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Mutants of bacteriophage lambda able to grow on the restricting host *Escherichia coli* strain W

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1. INTRODUCTION

Wild-type bacteriophage λ, its clear plaque and virulent mutants do not form plaques on *Escherichia coli* strain W. The host specificity of the phage does not appear to affect its plating ability since λ.K, λ.B and λ.C (obtained by growing phage λ on the hosts *E. coli* K, B and C) and phage λ carrying P1 host specificity all fail to plate efficiently on strain W. Phage λ is adsorbed normally by strain W but nevertheless the efficiency of plating (e.o.p.) is extremely low. When large quantities of λ are plated on strain W the bacteria are lysed and phage can be harvested from the lytic areas produced. This phage when replated on strain W also lyses the bacteria but the efficiency of plating is very little improved. However after several such enrichment cycles the e.o.p. increases and individual plaques can be obtained. These plaques contain mutants of λ which are able to plate on W with an efficiency of 1·0. Three such mutants have been isolated from stocks of λ.C and λ.K and in this paper we shall describe some of their properties.

2. MATERIAL AND METHODS

Bacteria. Escherichia coli strain W (Davis, 1950); *E. coli* C (Bertani & Weigle, 1953); *E. coli* B is strain B251 (Arber & Dussoix, 1962); *E. coli* K is strain C600 (Appleyard, 1954); *E. coli* $Kr^{-}m^{-}$ (Colson, Glover, Symonds & Stacey, 1965).

Bacteriophages. Phage λ, a clear plaque mutant λ*c* and a virulent mutant λ*v* (Jacob & Wollman, 1954).

Media. (See Glover, 1962.)

Phage techniques. The general phage techniques are as described by Adams (1950). Special techniques relating to λ are those described by Arber (1960).

Density gradient centrifugation. Phage suspensions were added to 10 M CsCl solutions and diluted with broth until the required density was reached. Three millilitre portions of these suspensions were added to $\frac{1}{2}$ in. × 2 in. cellulose nitrate tubes and overlaid with liquid paraffin. The tubes were centrifuged in the SW39L head of a Spinco ultracentrifuge for 24 hours at 25,000 r.p.m. at 20°C. After centrifugation the bottom of each tube was pierced with a needle and the drops collected in 0·5 ml. volumes of broth contained in plastic trays. Approximately seventy fractions were obtained from each tube. Fractions were taken from the top, bottom and middle of the tube to measure the refractive index of the CsCl solution and from these measurements the density was calculated (Weigle, Messelson & Paigen, 1959).

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3. RESULTS AND DISCUSSION

The e.o.p. of bacteriophage λ on *E. coli* strain W is extremely low. When large quantities of phage λ (more than 10^8 particles) are plated on strain W the phage is adsorbed and an area of lysis is produced. Phage was harvested from these lytic areas and replated on W bacteria; again the bacteria were lysed but the e.o.p. was very little improved. After several such enrichment cycles dilutions of the W-grown phage produced individual plaques on strain W. These plaques contained phage which, quite unlike wild-type λ, is able to plate on W with an efficiency of 1·0. We have designated this phage λ*w* to indicate that it can plate on *E. coli* W. Phage λ*w* was isolated by this enrichment procedure

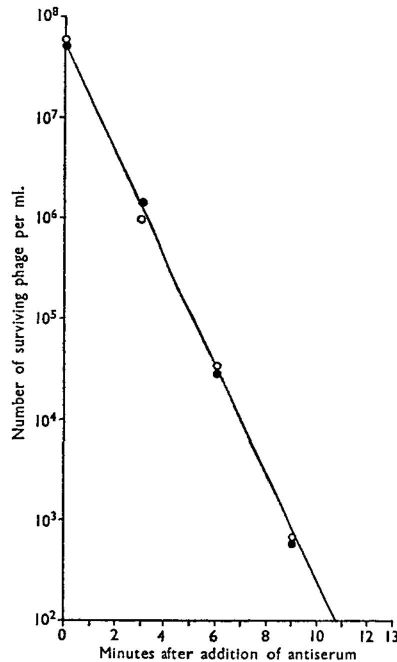

Fig. 1. The inactivation of phage λ and λ*w* by antiserum prepared against phage λ. Suspensions of bacteriophage in buffer were treated with antiserum and samples assayed for the number of surviving phage particles at 3-min. intervals.

