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NOTES

Growth of Bacteriophage H on Male and Female Strains of *Escherichia coli*

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Phage H propagated on *Yersinia pestis* was reported by Molnar and Lawton to be rapidly adsorbed to female but not to male strains of *Escherichia coli*. In contrast, we find phage H adsorbs to all *E. coli* strains tested (both male and female) and forms plaques on a wide variety of male strains. Phage H appears to be related to the T3-T7 group of coliphages.

Molnar and Lawton (6) reported that phage H of *Yersinia pestis* shows sex specificity in its ability to adsorb to *Escherichia coli* bacteria. These workers presented evidence that phage H was adsorbed to female (F^-) strains of *E. coli* but was not adsorbed to male (F^+, F′, or Hfr) strains of *E. coli*. Hence we hoped to use phage H as a reagent to discriminate male and female strains of *E. coli*. However, we found that phage H forms plaques on many male strains of *E. coli* and adsorbs to all *E. coli* strains tested, regardless of sex.

The broth (R broth) used in these experiments is composed of 0.1% yeast extract (Difco), 1% tryptone (Difco), and 0.8% NaCl. Solid medium was made by supplementing R broth with 1.5% agar (BBL). The minimal medium was the C medium of Roberts (8) supplemented with glucose (0.1%) and L-amino acids (1 mM) as required. The dilution medium was T broth (1% tryptone and 0.5% NaCl) or C medium.

Y. pestis bacteriophage H was obtained from W. Lawton. MS2, a male-specific RNA phage, is a stock collection of our laboratory and was used to test strains for the presence of the F factor. Phage T7 was the gift of W. Summers. The bacterial strains were derivatives of either *E. coli* K-12 or B (Table 1). F′ strains were constructed so that bacterial genes associated with the F factor were required for growth on minimal medium.

Phage stocks were prepared by growing a culture of either JC1553 or *E. coli* B in broth at 37 C overnight. The culture was diluted about 50-fold into 10 ml of fresh broth and grown with shaking to about 10^8 cells per ml (as determined by turbidity at 600 nm). A single plaque taken from a plate (using a lawn of the same bacterial strain) was added to the liquid culture. The incubation was continued for 2.5 h (lysis was visible after 1 h). The culture was sterilized with a few drops of chloroform, and these crude lysates were titered by the agar overlay technique (1) on a lawn of the same bacterial strain. The lysates were stored at 4 C over chloroform. In most experiments we used phage lysates grown on JC1553. Phage lysates grown on *E. coli* B gave similar results.

Upon receipt of phage H, we prepared fresh crude lysates as described above and checked several preparations for sex specificity. To our surprise samples of phage spotted on the male strain (F101/AB2463) inhibited bacterial growth to the same extent as phage spotted on a female strain (JC 1553), while the growth of an Hfr strain (C3000 HAL) was unaffected. To further examine the generality of this observation, we tested the ability of phage H to inhibit the growth of a number of male and female strains by replica plating bacterial colonies onto plates spread with phage. We found that the growth of a number of F′ strains was inhibited by phage H whereas all Hfr strains tested were unaffected (Table 2).

The results with the spot tests and the replica platings suggested that, whereas all F^- strains are sensitive to the phage, a number of F′ strains are also sensitive. The marked growth inhibition observed with some of the F′ strains indicated not only an ability to adsorb the phage and be killed but also a capacity to replicate the phage. To quantitate the ability of the sensitive strains to act as a host for phage H we carried out efficiency of plating determinations by the

