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SUBJECT OF INVESTIGATION

GENETIC ANALYSIS OF MICROORGANISM BY MIXED INFECTION OF ACTIVE PHAGE PARTICLES AND PHAGE CR-BACTERIAL DNA

RESPONSIBLE INVESTIGATOR

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During the last year, several fundamental experiments were carried out and the following results were obtained.

Phage A, cultured by Sg in typically controlled by this host and varied to be able to produce plaque on Sg, is such good efficiency as when plated on Sg. This restricted phase A (Sg) can adsorb on Sg and inject its DNA into the interior of Sg cell.

Glucose and mannose can specifically inactive the purified A plus plaque particles.

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1. Microorganisms - Genetics

The Jikei Univ, School of Medicine (Japan) GENETIC ANALYSIS OF MICROORGANISM BY MIXED INFECTION OF ACTIVE PHAGE PARTICLES AND PHAGE OR BACTERIAL DNA by Isamu Kondo.


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Glucose and mannose can specifically inactive the purified A plus plaque particles.
These monoasparaccharides are also two of four terminal component sugars of salmonella antigens 0(12) which is the receptor for this phage.

Methods of extraction and purification of a, phage DNA have been greatly improved by using the synthetic medium devised for Sg and the refrigerated centrifuge supplied under this contract.

With this newly obtained sample the sedimentation rate of a, phage DNA could be calculated 9.7 x 10^-13.

Misinfection of this sample of a, DNA and the active a10s phage particles belonging to the different serological type could also yield some new type phages corresponding to the recombinant, if any, between a, and a10s, one of which was very different from those previously obtained (Author).

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During the last one year several fundamental experiments were carried out and following results were obtained.

Phage $\text{A}$, cultured by $S_4$, is typically controlled by this host and varied to be unable to produce plaques on $S_4$ in such good efficiency as when plated on $S_5$. This restricted plaque can adsorb on $S_4$ and inject its DNA into the interior of $S_4$ cell. Glucose and mannose can specifically inactivate the purified phage particles. These monosaccharides are also the terminal component sugars of salmonella antigen O(12), which is the receptor for this phage. Methods of extraction and purification of $\text{A}$ phage DNA have been greatly improved by using the synthetic medium devised for $S_4$ and the refrigerated centrifuge supplied under this contract.

With this newly obtained sample the sedimentation rate of $\text{A}$ phage DNA could be calculated $9.7 \times 10^{-13}$ times the rate of $S_5$. The restriction of $\text{A}$ DNA corresponding to the different serologial type could also yield some new type phages corresponding to the recombinant, if any, between $\text{A}$ and $\text{A}_0$, one which was very different from those previously obtained.
GENETIC ANALYSIS OF MICROORGANISM BY MIXED INFECTION OF ACTIVE PHAGE PARTICLES AND PHAGE OR BACTERIAL DNA

Isamu Kondo
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1. A statement of the problem

In the previous experiment, it could be observed that S. gallinarum (Sg) doubly infected with active phage α10s particles and purified DNA extracted from phage α1 yielded a few new type phages corresponding to the recombinant type between α10s and α1 with a majority of normal α10s progeny phages. In these experimental results, the following points would be worth notice.

a. The genetic activity of phage DNA is directly shown on the level of purified DNA.

b. It was shown for the first time in this experiment that some recombinant type phages could be obtained even between two serological unrelated phages. (Phage α1 is quite different type phage serologically from phage α10s, and the cross between α1 and α10s by doubly infection of Sg with both active phage particles have not yet succeeded.)

c. The genome controlling the lysogenic conversion of salmonella C(1) antigen was shown to be contained in the DNA sample extracted from α1 phage particles. Furthermore it was indicated that this genome could enter into the bacterial cell of Sg upon the mixinfection with α10s active phage particles and it might be incorporated into the phage particle or even the phage chromosome of α10s.

However, this experiment was preliminary and there were too much problems which should be critically analyzed.

My chief research required in this Contract is a genetic and chemical analysis of this phenomenon particularly to analyze the genetic factor concerning the lysogenic conversion of salmonella C(1) antigen on the level of DNA by means of mixinfection of phage DNA and active phage particles.

2. Outline of experimental procedure

During the last one year, following rather basic experiments necessary for the development of the above experiment were chiefly concerned.

a. Preparation of synthetic medium for S. gallinarum.

b. Further studies on the genetic characters of phage α1, transducing ability and variation controlled by host cell Sg.

c. Analyses of the receptor substance of Sg for the attachment of phage α1 and the specific inactivation of α1 phage by some monosaccharides.

d. Preparation of the more effective samples of α1 phage DNA, and its biological and physical analyses.
3. Materials and methods

Materials chiefly employed in these experiments:

Phage a1. The temperate phage liberating from S. paratyphi A 1015.

