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Pasteurella Bacteriophage Sex Specific in *Escherichia coli*

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Phage H, thought to be specific for *Pasteurella pestis*, was shown to plate efficiently on F^- strains of *Escherichia coli* but not on F^+, F′, or Hfr strains. The phage was adsorbed rapidly to F^- strains but was not adsorbed to strains carrying F. Comparison with seven other reported female-specific phages showed that, although phage H was similar to the other phages in some characteristics, the exceptionally low efficiency of plating ($<10^{-9}$) on F-containing cells makes phage H a particularly useful female-specific phage.

During an investigation into the use of phage to eliminate donor cells in matings between *Pasteurella pseudotuberculosis* F′ *lac* and *P. pseudotuberculosis* F^-, we found that a certain phage lysed F^- but not F′ *lac* strains. Although this phage, which we designated H, is currently used to aid in the identification of *Pasteurella pestis*, its host range at 37 C included *Escherichia coli*. Therefore, we were able to confirm the specificity of phage H for F^- cells in *E. coli* K-12, to characterize phage H, and to compare it with other reported female-specific phages.

MATERIALS AND METHODS

Phages. The *Pasteurella* phage used in this study was obtained from D. C. Cavanaugh of the Walter Reed Army Research Institute, Washington, D.C. He had isolated it from sewage obtained from the Hooper Foundation in California; it is currently used in the rapid identification of *P. pestis* (3). The phage that he isolated appears to have properties similar to those of phage P (7), which was being used at the Hooper Foundation at the time. Since there is no proof of the identity of the two phages, we arbitrarily designated the phage isolated by Cavanaugh as phage H.

Phages MS2 and T3 were obtained from stock collections in this laboratory.

Bacterial strains. *P. pestis* strain TRU was used to propagate phage H. *E. coli* K-12 strain 23.10.S met^- F′*lac* was obtained from R. C. Clowes. The origin of *E. coli* strain K57 was described by Kondo and Mitsuhashi (10). *E. coli* strains AB-1518 (F′14) and AB-6 (F^-) were supplied by E. A. Adelberg. All other strains of bacteria were from our stock collection.

Media and cultural conditions. Cultures were grown in 5 ml of Brain Heart Infusion Broth (BHI; Difco) in test tubes (18 × 150 mm) incubated in a slanted position on a reciprocal shaker (5-cm stroke, 100 excursions per min) at 37 C. For phage assays, approximately 10^8 cells in the late logarithmic phase were used to prepare lawns.

TY medium, composed of 10 g of Bacto-tryptone, 5 g of yeast extract (Difco), 10 g of NaCl, and 2 mg of $MnCl_2 \cdot 4H_2O$ per liter and adjusted to *p*H 7.4, was used to propagate phage. Hard agar plates contained 25 ml of either Blood Agar Base (BAB; Difco) or the PA medium of Thorne (15). Soft agar contained 0.7% agar in either Heart Infusion Broth (HIB; Difco) or PA medium.

Propagation of phage. Large batches of phage were produced on the appropriate host growing in shaken flasks of TY broth at 37 C. The phage was usually added at a multiplicity of infection (MOI) of 0.1 to approx 4 × 10^7 cells, and incubation was continued until lysis ensued. Smaller batches of phage were produced by harvesting in 1% peptone the soft agar layer of plates showing confluent lysis. Usually cellular debris was removed by centrifugation and filtration through ultrafine fritted glass filters; occasionally lysates and plate harvests were sterilized by treatment with chloroform. Neither filtration nor chloroform treatment had any significant effect on the phage.

Assay of phage. We used the agar technique (1) to titrate phage suspensions and to determine plaque morphology. At first we used BAB plates overlaid with HIB soft agar, but phage H plaques were so large that titrations were inaccurate. Smaller and more distinctive plaques were obtained on PA agar, which made counts much more reliable. The standard diluent for phage suspensions was HIB mixed 1:1 with 0.033 M potassium phosphate buffer, *p*H 7.4. Plates were incubated at 37 C.

