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Male-Specific Bacteriophage MS2 Propagation in Fluorophenylalanine-Resistant *Escherichia coli* K12

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Mutation of *Escherichia coli* K12 HfrH to resistance to fluorophenylalanine resulted in changes in the plaque morphology of bacteriophage MS2 on this strain and led to an increased efficiency of propagation of the phage in liquid cultures. Evidence was obtained that the mutation resulted in inhibition of early lysis in infected cells and that lysis involved the production of a lysozyme. Genetic studies suggested that the observed pleiotropy of the resistance mutation was due to informational suppression.

Holloway and his co-workers (8, 34) have described mutants of *Pseudomonas aeruginosa* resistant to fluorophenylalanine (FPA) which are pleiotropically altered in their ability to propagate a number of bacteriophages. We have isolated a mutant of *Escherichia coli* K12 HfrH which gives plaques of the male-specific bacteriophage MS2 that are larger and clearer than those observed on the FPA-sensitive parent strain and which is also able to propagate the phage in liquid culture with increased efficiency. We have obtained evidence suggesting that this effect is due to the action of an informational suppressor of the kind described in *Pseudomonas* (29). In addition, we have found that MS2 progeny phage are released from infected *E. coli* cells by a process which involves the production of a lysozyme.

MATERIALS AND METHODS

Bacterial and bacteriophage strains are described in Table 1.

Media. Nutrient broth, nutrient agar, and soft agar have been described previously (3). Other media used were as follows (percent wt/vol): blood agar—nutrient agar plus fresh defibrinated horse blood, 5.0; 0.06 M phosphate buffer—K_2HPO_4, 1.04, and KH_2PO_4, 0.86, pH 6.5. FPA was incorporated into nutrient agar at 3 mg/ml and streptomycin at 200 μg/ml.

Propagation and titration of bacteriophage were as previously described (3).

One-step growth curves. An overnight shaken broth culture was diluted into fresh prewarmed broth and grown at 37 C for about 2 h to obtain approximately 1.5×10^8 cells per ml in late logarithmic phase. A 0.5-ml amount of this culture was added to 0.5 ml of MS2 phage to give a multiplicity of infection of 0.2, and the mixture was incubated at 37 C for 20 min, after which it was diluted to give a final count of 30 to 100 plaques per plate. Samples were then removed at 5-min intervals and assayed for PFU. Unadsorbed phage was determined by assay of the mixture at 20 min immediately prior to dilution.

Cell burst size. Single cell burst sizes were determined as described above, except that the mixture of phage and bacteria was diluted after adsorption to obtain 0.7 cells/ml, and 0.7-ml samples of this dilution were distributed into 50 separate tubes and incubated for a further 70 min (i.e., 90 min postinfection). At the end of this period 10-fold and 100-fold dilutions were plated and assayed for PFU.

Assay for lysozyme. Cells were lysed by shaking them with chloroform for 2 min at room temperature (30) and then allowing the chloroform to settle out at 4 C. The supernatant was removed and assayed for lysozyme by following the decrease in turbidity at 420 nm of a suspension of *Micrococcus lysodeikticus* prepared by washing an overnight blood agar culture with 0.06 M phosphate buffer (M. H. Richmond, Ph.D. thesis, University of Cambridge, 1957).

Isolation of Su. 3^+ strains. Cells were lysogenized with phage φ80 *p. Su. 3^+* by picking the growth from the turbid centers of the plaques (7) and purifying by restreaking.

RESULTS

Isolation and characterization of FPA^r mutants. Mutants of *E. coli* K12 HfrH resistant to fluorophenylalanine were isolated by picking the small colonies which grew inside the halo of clearing around a crystal of FPA which had been placed on a lawned nutrient agar plate. The colonies were purified by restreaking, and one such mutant was assigned the strain number of UB1057 prior to further study. Figure 1 shows that UB1057 is resistant to 3 mg of FPA per ml compared to the wild-type resistance level in HfrH of 2 mg/ml. When bacteriophage MS2 was titrated on UB1057 by the soft agar plate method (1) the phage formed larger clearer plaques than on HfrH (Fig. 2), although no differences in the efficiencies of plating of the

TABLE 1. *Strains of bacteria and bacteriophage*

Strain no.	Description	Origin
UB31	EC HfrH *lac*$^+$ prototroph	Microbial Genetics Research Unit, Hammersmith, 1962.
UB1057	UB31 FPA^R	This paper
UB1845	*Micrococcus lysodeikticus*	Laboratory collection
Bacteriophage MS2		T. D. Hennessey
Bacteriophage ϕ80 *p. Su. 3*$^+$		W. T. Brammar

