PHYSICAL AND SEROLOGICAL STUDIES OF PHAGES L_1 AND S_2 FROM *PSEUDOMONAS AERUGINOSA*[1]

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Abstract: Two virulent bacteriophages L_1 and S_2 of *Pseudomonas aeruginosa*, isolated from sewage, can be noted as new *P. aeruginosa* phages through the results of biological characterization.

INTRODUCTION

Two bacteriophages of *Pseudomonas aeruginosa* have been isolated in our laboratory and characterized as T-shaped, lytic and double-stranded DNA containing phages (Kao and Liu, 1978). Further studies regarding some physical and serological characteristics of these two phages are presented in this paper.

MATERIALS AND METHODS

Bacteria and bacteriophages

Pseudomonas aeruginosa Holloway strain 1 was used as the host strain for the studies. Phages L_1 and S_2 were isolated previously in this laboratory (Kao, 1976).

Media

Media used for the studies were prepared as previously described (Kao and Liu, 1978). Borate saline buffer (BSB) was prepared according to Campbell *et al.* (1970).

Preparation of antisera

Phages were purified as previously described (Kao and Liu, 1978). Antisera for both phages were prepared in rabbits by the use of subcutaneous injection of purified phages ranging in titers from 2×10^8 to 1×10^{11} PFU/ml at 4-5 day intervals for a total of 6-7 injection each. One week after the last injection, the sera were collected by cardiac puncture.

Neutralization reaction and K value determination

The neutralization reaction and the K value of the antisera were examined by the method described by Adams (1959).

Agar gel double diffusion method

Ouchterlony's method (1949) was followed. 1.0% BSB agar was coated on a slide as a supporter, the antigen and antibody were placed in separate wells cut into agar. The slide was incubated at 37°C for one day and then put into a refrigerator till a precipitate line occurred. The slides were stained with 0.1% amido black in 90% methanol and 10% acetic acid, then photographed.

Single-step growth experiments

According to the method of Adams (1959), phage lysates were sampled out at different time intervals to detect their titers.

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RESULTS

Serological relationship

Neutralization was tested by reacting each antiserum with both phages. Fig. 1 showed that the titer of phage L_1 fell about one log scale after one minute, and phage S_2 dropped slower than phage L_1. After ten minutes treatment, both phages were inactivated about 99%. The K value is listed in Table 1. The results indicate that phage L_1 and phage S_2 are serologically unrelated.

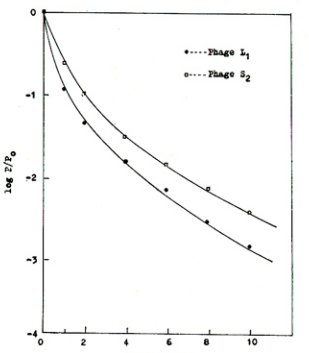

Fig. 1. Inactivation of phages with antisera.
P/P_0 is the ratio of the active phage titer to the initial phage titer.

Table 1. K value of antiphage sera against phages

Antiphage sera \ Phages	L_1	S_2
L_1	72	1.6
S_2	0.3	39

Agar gel double diffusion

Using the method described above, the results are shown in Fig. 2. There was only one line of precipitate formed in each test.

Single-step growth experiments

Both curves showing the typical shape observed in the experiments are illustrated in Fig. 3. The latent periods for phages L_1 and S_2 were 60 and 70 minutes; the burst sizes were 215 and 52 respectively.

Ultrasonic sensitivity

A 2 ml sample of a phage suspension was subjected to a 30 second burst by ultrasonic

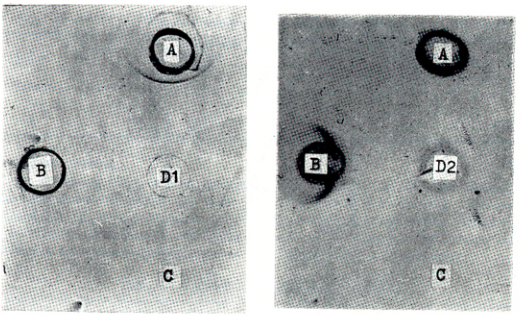

Fig. 2. Agar gel double diffusion of anti-phage sera.
A: phage L_1; B: phage S_2; C: 0.85% saline; D1: anti-phage L_1 serum; D2: anti-phage S2 serum.

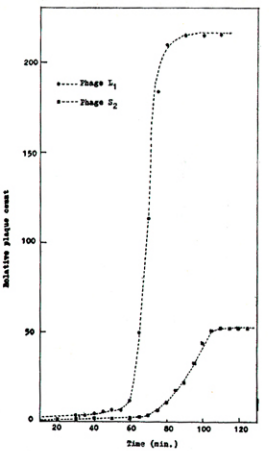

Fig. 3. Single-step growth of phages.