RESULTS

Characteristics of phage H. On BAB agar, phage H formed large clear plaques with large turbid halos when plated on *P. pestis* TRU. With prolonged incubation, the plaques continued to enlarge until the entire plate might be cleared; viable phage could be recovered from any area of the converged plaques. We purified the phage by three successive single-plaque isolations, always

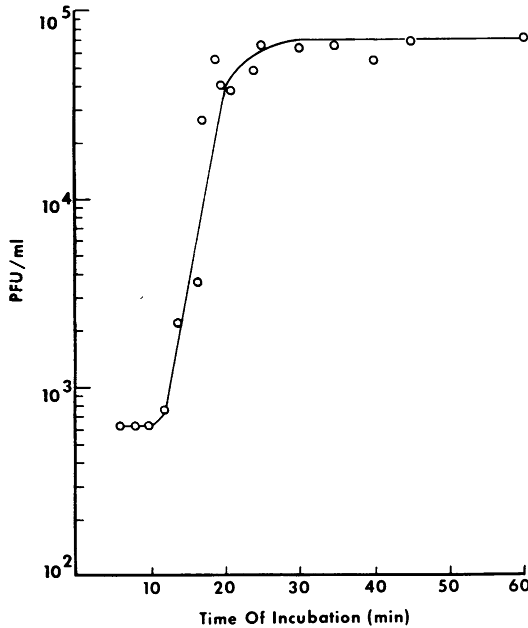

FIG. 1. *Single-step growth curve of phage H on E. coli AB-6.*

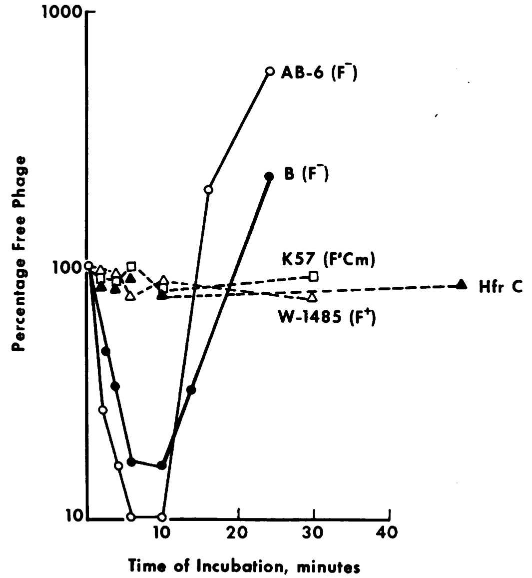

FIG. 2. *Adsorption of phage H to strains of E. coli of different sexual types.*

picking from the large clear center. A stock preparation was propagated on *P. pestis* TRU in BHI broth at 37 C. The filtered lysate had a titer of 4×10^{10} plaque-forming units (PFU) per ml and was stored at 5 C. Phage H was very stable even at high dilutions. Its nucleic acid was of the deoxyribonucleic acid (DNA) type, measured by the procedure of Burton (2).

A one-step growth curve of phage H was run by the procedure of Ellis and Delbruck (6) by using *E. coli* AB-6 as the host. The latent period was 10 to 12 min, and the burst size was approximately 100 (Fig. 1).

Phage H has a head approximately 50 nm in diameter and a short tail approximately 22 nm long (Fig. 3).

Host range of phage H. When spotted on lawns at 37 C, phage H attacked all the strains of *P. pestis* and roughly half the strains of *P. pseudotuberculosis* tested. In addition, it attacked all F^- strains of *E. coli* tested except strain 58-161/sp (F^-) but none of the F^+, F', or Hfr strains. The activity of phage H was not inhibited by the presence of λ prophage, *col* K3, or several R factors in F^- cultures. Although *col* I inhibited MS2 activity in one Hfr and one F^+ strain, it did not render these cultures sensitive to H phage.

Phage H did not attack the following bacterial strains: *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella dysenteriae*, *Proteus morganii*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Arizona arizona*, *Klebsiella pneumoniae*, and *Erwinia carotovora*. Some preparations at high concentrations attacked *Serratia marcescens*.

Specificity of phage H for F^- strains of E. coli. The female-specificity of phage H was verified by comparing the efficiency of plating (EOP) of phage H and phage MS2 on strains of *E. coli* of different sexual types. The EOP of MS2 was 1 compared with 10^{-9} for H phage on strains carrying the F factor as F^+, F', or Hfr (Table 1). On F^- strains, the EOP was $< 10^{-10}$ for MS2 and 0.1 to 1.0 for phage H with one exception. This again was on strain 58-161/sp(F^-), which was also insensitive to T3 according to Schell et al. (14). They proposed that this strain contained a defective F that had retained the gene controlling restriction but had lost those controlling other recognizable properties of the F factor. Meynell and Datta (13) showed that cultures of 58-161/sp(F^-) contained a small proportion of MS2-sensitive cells (determined by titer increase) and interpreted their results to mean the F factor was still present but in a repressed state.