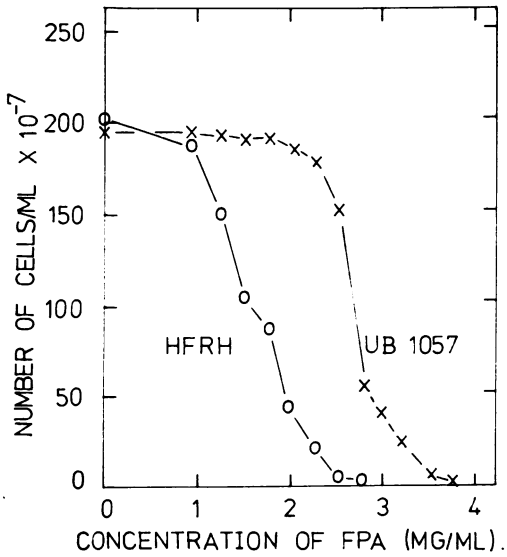

FIG. 1. *Determination of single cell minimal inhibitory concentrations of FPA. Dilutions (10-fold) of overnight shaken cultures were plated on nutrient agar plates with and without FPA. The plates were incubated overnight at 37C.*

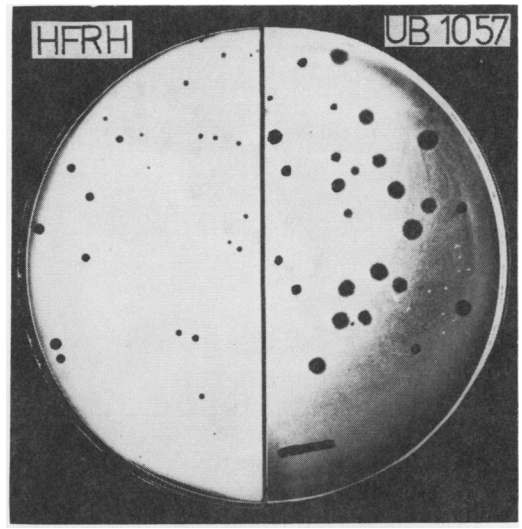

FIG. 2. *The plaque morphology in soft-agar layers of MS2 bacteriophage on HfrH and UB1057. The bar represents 1 cm.*

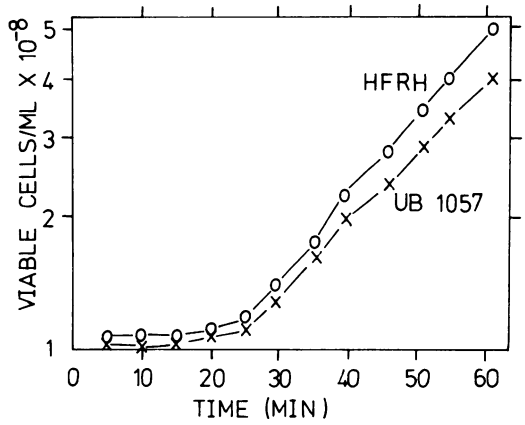

FIG. 3. *Growth rates of HfrH and UB1057. Overnight cultures were diluted 1/10 into nutrient broth, and the optical density at 675 nm (OD_{675}) was followed. OD_{675} was converted to viable cells per milliliter by using a standard curve.*

phage were apparent. On the other hand, propagation of phage MS2 in liquid cultures of UB1057 appeared to be more efficient in that the final titer was fivefold higher than that obtained in cultures of HfrH. Both strains were shown to have the same levels of sex piliation by electron microscopy and radioactive MS2 phage filtration assay (3), and both were equally efficient in MS2 phage adsorption. In addition, it was found that MS2 phage prepared on either strain, both on plates and in liquid cultures, retained the ability to produce plaques of different morphologies on UB1057 and HfrH irrespective of the number of cycles of propagation it had undergone.

Figure 3 shows that the doubling times of the two strains in nutrient broth are similar, about 20 min and 22 min for the FPA-sensitive and -resistant strains, respectively. We considered that this difference could not fully account for the differences in plaque morphology and phage propagation which we had observed. The infective cycles of bacteriophage MS2 in the two strains were therefore examined in detail.

