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The properties of a temperate bacteriophage Wϕ isolated from *Escherichia coli* strain W

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1. INTRODUCTION

In the course of experiments in which the growth of phage λ in *Escherichia coli* strain W was being studied (Glover & Aronovitch, 1967), it became apparent that strain W was lysogenic for a hitherto undescribed bacteriophage Wϕ. This phage plays an important role in the restriction of λ (Kerszman, Glover & Aronovitch, 1967). In this paper we shall describe some of its properties and the behaviour of several different strains of bacteria made lysogenic for Wϕ.

2. MATERIALS 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); Kr^-m^- (Colson, Glover, Symonds & Stacey, 1965); *Shigella dysenteriae* (Lennox, 1955).

Bacteriophages. Phage λ and a virulent mutant λv (Jacob & Wollman, 1954); Phage P1 (Lennox, 1955); Phage P2 kindly supplied by Dr G. Bertani.

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. (See Glover & Aronovitch, 1967.)

Anti-sera. Rabbit anti-sera were prepared against λ, P1 and Wϕ. Anti-P2 serum was a generous gift from Dr G. Bertani.

3. RESULTS AND DISCUSSION

(i) *Properties of Wϕ*

Log-phase cultures of *E. coli* W contain a phage Wϕ which forms plaques on *E. coli* C at an efficiency which we arbitrarily call 1·0. Such cultures usually contain about 10^6 plaque forming units (p.f.u.) per millilitre. The ultra-violet sensitivity of Wϕ is like that of λ (see Fig. 1). But ultra-violet radiation of cultures of strain W does not increase the yield of Wϕ. Repeated attempts have been made to cure strain W of its phage without success. However it is relatively easy to isolate strains of W which no longer produce the phage but which nevertheless do not plate it. Like the parent strain W these strains still restrict the growth of phage λ and are presumably lysogenic for a defective form of Wϕ. For this reason Wϕ was routinely grown on *E. coli* C on which it forms λ-like plaques 2–3 mm. diameter with turbid centres.

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The relationship of Wϕ to a number of other well-known phages was investigated serologically. Antisera were prepared against Wϕ, λ, P1 and P2 and used to inactivate each of the phages in turn. Anti-λ serum was completely without effect on Wϕ but P1,

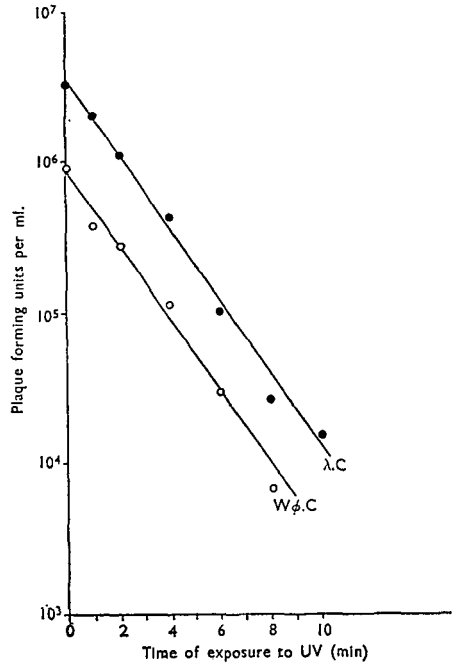

Fig. 1. Ultra-violet inactivation of phage λ and Wϕ. Samples of phage suspension were irradiated at a distance of 50 cm. from a Hanovia bacterial lamp for the time intervals shown. The number of surviving phage particles was assayed on *E. coli* C.

●——● Phage λ.C.
○——○ Phage Wϕ.C.

P2 and Wϕ appear to be antigenically related. The K values of the sera which are listed in Table 1 indicate that Wϕ is very closely related antigenically to P2 and much less closely related to P1.

Table 1. *The K values of anti-sera prepared against phages P1, P2 and Wϕ*

	Rabbit anti-sera		
Phage	Anti-Wϕ	Anti-P1	Anti-P2
Wϕ	634	73	20
P1	41	593	1
P2	460	69	29

Inactivation of the phages was measured at 37°C. in phage buffer. The K values of the antisera were calculated from the relationship:

$$K = 2{\cdot}3\frac{D}{t} \times \log\frac{p_0}{p}$$

D = final dilution of antiserum.
p_0 = phage titre at time zero.
p = phage titre at time t min.

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The buoyant density of Wϕ was measured by density gradient centrifugation using phage λ as a reference. It forms a single broad peak in a CsCl gradient lighter than λ and at about the same position as P2 (see Fig. 2).

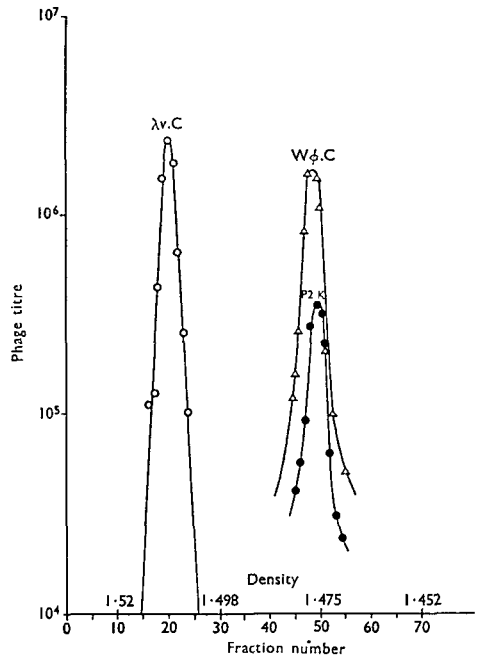

Figure 2. Titres of phages in the fractions collected after density gradient centrifugation.

