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**SUBJECT OF INVESTIGATION**

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

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Office of the Chief of Research and Development

United States Army

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<p>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. Final report No. 1, 20 Jan 62 - 19 Jan 63. 8 p. illus, table, 7 refs. (Contract DA 92-557-FEC-35778)Unclassified report.</p> <p>During the last year, several fundamental experiments were carried out and the following results were obtained.</p> <p>Phage <math>\phi_1</math> cultured by Sg is typically controlled by his host and varied to be unable to produce plaque on S<sub>4</sub> in such good efficiency as when plated on Sg. This restricted phage <math>\phi_1</math> (Sg) can adsorb on S<sub>4</sub>S and inject its DNA into the interior of S<sub>4</sub>S cell.</p> <p>Glucose and mannose can specifically inactivate the purified <math>\phi_1</math> phage particles.</p>	<p>UNCLASSIFIED</p> <p>1. Microorganisms - Genetics</p> <p>I. Title: Microorganisms-Genetics</p> <p>II. Kondo, Isamu (FEI), OCRD, DA, Wash, D. C.</p> <p>IV. Contract DA 92-557-FEC-35778</p> <p>Armed Services Technical Information Agency</p> <p>UNCLASSIFIED</p>	<p>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. Final report No. 1, 20 Jan 62 - 19 Jan 63. 8 p. illus, table, 7 refs. (Contract DA 92-557-FEC-35778)Unclassified report.</p> <p>During the last year, several fundamental experiments were carried out and the following results were obtained.</p> <p>Phage <math>\phi_1</math> cultured by Sg is typically controlled by his host and varied to be unable to produce plaque on S<sub>4</sub> in such good efficiency as when plated on Sg. This restricted phage <math>\phi_1</math> (Sg) can adsorb on S<sub>4</sub>S and inject its DNA into the interior of S<sub>4</sub>S cell.</p> <p>Glucose and mannose can specifically inactivate the purified <math>\phi_1</math> phage particles.</p>	<p>UNCLASSIFIED</p> <p>1. Microorganisms - Genetics</p> <p>I. Title: Microorganisms-Genetics</p> <p>II. Kondo, Isamu (FEI), OCRD, DA, Wash, D. C.</p> <p>IV. Contract DA 92-557-FEC-35778</p> <p>Armed Services Technical Information Agency</p> <p>UNCLASSIFIED</p>		
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These monosaccharides are also two of four terminal component sugars of salmonella antigen 0(12) which is the receptor for this phage.

Methods of extraction and purification of a<sub>1</sub> 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<sub>1</sub> phage DNA could be calculated 9.7 x 10<sup>-13</sup>.

Mixinfection of this sample of a<sub>1</sub> DNA and the active a10s phage particles belonging to the different serological type could also yield some new type phages corresponding to the recombinat, if any, between a<sub>1</sub> and a10s, one of which was very different from those previously obtained (Author).

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### Abstract

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

Phage  $\alpha_1$  cultured by Sg is typically controlled by this host and varied to be unable to produce plaque on S<sub>4</sub>S in such good efficiency as when plated on Sg. This restricted phage  $\alpha_1$ (Sg) can adsorb on S<sub>4</sub>S and inject its DNA into the interior of S<sub>4</sub>S cell.

Glucose and mannose can specifically inactivate the purified  $\alpha_1$  phage particles. These monosaccharides are also two of four terminal component sugars of salmonella antigen O(12) which is the receptor for this phage.

Methods of extraction and purification of  $\alpha_1$  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  $\alpha_1$  phage DNA could be calculated  $9.7 \times 10^{-3}$ .

Mixinfection of this sample of  $\alpha_1$  DNA and the active  $\alpha_{10s}$  phage particles belonging to the different serological type could also yield some new type phages corresponding to the recombinant, if any, between  $\alpha_1$  and  $\alpha_{10s}$ , one of 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

Professor of Bacteriology  
The Jikei University School of Medicine

## Table of Contents

- (1) A statement of the problem
- (2) Outline of experimental procedure
- (3) Experimental materials and methods
- (4) Results of experiments
  - a. Preparation of the synthetic medium for Sg
  - b. Further studies on the genetic characters of a<sub>1</sub> phage
  - c. Analyses of the receptor substance of Sg for Sg, and the specific inactivation of a<sub>1</sub> by some monosaccharides
  - d. Preparation of more effective sample of a<sub>1</sub> phage DNA and its biological and physical analyses.
- (5) Summary of experimental results
- (6) Discussion
- (7) List of References
- (8) Figures of sedimentation patterns of a<sub>1</sub> phage and a<sub>1</sub> phage DNA

## 1. A statement of the problem

In the previous experiment, it could be observed that *S. gallinarum* (Sg) doubly infected with active phage  $a_{10s}$  particles and purified DNA extracted from phage  $a_1$  yielded a few new type phages corresponding to the recombinant type between  $a_{10s}$  and  $a_1$  with a majority of normal  $a_{10s}$  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  $a_1$  is quite different type phage serologically from phage  $a_{10s}$ , and the cross between  $a_1$  and  $a_{10s}$  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  $a_1$  phage particles. Furthermore it was indicated that this genome could enter into the bacterial cell of Sg upon the mixinfection with  $a_{10s}$  active phage particles and it might be incorporated into the phage particle or even the phage chromosome of  $a_{10s}$ .