Adsorption of phage H. Mixtures of log-phase cells of *E. coli* of different sexual types and phage at an MOI of 0.1 were incubated at 35 C. Samples were removed at various time intervals, the cells were sedimented by centrifugation, and the supernatant fluid was assayed for remaining phage (Fig. 2). There was little or no adsorption to strains Hfr C, W-1485 F^+, or K57 $F'Cm$; however, there was rapid adsorption to the F^- strains

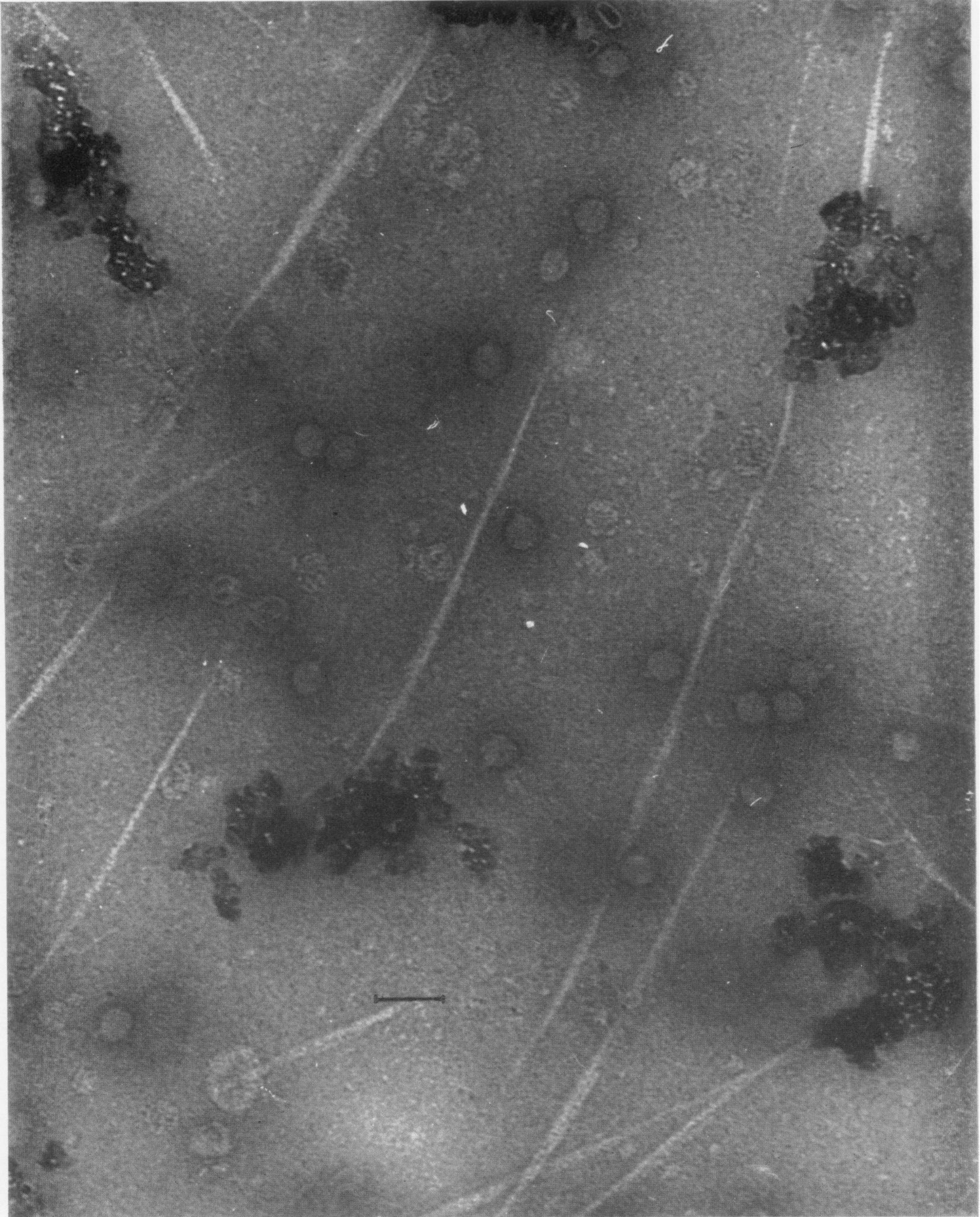

FIG. 3. *Electron micrograph of phage H.* × *140,000. Scale, 100 nm.*

AB-6 and B, followed by a rapid increase of free phage after 10 min.