Phage a10. The temperate phage liberating from S. paratyphi A No. 10 strain.

Sg. S. gallinarum Akita strain, nonlysogenic, good indicator for phage a1 and a10s.

Phage a1 produces a middle large plaque and phage a10s a pin-hole plaque on this indicator.

Sa/S. S. typhimurium var cohpenhagen, artificially delysogenized strain by ultraviolet light irradiation, specific indicator for a1 and a10s.

Sa/r Resistant strain to a1 phage derived from Sa. Phage a10s alone produces a pin-hole plaque on this specific indicator.

Experimental methods will be described respectively in the item of experimental results.

4. Experimental Results

The results of the above experiments are respectively summarized as follows.

a. Preparation of synthetic medium for S. gallinarum

It is desirable to use chemical defined medium for phage experiments, especially preparation of purified phage DNA or experiment using isotope chemicals. So far examined, the following chemical constitution of medium seems to satisfy the growth requirement of Sa as well as Sa/S.

Adenine 20 mg
Aspartic Acid 20 mg
Tryptophane 19 mg
Leucine 20 mg
Cystine 26 mg
MgSO4 0.2 g
(NH4)2SO4 2.0 g
KH2PO4 9.0 g
K2HPO4 21.0 g
Glucose (40%) 5 ml
(Agar) 11 g
Aq. dest. 1000 ml
It is interesting and also favorable for our experiment that the medium contains adenine as indispensable growth factor for Sg, since adenine is one of the bases of DNA and can be directly incorporated into the chromosomal component of phage or bacterium.

b. Further studies on the genetic characters of a1

Host controlled variation:

Phage a1 cultured by Sg forms plaque on indicator Sg in good efficiency of plating (EOP) but scarcely produces on S4S. On the other hand, phage a1 cultured by S4S can form plaque either on Sg or S4S with almost same eop.

This restricted phage a1(Sg), however, can also adsorb to S4S as well as to Sg. The K value, velocity constant of adsorption, of a1(Sg) is a little lower (4.7 x 10^-9 ml/min) than that of a1(S4S) (2 x 10^-9 ml/min) in this case.

Transduction experiment using the restricted phage a1(Sg) and auxotrophic strains of S4S:

It is of some interests to examine whether a1(Sg) can or can not inject its DNA into S4S cell. Under this question transduction experiments were carried out using a1 phage cultured by Sg and auxotrophic strains of S4S.

The results showed that some of genetic marker of Sg (proline and iso-leucine valine markers) could be incorporated into the capsid of a1 phage and be injected into the auxotrophic S4S cells in which transduction was succeeded without development of vegetative or pro-phage. Since the rate of transduction of these markers was shown to be almost same irrespective of whether they were transduced from Sg to S4S by a1(Sg) or from S4S to Sg by a1(S4S), the injection itself of these genomes upon the infection of a1(Sg) to S4S seemed to be carried out at the same rate as upon the infection of a1(S4S) to Sg or S4S and a1(Sg) to Sg.

Another evidence was found that indicated the injection of phage DNA of a1(Sg) into S4S cell. About five minutes after the infection of S4S by a1(Sg) phage, the infected S4S cell was shown to be agglutinable by anti-O(1)-serum, indicating that O(1) antigen was formed on the infected S4S cell under the control of injected a1 phage genom. However, of course the progeny cells of S4S infected by a1(Sg) were not agglutinatable by anti-O(1)-serum. Consequently the above mentioned conversion is temporary one restricted to the infected cell itself, and the converting genom can not be transmitted into its progeny cells.
c. Analyses of the receptor substance of Sg for a1 phage, and the specific inactivation of a1 by some monosaccharides:

Concerning the phage P22 which seems to be very similar to phage a1, Lederberg has reported that the receptor for this phage would be overlapped with salmonella antigen 0(12). On the other hand, it has been revealed by recent developments in bacteriochemistry of salmonella antigens that at least the sugar component of salmonella antigen 0(12) is composed of two branched chain respectively terminating with glucose and rhamnose and the glucose chain has such a sequence as d-glucose-galactose-mannose-rhamnose.

These things considered, it will be supposed that the receptor for a1 phage has some relations in its molecular configuration to the terminal sugar component of salmonella antigen 0(12).