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, especially 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  $a_1$ , transducing ability and variation controlled by host cell Sg.

c. Analyses of the receptor substance of Sg for the attachment of phage  $a_1$  and the specific inactivation of  $a_1$  phage by some monosaccharides.

d. Preparation of the more effective samples of  $a_1$  phage DNA, and its biological and physical analyses.

### 3. Materials and methods

Materials chiefly employed in these experiments:

- Phage  $a_1$  ..... the temperate phage liberating from  
S. paratyphi A 1015.  
Phage  $a_{10s}$ .... the temperate phage liberating from  
S. paratyphi A No. 10 strain.  
Sg. .... S. gallinarum Akita strain, nonlysogenic,  
good indicator for phage  $a_1$  and  $a_{10s}$ .  
Phage  $a_1$  produces a middle large plaque  
and phage  $a_{10s}$  a pin-hole plaque on this  
indicator.  
S4S ..... S. typhimurium var copenhagen, artific-  
ially delysogenized strain by ultraviolet  
light irradiation, specific indicator for  
 $a_1$  and  $a_{10s}$ . also good  
Sg/2 ..... Resistant strain to  $a_1$  phage derived from  
Sg. Phage  $a_{10s}$  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 Sg as well  
as S4S.

Adenine	20 mg
Aspartic Acid	30 mg
Tryptophane	19 mg
Leucine	20 mg
Cystine	26 mg
MgSO <sub>4</sub>	0.2 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	2.0 g
KH <sub>2</sub> PO <sub>4</sub>	9.0 g
K <sub>2</sub> HPO <sub>4</sub>	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 a<sub>1</sub>

Host controlled variation:

Phage a<sub>1</sub> cultured by Sg forms plaque on indicator Sg in good efficiency of plating (EOP) but scarcely produces on S<sub>4</sub>S. On the other hand, phage a<sub>1</sub> cultured by S<sub>4</sub>S can form plaque either on Sg or S<sub>4</sub>S with almost same eop.

This restricted phage a<sub>1</sub>(Sg), however, can also adsorb to S<sub>4</sub>S as well as to Sg. The K value, velocity constant of adsorption, of a<sub>1</sub>(Sg) is a little lower ( $4.7 \times 10^{-10}$  ml/min) than that of a<sub>1</sub>(S<sub>4</sub>S) ( $2 \times 10^{-10}$  ml/min) in this case.

Transduction experiment using the restricted phage a<sub>1</sub>(Sg) and auxotrophic strains of S<sub>4</sub>S:

It is of some interests to examine whether a<sub>1</sub>(Sg) can or can not inject its DNA into S<sub>4</sub>S cell. Under this question transduction experiments were carried out using a<sub>1</sub> phage cultured by Sg and auxotrophic strains of S<sub>4</sub>S.

The results showed that some of genetic marker of Sg (proline and iso-leucine valine markers) could be incorporated into the capsid of a<sub>1</sub> phage and be injected into the auxotrophic S<sub>4</sub>S 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 S<sub>4</sub>S by a<sub>1</sub>(Sg) or from S<sub>4</sub>S to Sg by a<sub>1</sub>(S<sub>4</sub>S), the injection itself of these genomes upon the infection of a<sub>1</sub>(Sg) to S<sub>4</sub>S seemed to be carried out at the same rate as upon the infection of a<sub>1</sub>(S<sub>4</sub>S) to Sg or S<sub>4</sub>S and a<sub>1</sub>(Sg) to Sg.