●——● Phage λ assayed on *E. coli* K.
○——○ Phage λ*w* assayed on *E. coli* W.

from stocks of λ wild-type, λ*c* and λ*v*. Rarely, when more than 10^{10} particles of λ were mixed with an excess of W bacteria and plated out a few plaques were obtained. These plaques contained λ*w* phage.

Since λ*w* was isolated usually only after several cycles of growth in strain W it was important to establish that, λ*w* was in fact phage λ and not a contaminating phage. For this reason the properties of λ*w* and λ were compared in a number of respects. Phage λ*w* produces λ-like plaques on strain W. The plaques of λ*w* isolated from a stock of wild-type λ have turbid centres like those of a typical temperate phage. The plaques of λ*w* isolated from stocks of λ*c* and λ*v* are clear. Phage λ and λ*w* share common receptors since both adsorb to λ-sensitive strains and neither adsorb to λ-resistant strains. Bacteria lysogenic for phage λ are immune to infection by λ*w* and λ*w* lysogens are immune to λ

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so it is clear that λ and λw are sensitive to the same repressor. Antiserum prepared against phage λ inactivates both λ and λw at the same rate (see Fig. 1).

Three different λw phages have been isolated. When W-grown all of them plate with an efficiency of 1·0 on strain W but they can be distinguished by their plating efficiencies on other indicator strains. λw-*1* is not able to plate on *E. coli* C, λw-*2* plates on all the indicators tested and λw-*3* plates with a low efficiency on *E. coli* Kr^-m^- (Table 1). Furthermore λw carries the host-specificity of the bacterial strain in which it is grown (Table 2). The plating efficiency of λw-*2*.W on the indicator strains is different from the e.o.p.

Table 1. *The approximate efficiencies of plating of* λw *mutants on different strains of* E. coli

	Plating bacteria		
Phage	W	C	Kr^-m^-
λw-*1*.W	1·0	$< 1 \times 10^{-10}$	1·0
λw-*2*.W	1·0	1·0	1·0
λw-*3*.W	1·0	1·0	1×10^{-5}

of λw-*2*.B, λw-*2*.K and λw-*2*.C. Thus a W-specific host modification can be recognized which is different from that conferred by B, K and C.

The evidence summarized above demonstrates unequivocally that λw is identical with λ in all the respects investigated save for its ability to plate on strain W and certain other indicator strains and it is clearly not a contaminant.

E. coli strain W is lysogenic for a hitherto unknown temperate phage (Glover & Kerszman, 1967) and the possibility therefore remained that λw mutants were in fact recombinants between phage λ and the prophage carried by W strains. To test this possibility two different experiments were carried out. First, the indirect selection of λw from λ without passage through W and second, the buoyant densities of λ, λw and the temperate phage from W were compared.

Table 2. *The approximate efficiencies of plating of host-modified phage* λw-2 *on different strains of* E. coli

	Plating bacteria			
Phage*	W	C	B	K
λw-*2*.W	1·0	1·0	1×10^{-3}	1×10^{-3}
λw-*2*.C	1×10^{-3}	1·0	1×10^{-4}	1×10^{-4}
λw-*2*.B	1×10^{-4}	1·0	1·0	1×10^{-4}
λw-*2*.K	1×10^{-4}	1·0	1×10^{-4}	1·0

* Following the notation of Arber & Dussoix (1962) the host specificity of a phage is represented by a symbol for the phage followed by a symbol for the strain in which it was last grown.

