (strain No 16)</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>1 (strain Saisava)</td>
<td>2</td>
</tr>
<tr>
<td>V</td>
<td>9</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>42</strong></td>
<td><strong>11</strong></td>
<td><strong>28</strong></td>
</tr>
</tbody>
</table>

*At a dilution of 10⁻³, a confluent lysis in Past. pestis strain A 1122, EV 76, and TWJ.

As the chart shows, the suspensions of bacteriophage Y 11 (types I - IV) or 29 Past. pstbc. strains (types I - V), concentrated over Past. pestis strain A 1122 or Past. pstbc. strain No 21, showed a reaction. The degree of lytic action of these and other phage suspensions over Past. pstbc. strains No. 1779II, IkegakiIV, and 25V, of the sensitivity of the individual strains of Past. pstbc. types I - V to the various concentrations of the Y-phage was not uniform, so that it was impossible to determine regularities.

Bacteriophage Y, concentrated over Past. pestis strain A 1122, lead to a confluent lysis in only two strains of Past. pstbc. type III (No 2200 and H 21) at a dilution of 10⁻³, while two other strains of type III, or five of 14 strains of type I, and 1 strain each of types II and IV, showed sporadic plaques only with the concentrated or 1:10 diluted phage suspension. The concentration of bacteriophage Y, gained over Past. pstbc. strain No 21 and diluted up to 10⁻³, released a confluent lysis in all type III Past. pstbc. strains. This phage concentration produced no reaction in 4 Past. pstbc. strains type I (No 1323, 842, H 19, 1147), 4 of type II (No 255, 256, 257, 798), and 2 strains of type IV (No 32 and Saisava). Consequently, questions II and III may be answered as follows:

The differences in the lytic action of the two PST-phage concentrations gained over Past. pstbc. strain No 21 or Past. pestis strain A 1122 on Past. pstbc. strains type I - V are primarily quantitative.
With the exception of 2 strains (No 7811 and 23411), all Paste. pestis strains with two different concentrations of the PST-phage strain showed a reaction; however, the titer of the PST-phage suspension over Paste. pestis was lower.

The differences in the lytic action of the two Y-phage concentrations on Paste. pestis strains types I - V are not only quantitative, but also qualitative. A comparison of the action of the two phage concentrations shows that following the concentration of the Y-phage over Paste. pestis strain No 2, there was a considerably greater number of Paste. pestis strains types I - V than with the concentration gained over Paste. pestis strain A 1122. The application of the Y-phage to Paste. pestis strain No 2, a heterologous strain, lead, as it was to be expected, to an increase of its lytic properties, unlike it was the case with Paste. pestis strains types I - V.

Tests to Question IV.

We do not intend to go into the individual experiments, since they showed no definite proof of an adaptation of the PST-phage, concentrated separately over Paste. pestis strain No 1611 or 3211, to S. typhimurium (No 9) and S. schottmuelleri (No 3), or S. enteritidis (No 64) and S. gallinarum pullorum (No 74). In a number of experiments with a filtrate of 20 S. typhimurium cultures on proteose agar plates, not injected with the PST-phage, the same lytic action was observed in 4 of the 21 Salmonella strains tested as in the third filtrate passage of S. typhimurium cultures which had been impregnated with PST-phages, no virulent bacteriophages were found in any of the numerous filtrate passages against Salmonella strains. These observations, however, cannot be generalized as long as we have no further results in other Paste. pestis bacteriophage strains or Salmonella strains.

Tests to Question V.

A transfer of the Salmonella 01 phage and the Salmonella typhimurium phage No 1 to Paste. pestis strain No 21, 1611, 53111, 2200111, Ikogak1111, 3211, 2311, 74R (rough strain of Paste. pestis type I; Thai 1954), or to Paste. pestis strain A 1122, TW, and EV 76, failed in ten and more passages on liquid and solid culture-medium, even with the aid of UV-rays or culture coverings.

Consequently, the initial question regarding the two Salmonella phages used cannot be answered in the affirmative; however, until we have further observations of other Salmonella phages with other experimental techniques, it is impossible to draw definite conclusions.

Summary.

This is a report of studies done with a Paste. pestis bacteriophage (PST-phage) and a Paste. pestis bacteriophage (bacteriophage Y), based on five main points. Under the conditions individually described, the
42 strains of Past. pstbc. type I - V tested reacted to PST-phage, and within the Pasteurella genre 3 pestis strains showed a reaction, while the Past. multocida and Past. tularensis strains showed none. In countries with no occurrence of pestilence, the PST-phage can be a valuable aid for type diagnosis. The concentration of the PST-phage over Past. pestis resulted in a decrease of the lytic action in Past. pstbc. strains without diminishing the number of Past. pstbc. type I - V strains gained. The concentration of bacteriophage Y over Past. pstbc. strains was, on the other hand, a result of the increase in the lytic action and the number of Past. pstbc. Type I-V strains. Definite proof of an adaptation of the PST-phage to Salmonella strains and the Salmonella phage to Past. pstbc. and Past. pestis strains could not be found.

Bibliography.


Gunnison, J. B., A. Larson, and A. S. Lazarus: Rapid differentiation between Past. pestis and Past. pseudotuberculosis by action of