○——○ Phage λv.C assayed on *E. coli* C$W\phi^r$.
△——△ Phage Wϕ.C assayed on *E. coli* Cλ^r.
●——● Phage P2.K assayed on *E. coli* K.

In spite of these similarities between Wϕ and P2 they are clearly not co-immune since Wϕ plates on C(P2) and the P2 plates on C(Wϕ) and on strain W which carries the Wϕ. Similar tests have also shown that Wϕ and P1 are not co-immune. The plating efficiency of Wϕ on a number of indicator strains is shown in Table 2. These results clearly indicate the difference between λ, P1, P2 and Wϕ.

Table 2. *The approximate plating efficiencies of λ, P1, P2 and Wϕ on different strains of* E. coli

	Plating bacteria					
Phage*	C	C(P1)	C(P2)	C(Wϕ)	C$W\phi^r$	*Shigella*
Wϕ.C	1·0	1·0	1·0	Immune	Resistant	1·0
λ.C	1·0	10^{-4}	$< 10^{-8}$	See Table 4	1·0	$< 10^{-8}$
P1.C	1·0	Immune	1·0	1·0	1·0	1·0
P2.C	1·0	10^{-4}	Immune	1·0	Resistant	1·0

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

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A point of some interest is that P2 does not plate on a strain of *E. coli* C made resistant to Wϕ. In fact, simple tests show that P2 and Wϕ do not adsorb to C Wϕ^r so that these phages appear to share a common receptor.

Preliminary electron micrographs show that Wϕ is a tadpole-like phage rather like T1 and P2 (Bertani, 1958). It has a head approximately 65×65 mμ and a tail approximately 140 mμ long with a contractile sheath.

(ii) *The properties of Wϕ lysogens*

Suspensions of Wϕ were prepared by spontaneous lysis of strain W and from a single plaque of Wϕ on *E. coli* C and plated on C, K and Kr^-m^-. The e.o.p. of these suspensions on K and Kr^-m^- was about 10^{-6} compared to 1·0 on C. A suspension of phage was prepared from a single plaque on K and replated on C, K and Kr^-m^-. Table 3 shows that the e.o.p. of this suspension was 1·0 on all three strains. However, this change in the e.o.p. of Wϕ after growth in K was not due to host modification because after several cycles of growth in C this phage retains its ability to plate on K, rather it is a mutant Wϕk. In fact the only plaques obtained when suspensions of Wϕ were plated on K were produced by Wϕk mutants. The reason why Wϕ isolated either directly from strain W or from plaques on C does not plate on K has not been investigated.

Table 3. *The approximate e.o.p. of Wϕ and its mutant Wϕk on* E. coli *K and C*

	Plating bacteria		
Phage	K	C	Kr^-m^-
Wϕ.C	$< 10^{-6}$	1·0	$< 10^{-6}$
Wϕk.K	1·0	1·0	1·0
Wϕk.C	1·0	1·0	1·0

Table 4. *The approximate e.o.p. of phage λ on strains of* E. coli *lysogenic for Wϕ*

	Plating bacteria				
Phage	K	K(Wϕk)	C	C(Wϕk)	C(Wϕ)
λ.K	1·0	1·0	1·0	1·0	$< 10^{-8}$

Wϕ and its mutant Wϕk were used to prepare the following lysogenic strains, C(Wϕ), C(Wϕk) and K(Wϕk). Phage λ does not form plaques on strain W which carries the Wϕ prophage so it was of obvious interest to test the e.o.p. of λ on these new Wϕ lysogenic strains. The results of these tests which are summarized in Table 4 indicate that strains lysogenic for the mutant Wϕk do not restrict the growth of λ but that bacteria lysogenic for Wϕ may do so. It has been shown that the DNA of phage λ is degraded in W(Wϕ) (Kerszman, Glover & Aronovitch, 1967). Therefore Wϕk could be regarded as a mutant of Wϕ which has lost the ability to direct the degradation of λ DNA. However not all C(Wϕ) isolates behave in the same way, some strains of C when made lysogenic for Wϕ plate λ almost as efficiently as non-lysogenic strains, others display intermediate patterns of behaviour. In respect of the biological properties listed in section (i) and in serological tests and by density gradient centrifugation Wϕ and Wϕk do not differ. The reason for the differences in behaviour among different C(Wϕ) isolates is under investigation.

SUMMARY

Escherichia coli strain W was found to be lysogenic for a temperate phage Wϕ. This phage, which plates on *E. coli* C, forms λ-like plaques 2–3 mm. diameter with turbid centres. It is serologically unrelated to λ but is closely related to P2 which it resembles in the electron microscope. Its buoyant density in CsCl has been measured and it is different from λ but similar to P2. *E. coli* C made lysogenic for Wϕ restricts the growth of λ, and elsewhere (Kerszman, Glover & Aronovitch, 1967) it has been shown that the DNA of phage λ is degraded shortly after infection of bacteria lysogenic for Wϕ. A mutant of Wϕ has been isolated which has lost the property of restricting the growth of λ.

We wish to thank Dr D. E. Bradley who kindly took the electron-micrographs of Wϕ. One of us (G. K.) is grateful to the British Council for a scholarship during the academic year 1965–66.

During the course of this work we learned that a similar phage had been isolated by Dr Lewis Pizer and we are grateful to him for a copy of a manuscript prior to publication (Pizer, Miovic & Pylkas, 1967).

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