To select λw indirectly from λ, confluent lysis plates of λv on *E. coli* C were replicated onto *E. coli* W. If sufficient phage was transferred in the replication procedure a few small plaques were observed on the lawns of W bacteria. Phage was harvested from the lytic areas on C corresponding in position to the plaques on W. This phage was replated on C and again replicated onto W. At each step there was an increase in the number of small plaques on W. This increase was maintained until the plaques on W represented about a 10^{-3} fraction of the plaques on C, beyond this point the enrichment procedure failed. The reason for this failure is apparent from the observation that λw-*2*.C

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plates at an efficiency of 10^{-3} on strain W (see Table 2). Thus at most only one in a 1000 λw phage particles replicated from C onto W is able to form a plaque. Clearly if from each plaque on C at least 1000 phage particles can be transferred by replication to W the number of plaques on C and W will be equal. Obviously the plaque replication procedure is less efficient than this.

The buoyant densities of λ and the temperate phage from strain W are quite different (Glover & Kerszman, 1967). Thus if λw was not a mutant of λ but rather a recombinant between λ and the W prophage involving a considerable amount of the genomes of the

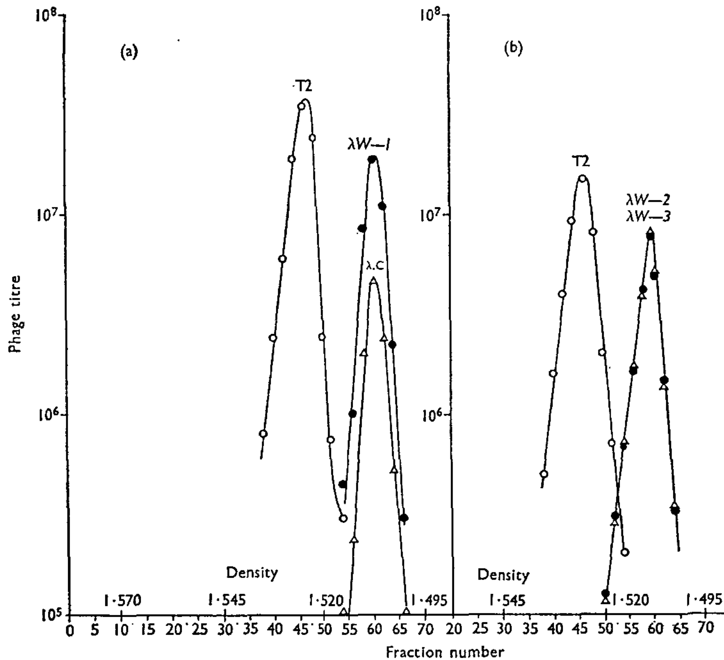

Fig. 2. Titres of phages in the fractions collected after density gradient centrifugation.

(*a*) ○——○ Phage T2 assayed on *E. coli* B.
△——△ Phage λ.C assayed on *E. coli* C.
●——● Phage λw-*1*.W assayed on *E. coli* W.

(*b*) ○——○ Phage T2 assayed on *E. coli* B.
△——△ Phage λvw-*2*.W clear plaques on *E. coli* W.
●——● Phage λw-*3*.W turbid plaques on *E. coli* W.

two phages, the buoyant density of λw would be different from that of λ. In Fig. 2 the buoyant densities of λ and three λw phages are compared using T2 as a reference phage. It is clear that the buoyant densities of λ, λw-*1*, λw-*2* and λw-*3* are the same.

SUMMARY

Escherichia coli strain W adsorbs phage λ very efficiently but the phage does not form plaques on this strain because the DNA of λ is broken down in a majority of the infected cells shortly after adsorption. In a 10^{-3} to 10^{-4} fraction of the infected cells λ grows and small bursts of phage are produced. This phage does not carry the W-specific host modification and is unable to complete a second round of infection in W (Kerszman,

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Glover & Aronovitch, 1967). λw mutants have been isolated which are able to escape this restriction process and which plate on W with an efficiency of 1·0 and when grown in W these mutants carry a W-specific host modification.

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