In fact, it could be observed that glucose and mannose could both inactivate the purified a1 phage although other components, galactose and rhamnose, were not effective for this phage inactivation.

To analyze further this interesting phenomenon some studies were carried out. The resulting data are summarized as follows.

i) The inactivating effect of glucose and mannose can be observed on the purified a1 phage but not on crude lysate by phage a1.

ii) Glucose effect is less than that of mannose. Purified a1 phage sample incubated in 1 mol solution of d-mannose at 37°C for 3 hours showed titer dropped to about $10^{-6}$ of its original one. In the case of d-glucose the inactivation rate is about $10^{-3}$ at the same condition.

iii) A positive control experiment was carried out using Pc phage, one of the salmonella virulent phages isolated in our department. Pc is known by our previous studies to adsorb to almost all salmonella bacilli possessing 0(12) antigen. And it is also specifically inactivated by the culture filtrate of these salmonella bacilli in which the 0(12) antigenic substance would have been eluted from the surface of these bacilli. (M. Terada and I. Kondo; 1959, Japan. Jour. of Microbiol. vol 3, no. 2)

In this time experiments it was revealed that Pc phage was also inactivated by d-glucose and d-mannose. It is of some interests that the inactivating effects of these sugars are respectively about $10^{-4}$ and $10^{-3}$, and this relation is just the reverse of that observed in a1 phage. Incidentally Pc phage is not also inactivated by galactose and rhamnose.
iv) As another control, T2 and T3 phage were examined. The results of examination show that T2 phage is not affected at all by almost all hexoses while T3 is inactivated by galactose and arabinose as well as glucose and mannose. The latter data are interesting in consideration of the evidence that the receptor substance for this phage is recently reported to contain galactosamine and glucosamine, and on the other hand the sugar effect of arabinose and galactose in the blockage of the antiserum against human blood type antigen are put into the same one category by Meikaela's classification of monosaccharide.

These results of experiments on the phage inactivation by monosaccharide were summarized and reported at 10th General Meeting of Japanese Society of Virologist, and now they are in press.

d. Preparation of more effective sample of a1 phage-DNA and its biological and physical analyses.

Methods of the purification of phage a1 and the extraction of DNA from this phage could be greatly improved by using the refrigerated centrifuge supplied under this contract. The more pure phage sample could be obtain by using alternatively high speed centrifugation by Sharples or Spinco type ultracentrifuge and low speed centrifugation by this refrigerated centrifuge. Especially low speed centrifugation by this instrument at low temperature for enough long time made it possible to purify the sample without dropping its biological activities.

Figure 1 indicates the sedimentation pattern of the purified a1 phage particles at 30,000 rpm centrifugation by Spinco E type centrifuge. There can be observed only one unique peak. Also Fig. 2 - 5 show the pattern of sedimentation of purified phage-DNA extracted from a1(S4S) taken at the moment of 0, 10, 20 and 30 minutes after the speed meter just indicates 60,000 rpm. The sedimentation rate of a1 phage calculated from these pattern is 9.7 x 10^{-13} s.

It was also known during the course of experiments that the a1 phage particles enough purified by means of the combination of the refrigerated centrifuge and Sharples or Spinco type ultracentrifuge were much more sensitive to freezing and thawing method for rupturing of the phage coats than that purified by the previous method with Sharples type ultracentrifuge alone.
For instance, it needed at least about sixty times of freezing and thawing in 5 mol. NaCl solution to drop the titer of infective particles until to $10^{-5}$ for the $a_1$ phage sample purified by the previous method, but it is enough accomplished with only ten times of the same treatment for the sample purified by the new method.

Biological analyses of the above DNA sample are now carried on, and so far examined the previous results which we reported in Japanese Journal of Genetics vol 34, No. 8 (1959) are also verified with these new phage DNA samples, i.e. the micoinfections of these $a_1$ DNA and the active phage particles $a_1a_0$ yielded a variety of new type phages corresponding to the recombinant type between $a_1$ and $a_1a_0$.

However, one of these new type phages is a little different from those previously reported. It produces plaque larger than that of phage $a_1$ on both indicators either Sg or Sg/z while the new type phages previously obtained are all those producing a plaque larger than $a_1a_0$ plaque and smaller than $a_1$ plaque. Furthermore it seems to be more virulent being able to produce a plaque on Sg($a_1a_0$), Sg lysogenized by $a_1$ phage.