TABLE 1. *Bacterial strains*

Designation	Sexuality	Pertinent markers	Source
E. coli B strains:			
E. coli B	F^-	Wild-type	M. J. Bessman
E. coli B S-1 (SY 106)	F^-	$T1^r$	W. Summers
E. coli K-12 strains:			
JC 411	F^-	*leu*$^-$, *his*$^-$, *metB*$^-$, *argG*$^-$, *lac*$^-$, *str*$^-$, $T1^r$	B. Low
JC 1552	F^-	*trp*$^-$ mutant of JC 411	B. Low
JC 1553	F^-	*recA*1 mutant of JC 411	B. Low
MA 124	F^-	*spc*$^-$, *gal*$^+$ derivative of JC 411	B. Low
F101/AB 2463	F′	*leu*$^+$/*leu*$^-$, *thr*$^+$/*thr*$^-$, *ara*$^+$/*ara*$^-$, *proA*$^-$, *his*$^-$, *argE*$^-$, *thiA*$^-$, *recA*13	B. Low
F104/AB 2463	F′	Markers as in F101/AB 2463	B. Low
F103/JC 1552	F′	*his*$^+$/*his*$^-$, other markers as in JC 1552	B. Low
F110/JC 1553	F′	*metB*$^+$/*metB*$^-$, other markers as in JC 1553	B. Low
F101/JC 1553	F′	*leu*$^+$/*leu*$^-$, other markers as in JC 1553	By mating F101/AB 2463 with JC 1553
F116/KL 110	F′	*thy*$^+$/*thy*$^-$, other markers as in JC 1553	B. Low
AB 1518	F′	*F*14 *ilvE*$^-$, *argH*$^-$/*ilvC*7, *argE*3, *proA*2, *his*-4, *thi*-1	E. Adelberg
JC 182	Hfr	Double male, *purF*$^-$	B. Low
JC 12	Hfr	*purF*$^-$, *metB*$^-$	B. Low
Hfr Hayes	Hfr	*thiA*$^-$	B. Low
C3000 HAL	Hfr	*his*$^-$, *arg*$^-$, *lys*$^-$, *thiA*$^-$	(7)

TABLE 2. *Ability of phage H to replicate on various strains of E. coli*[a]

Bacterial strain	Filter paper		Replica plating		Efficiency of plating	
	ΦH	MS 2	ΦH	MS 2	ΦH	MS 2
F^- JC 1553	+	−	+	−	1.0[b]	$<10^{-9}$
JC 1552			+	−		
AB 2463			+	−		
MA 124	+	−	+	−		
E. coli B			+	−	1.5	$<10^{-9}$
F′ F101/AB 2463	+	+	+	+	0.31	0.81
F101/JC 1553	−	+	−	+	<0.1[c]	0.96
F104/AB 2463			+	+		
F103/JC 1552			−	+	1.4×10^{-7}	
F110/JC 1553			+	+	0.26	0.21
F116/KL 110			+	+		
Hfr C3000 HAL	−	+			$<10^{-7}$	1.0
JC 182					$<10^{-7}$	0.9
JC 12					<0.1[c]	0.75
Hayes Hfr					$<10^{-7}$	1.2

[a] A plus indicates that the strain was sensitive to the phage, a minus indicates resistance to the phage. For replica plating, approximately 100 single colonies of each bacterial strain used were streaked on a master broth plate and incubated at 37 C until growth was visible (~4 h). The master plate was then replica plated onto a broth plate control, a phage H spread plate (~10^9 plaque-forming units per plate), and an MS 2 spread plate (~10^{10} plaque-forming units per plate). Replica plates were incubated at 37 C and examined for bacterial growth within 4 to 6 h. Filter paper spot tests were carried out according to Benzer (2).

[b] JC 1553 was used as the standard strain in determining reference phage titer for these determinations.

[c] Plaques too minute to count quantitatively.

method of Adams (1) (Table 2). A number of F′ strains were found to be competent hosts for phage H exhibiting plating efficiencies as high as 0.3 (the value observed with F⁻ hosts) (Table 2).

We then sought to confirm Molnar and Lawton's observation that male *E. coli* strains resistant to phage H do not adsorb phage H. Strains C3000 HAL (Hfr) and F101/JC1553 were selected for the test because of their insensitivity to the phage. We found that both of these male strains adsorb phage H efficiently (Fig. 1). We then tested the ability of the original stock of phage H as provided by Molnar and Lawton to grow on male strains of *E. coli*. The results obtained with these phage are identical to those reported in Table 2. Finally we examined the ability of phage H to adsorb to AB1518, an F′ strain used in the original studies of Molnar and Lawton. In contrast to their observations, we found AB1518 to efficiently adsorb phage H in R broth (greater than 80% adsorbtion within 10 min).