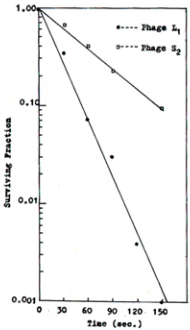

Fig. 4. Ultrasonic sensitivity of phages.

oscillation with a Bronson sonifier. A setting equivalent to 50 watts was used throughout. The samples were chilled in ice-water prior to and during sonication to reduce heat denaturation. Then they were diluted and assayed for surviving phages. As shown in Fig. 4, phage S_2 was more resistant to sonication than phage L_1. After treatment for 2 minutes, about 99% of L_1 phages and 85% of S_2 phages were inactivated.

Effect of citrate

Phage suspensions were plated on media containing 0.1% and 1.0% citrate respectively, and on a plate without citrate to be used as a control. The results are shown in Table 2. Both phages were susceptible to citrate inhibition of growth. When plated on media containing 1.0% citrate, phage L_1 was completely inactivated; while 30% of phage S_2 continued to grow.

Table 2. Effect of citrate on phages

Phages	Surviving fraction (%)		
	Control	0.1% Citrate	1.0% Citrate
L_1	100	49	0
S_2	100	69.4	29.6

Osmotic shock studies

A 0.1 ml of phage suspension was added to 10 ml PB containing 4 M NaCl (or 2 M sucrose). After incubation at 37°C for 30 and 90 minute periods, samples were shocked by diluting (1/100) from 4 M NaCl (or 2 M sucrose) directly into PB and examined for surviving phages. Table 3 indicates phage S_2 was very sensitive to 4 M NaCl and 2 M sucrose, especially the latter; while phage L_1 was slightly inactivated by salt exposure, and not affected by sucrose exposure.

Table 3. Osmotic shock studies of phages

Phages	Inactivation after dilution from (%)			
	4 M NaCl		2 M Sucrose	
	30 min.	90 min.	30 min.	90 min.
L_1	15.1	22.5	0	0
S_2	71.1	79.5	99.9	99.9

Effect of freeze-thawing

Tubes containing 10 ml of phage suspension were maintained at −34°C in a freezer. After samples were completely frozen, they were put in 37°C incubator for thawing. This procedure was repeated 5, 10, 15 and 20 times, then samples were diluted and plated. The results are given in Table 4. Both phages were slightly affected by the treatment, but it seems that they can be frozen and thawed repeatedly without any appreciable loss of infectivity.

Table 4. Effect of freeze-thawing on phages*

No. of times (frozen and thawed)	Plaque-forming units/ml	
	L_1	S_2
0	6.0×10^9	6.4×10^7
5	3.4×10^9	3.3×10^7
10	2.0×10^9	2.2×10^7
15	1.5×10^9	1.3×10^7
20	1.1×10^9	1.2×10^7

* Frozen (−34°C) and thawed (37°C).

DISCUSSION

Tris buffer was first used to prepare CsCl solution for purifying phages, however, the titers of both purified phages decreased to a very low level. Reese (1974) studied the *Pseudomonas aeruginosa* phage and suggested that phage adsorption could be carried out only in the presence of Na^+, Mg^{++} and Ca^{++} ions. After the modified Weigles buffer containing 0.1 M NaCl and 0.5 mM $MgSO_4$ was substituted for the purification procedures, the titers of both purified phages rose to the normal level. Plating in media containing citrate has been used as an indication of the calcium requirement for phage growth (Friedman and Cowles, 1953). As the results (Table 2) indicate, both phages of L_1 and S_2 required calcium for growth.

The results of agar gel double diffussion (Fig. 2) reveal that only one kind of antibody is produced against a phage, but this is not in accordance with the usual polyantigenicity property of phages (Goodheart, 1969). Perhaps this is because Ouchterlony's method (1949) was used to detect the kinds and titers of antibodies in a given antiserum, but its sensitivity was not great. And in order to give a visible line of precipitate, the antigen or antibody involved must be over ten μg/ml in quantity, therefore perhaps the insufficient amount of phages or sera used explains why only one kind of antibody was produced by L_1 or S_2.

The adsorption rate of phage L_1 was slower than phage S_2, while the titer of the former lysate was larger than the latter (Kao and Liu, 1978). Fig. 3 shows that the burst size of L_1 is larger than S_2.

The serological experiments, single-step growth curves and physical inactivation studies o

phages L_1 and S_2 gave different resuls. Phage S_2 is probably a new *P. aeruginosa* phage, since the size of this phage is larger than any other known *Pseudomonas* phage, besides, its physical and physiological properties are different (O'Callaghan *et al.*, 1969; Holloway and Krishnapillai, 1975). The size of phage L_1 is rather close to the *Pseudomonas* phages, PO4 and M6, but the similarity of L_1 to PO4 and M6 can readily dismissed because PO4 has an octahedral head (Bradley, 1973), while L_1 has an elliptical one, and M6 contains three fibers each with a knob at its tip (Bradley and Pitt, 1974), while L_1 only has a knob at the end of its tail. Therefore, it is deduced that L_1 can also be a new *P. aeruginosa* phage. In order to confirm that both of these phages are new to science, further studies are needed.

ACKNOWLEDGEMENT

The authors thank Dr. Charles E. Devol for reading this manuscript.

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