The biological and genetic analysis on this curious new type phage $x$ will be chiefly carried on in the nearest future.

f. Summary of experimental results

By the experiments during the last one year, the following evidences could be known.

a. Remarkable host controlled variation can be observed in $a_1$ phage cultured by Sg. The plating efficiencies of $a_1$(Sg) on indicator Sg and Sg/z are respectively 1 and $10^{-5}$ while $a_1$(Sg/z) can produce a plaque on these both indicators with almost the same e.o.p.

b. The restricted phage $a_1$(Sg), however, can adsorb on Sg cell and inject its DNA into the interior of the infected cell. Consequently the somatic antigen $O(1)$ is produced on the cell surface on SgS infected by this restricted phage. However, this antigenic conversion is temporary, observed on the infected cells under the control of the injected converting genome, and the ability of the production of $O(1)$ antigen cannot transmitted to their progeny cells.

c. The receptor substance for the adsorption of $a_1$ phage is closely related with salmonella $O(12)$ antigen. And it is known that glucose and mannose, two of four sugar components of $O(12)$ antigen, can inactivate this phage, while
other two sugar, galactose and rhamnose can not affect at all. The specificity of this sugar effect is shown by various control experiments.

3. Methods of the purification of phage sample and the extraction of phage DNA could be greatly improved by using the refrigerated centrifuge supplied under this contract.

The sedimentation rate of \( \alpha_1 \) phage is calculated 9.7s from the sedimentation pattern of this phage analyzed by the ultracentrifuge of Spinco F type.

a. Mixinfection of \( S_5 \) with the purified DNA extracted from \( \alpha_1 \) phage and the active particles of \( \alpha_{10} \) phage can yield several new type phages besides a majority of \( \alpha_{10} \) progeny phage. One of them is shown to be different from those obtained in the previous experiment. At least it can produce a plaque larger than that of \( \alpha_1 \) phage on both indicators, \( S_5 \) and \( S_5/2 \), and even on \( S_8(\alpha_{10} \).

6. Discussion

The host controlled variation observed in \( \alpha_1 \) phage cultured by \( S_5 \) is very typical. The evidence that the restricted phage \( \alpha_1(S_5) \) can adsorb on the cells of \( S_5 \) and inject its DNA into the interior of \( S_5 \) call without the development of infective phage particles are of some utility values for this research. For instance, mixinfection of \( S_5 \) with the DNA extracted from the nonrestricted phage \( \alpha_1(S_5) \) and the active particles of the restricted phage \( \alpha_1(S_5) \) will be one of promising experiments to analyze the nature of the host controlled variation and the lysogenic conversion of salmonella \( O(1) \) antigen.

The specific inactivating effect of glucose and mannose to \( \alpha_1 \) phage is also interesting although it is rather beyond the frame of this research. However, the relation between sugar components of salmonella \( O(1) \) antigen and \( O(12) \) antigen is worth notice for the biochemical analysis of the lysogenic conversion concerning \( O(1) \) antigen by \( \alpha_1 \) phage.

As mentioned above, the sugar component of the \( O(12) \) antigen, the receptor for \( \alpha_1 \) phage, is glucose-galactose-mannose while the \( O(1) \) antigen is also composed of glucose, galactose, mannose and rhamnose with the same sequence. The only difference is the linkage between the terminal glucose and the second sugar-galactose, which is 1-6 in the former and 1-4 in the latter. There will naturally raise the question: Is there some close correlation between the \( O(1) \) antigen converted by \( \alpha_1 \) phage and the antigen \( O(12) \) as the receptor for this phage? And is it an essential part of the lysogenic conversion of salmonella \( O(1) \)?
antigen that the $a_1$ phage genome injected through the receptor, $O(12)$ antigen, induces the production of such an enzyme as converting the 1-6 linkage between glucose and galactose in the $O(12)$ antigen to 1-4 linkage as observed in the $O(1)$ antigen.

The improvement of the method of extraction and purification of $a_1$ phage DNA seems to be shown in the sedimentation pattern of $a_1$ phage particles and $a_1$ phage DNA in Figures 1-5. The sedimentation rate of $a_1$ phage DNA, $7.9 \times 10^{-15}$, is a little smaller than those reported of $T$ phage.

As for this evidence, it will be a future problem to analyze whether this smaller $s$ value indicates the specificity of $a_1$ phage DNA itself or the rather drastic method (freezing and thawing in 5 mol salt solution) employed in this experiment.
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Fig. 1. Sedimentation pattern of a1 phage. Photograph is taken at 31820 rpm.

Fig. 2-5. Sedimentation patterns of a1 phage DNA. Photographs 2-5 are respectively taken 0, 10, 20 and 30 min. after the moment just the speed meter indicates 60,000 rpm.