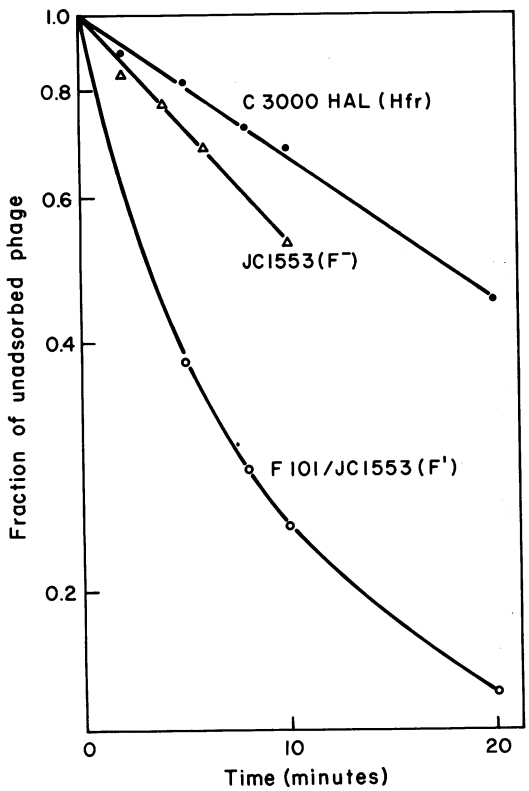

FIG. 1. *Adsorption of phage H to various bacterial strains was determined as described by Adams (1). Mixtures of log-phase cells (2 × 10⁸ cells per ml) and phage H (grown on E. coli B) (2 × 10⁷ plaque-forming units per ml) were incubated with shaking at 37 C. At various times, 0.1-ml samples were diluted into 10 ml of ice-cold R broth. Samples (1 ml) of dilute suspension were centrifuged to sediment cells. The supernatant fluid was titered for free phage by agar-overlay technique (1) using JC1553 as indicator for all experiments.*

The ability of phage H to adsorb to male bacteria and to replicate on a variety of male (F containing) strains prompted us to compare the phage H host adsorption sites to those of phage T7 (a female-specific phage similar in size and morphology to phage H). We isolated mutants of *E. coli* B which were resistant to phage H by the standard method (3). The mutants were then tested for T7 sensitivity. Seventeen out of 43 phage H-resistant mutants were found also to be resistant to T7. These data indicate that the two phages share at least one common receptor site on the host. Phage T3 and T7 are known to be closely related (4). Mutants of *E. coli* isolated as resistant to coliphage T3 give a similar proportion of mutants which are also resistant to T7 (3).

We have shown that phage H and coliphage T7 share a common adsorbtion site and that phage H, like coliphage T7 (5), is restricted in replication but not adsorbtion by male strains of *E. coli*. Other common characteristics between the T3-T7 phage group and phage H have been observed. These similarities are a DNA chromosome (6), particle size and morphology (6), plaque size and morphology (6), latent period and burst size (6), and an ability to form plaques on a lawn of *Y. pestis* strain TRU (6). From these data it appears quite likely that phage H is closely related to the T3-T7 group of coliphages. A similar conclusion was reached by Williams and Meynell who proposed, on the basis of plating on female strains of *E. coli*, that phage H was closely related to coliphage φII. We have given a sample of phage H to W. Summers to use in comparative studies with F⁻-specific coliphages. By carrying out electron microscope examination of φII-H DNA heteroduplexes, his laboratory has demonstrated that phage H is indeed closely related to coliphage φII (I. Brunovskis, R. Hyman, and W. C. Summers, personal communication).

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