Comparison of phage H with other female-specific phages. Six *E. coli* phages and one *S. typhimurium* phage have been reported to be specific for F^- strains. The data reported for these phages are compared with the data that we have obtained on phage H in Table 2. Phage H appeared to be different from all of the other female-specific phages.

DISCUSSION

The results reported here have shown clearly that phage H does not plaque on strains of *E. coli* carrying the F factor. Some of its characteristics are similar to those reported for other female-specific phages (Table 2). All except phage tau produce large clear plaques on F^- strains and have a very short latent period. Tau is also exceptional in being a temperate phage, although T3 and T7 are said to give semitemperate mutants (11). Only ϕII approached the female specificity demonstrated by phage H based on the EOP on F^+ strains; however, ϕII was adsorbed to F^+ strains equally as well as to F^- strains.

Hertman (9) discovered a serological relationship between T3 and *P. pestis* phage Y. Although they were indistinguishable morphologically, T3 did not form plaques on *P. pestis*. He concluded that phage Y had an antigenic site in common with T3 plus another site functional in the infection of *P. pestis*. Although Hertman's description of phage Y showed similarities to phage H, the exact relationship of these two phages is unknown.

Phage H was not adsorbed by F^+, F′, or Hfr strains of *E. coli* but was rapidly adsorbed by F^- strains. A similar observation by Dettori et al. (5) with ϕ1 led them to postulate that the ϕ1 receptor was present on both F^+ and F^- cells but was functionally covered by F pili on F^+ cells. Our observation that *col* I inhibited F function (determined by MS2 sensitivity) but failed to

TABLE 1. *Influence of the F factor in strains of E. coli on the efficiency of plating of phage H*

Strain	Sexual type	EOP[a]	
		H	MS2
AB-6	F^-	1	$<10^{-10}$
AB-359	F^-	10^{-1}	$<10^{-10}$
C600 (λ^+)	F^-	10^{-1}	$<10^{-10}$
58-161/sp	F^-	$<10^{-9}$	$<10^{-10}$
58-161	F^+	$<10^{-9}$	1
W-1485	F^+	$<10^{-9}$	1
W-4520 (F′8)	F′	$<10^{-9}$	1
W-3747 (F′13)	F′	$<10^{-9}$	1
AB-1518 (F′14)	F′	$<10^{-9}$	1
23.10.S (F′*lac*)	F′	$<10^{-9}$	1
Hfr C (λ^+)	Hfr	$<10^{-9}$	1
P4 X 6 (λ^+)	Hfr	$<10^{-9}$	1

[a] Titer of phage H·TRU on *E. coli* AB-6 was 10^{10} PFU/ml and was assigned an EOP of 1. The titer of MS2 on Hfr C was 10^{11} PFU/ml and was assigned an EOP of 1.

TABLE 2. *Comparison of phage H with other reported sex-specific phages*

Phage	Ref	Latent period	Burst size	Adsorption to F^+ compared to F^-	EOP on F^+	Comments[a]
ϕ1	(5)	—[b]	—	Poor	10^{-3}	
W31	(16)	12 min	106 in F^- 56 in F^+	Equally good	10^{-1} to 10^{-2}	Not restricted in stable Hfr strains, nor by *col* E2, λ prophage, or two R factors. Restricted by *col* B and *col* I.
tau	(8)	120 min after UV[c]	200	Equally good	—	Temperate; forms plaques on F^+ but not on F^- strains.
T7	(12)	12 min	250 in F^- 102 in F^+	Equally poor	10^{-2}	F restriction inhibited by presence of an R factor along with other expressions of F.
T3	(14)	13 min	200 in F^- low in F^+	Poor	10^{-5}	Attacks only one strain (TRU) of *P. pestis*. Host range mutants that can lyse male cells were obtained after growth in AB-6 (but not B).
ϕII	(4)	15–20 min	150 in F^- 2 in F^+	Equally good	10^{-9}	On *E. coli* B, EOP was 1 as on F^- strains of K-12.
SP6	(17)	15 min	20	Equally good	10^{-6}	Specific for *Salmonella*; does not adsorb to *E. coli*. Kills male cells but no progeny released.
H		10–12 min	100–200 in F^-	Poor	$<10^{-9}$	Attacks all strains of *P. pestis*. In contrast to T3 (*see above*), no host range mutants could be obtained after growth in AB-6.

[a] All the female-specific phages (except that plaque morphology was not described for tau and SP6) produced large clear plaques with large turbid halos on F^- strains and, when present, very small plaques on strains harboring the F factor.

[b] Not reported.

[c] Ultraviolet irradiation.

render the cells sensitive to phage H did not support Dettori's hypothesis.

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