Another evidence was found that indicated the injection of phage DNA of a<sub>1</sub>(Sg) into S<sub>4</sub>S cell. About five minutes after the infection of S<sub>4</sub>S by a<sub>1</sub>(Sg) phage, the infected S<sub>4</sub>S cell was shown to be agglutinable by anti-O(1)-serum, indicating that O(1) antigen was formed on the infected S<sub>4</sub>S cell under the control of injected a<sub>1</sub> phage genome. However, of course the progeny cells of S<sub>4</sub>S infected by a<sub>1</sub>(Sg) were not agglutinated by anti-O(1)-serum. Consequently the above mentioned conversion is temporary one restricted to the infected cell itself, and the converting genome can not be transmitted into its progeny cells

c. Analyses of the receptor substance of Sg for a<sub>1</sub> phage, and the specific inactivation of a<sub>1</sub> by some monosaccharides:

Concerning the phage P22 which seems to be very similar to phage a<sub>1</sub>, Lederberg has reported that the receptor for this phage would be overlapped with salmonella antigen O(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 O(12) is composed of two branched chain respectively terminating with glucose and rhamnose and the glucose chain has such a sequence as α-glucose-galactose-mannose-rhamnose.

These things considered, it will be supposed that the receptor for a<sub>1</sub> phage has some relations in its molecular configuration to the terminal sugar component of salmonella antigen O(12).

In fact, it could be observed that glucose and mannose could both inactivate the purified a<sub>1</sub> 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 a<sub>1</sub> phage but not on crude lysate by phage a<sub>1</sub>.

ii) Glucose effect is less than that of mannose. Purified a<sub>1</sub> phage sample incubated in 1 mol solution of d-mannose at 37°C for 3 hours showed titer dropped to about 10<sup>-4</sup> of its original one. In the case of d-glucose the inactivation rate is about 10<sup>-4</sup> 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 O(12) antigen. And it is also specifically inactivated by the culture filtrate of these salmonella bacilli in which the O(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<sup>-6</sup> and 10<sup>-3.5</sup>, and this relation is just the reverse of that observed in a<sub>1</sub> 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 Mekaala'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  $a_1$  phage-DNA and its biological and physical analyses.

Methods of the purification of phage  $a_1$  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 obtained 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  $a_1$  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  $a_1(S_4S)$  taken at the moment of 0, 10, 20 and 30 minutes after the speed meter just indicates 60,000 rpm.

The sedimentation rate of  $a_1$  phage calculated from these pattern is  $9.7 \times 10^{-13}$  s.

It was also known during the course of experiments that the  $a_1$  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  $10^{-7}$  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 were reported in Japanese Journal of Genetics vol 34, No. 8 (1959) are also verified with these new phage DNA samples, i.e. the microinfections of these  $a_1$  DNA and the active phage particles  $a_{10}$ s yielded a variety of new type phages corresponding to the recombinant type between  $a_1$  and  $a_{10}$ s.

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 i.e. Sg or Sg/z while the new type phages previously obtained are all those producing a plaque larger than  $a_{10}$ s plaque and smaller than  $a_1$  plaque. Furthermore it seems to be more virulent being able to produce a plaque on Sg( $a_{10}$ s), Sg lysogenized by  $a_{10}$ s 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 S4S are respectively 1 and  $10^{-7}$ , while  $a_1$ (S4S) 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 S4S 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 S4S infected by this restricted phage. However, this antigenic conversion is temporarily observed on the infected cells under the control of the injected converting genome, and the ability of the production of O(1) antigen can not be 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.

d. 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  $a_1$  phage is calculated 9.7s from the sedimentation pattern of this phage analyzed by the ultracentrifuge of Spinco E type.

e. Mixinfection of Sg with the purified DNA extracted from  $a_1$  phage and the active particles of  $a_{10s}$  phage can yield several new type phages besides a majority of  $a_{10s}$  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  $a_1$  phage on both indicators, Sg and Sg/z, and even on Sg( $a_{10s}$ ).

## 6. Discussion

The host controlled variation observed in  $a_1$  phage cultured by Sg is very typical. The evidences that the restricted phage  $a_1$ (Sg) can adsorb on the cells of S<sub>4</sub>S and inject its DNA into the interior of S<sub>4</sub>S cell without the development of infective phage particles are of some utility values for this research. For instance, mixinfection of S<sub>4</sub>S with the DNA extracted from the nonrestricted phage  $a_1$ (S<sub>4</sub>S) and the active particles of the restricted phage  $a_1$ (Sg) 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  $a_1$  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  $a_1$  phage.

As is stated above, the sugar component of the O(12) antigen, the receptor for  $a_1$  phage, is  $\alpha$  glucose-galactose-mannose-rhamnose while the O(1) antigen is also composed of  $\alpha$  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 interrelation between the O(1) antigen converted by  $a_1$  phage and the antigen O(12) as the receptor for this phage? And is it a essential part of the lysogenic conversion of salmonella O(1)

antigen that the  $a_1$  phage genom 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 -  $7.9 \times 10^{-13}$  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



Fig 2



Fig 4



Fig 3



Fig 5

Fig. 1. Sedimentation pattern of a<sub>1</sub> phage. Photograph is taken at 31820 rpm.

Fig. 2-5. Sedimentation patterns of a<sub>1</sub> 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.