One-step growth curves. Figure 4 shows that the latent period for both HfrH and UB1057 was similar (about 45 min), whereas the rise period was about 35 min for HfrH and about 50 min for UB1057. The mean burst sizes for the two strains were calculated to be 1,100 PFU/ml

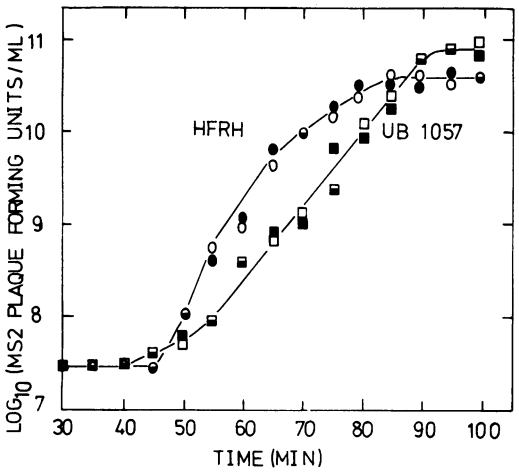

FIG. 4. *One-step growth curve of MS2 on HfrH and UB1057. Symbols: O, HfrH day 1; ●, HfrH day 2; □, UB1057 day 1; ■, UB1057 day 2. Each point represents the average of two readings.*

for HfrH and 2,100 PFU/ml for the FPA^r mutant. These results suggest that the mutation to FPA resistance resulted in an inhibition of early lysis in infected cells when compared to the FPA-sensitive parent. This possibility was tested further in single-cell burst experiments.

Single-cell burst experiments. Figure 5 shows the distribution of burst sizes of MS2-infected cells determined at 90 min postinfection. It can be seen that the maximum burst sizes of cells of HfrH and UB1057 are similar (approximately 9,000 PFU/cell) and that the FPA-resistant strain displays less heterogeneity in burst sizes than the FPA-sensitive parent, leading to a higher mean single-cell burst size for UB1057 (3,720 PFU/cell) than for HfrH (2,100 PFU/cell). Single-cell burst experiments performed at other times in the infective cycle confirmed this picture; for example, Figure 6 shows that at 70 min postinfection about twice as many cells of HfrH had lysed as of the mutant UB1057.

Assay for lysozyme. Bacteriophage MS2 is released from infected cells via lysis (18) but the details of this process are poorly understood. We investigated the possibility that the phage-directed lysis involves the production and utilization of a lysozyme, and that changes in this process might account for the differences in plaque morphology and phage propagation which we observed.

Figure 7 shows that a lysozyme was indeed produced by MS2-infected cells, and that production of this enzyme was delayed in the FPA-resistant mutant relative to the parent strain. The maximum amounts of lysozyme produced by both strains were, however, similar (approximately 3.5×10^{-8} μg/cell).

Effect of mutation to high-level strep-

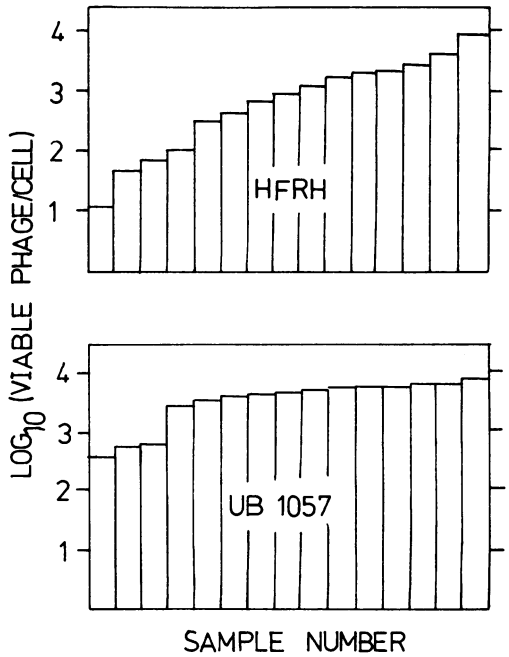

FIG. 5. *Single-cell burst sizes of HfrH and UB1057 at 90 min postinfection with MS2.*

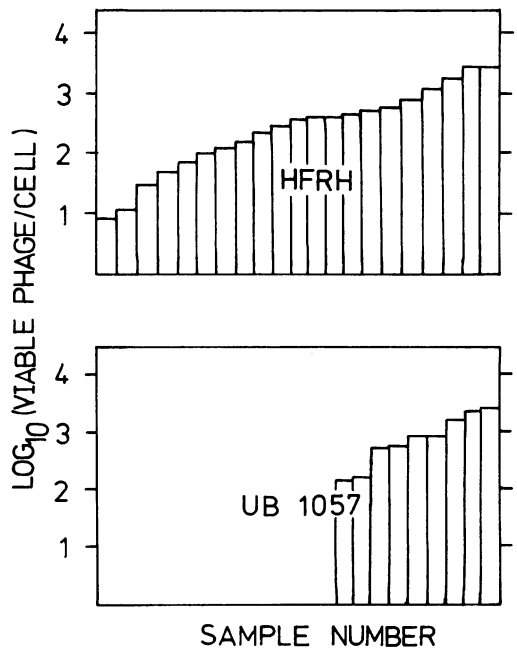

FIG. 6. *Single-cell burst sizes of HfrH and UB1057 at 70 min postinfection with MS2.*

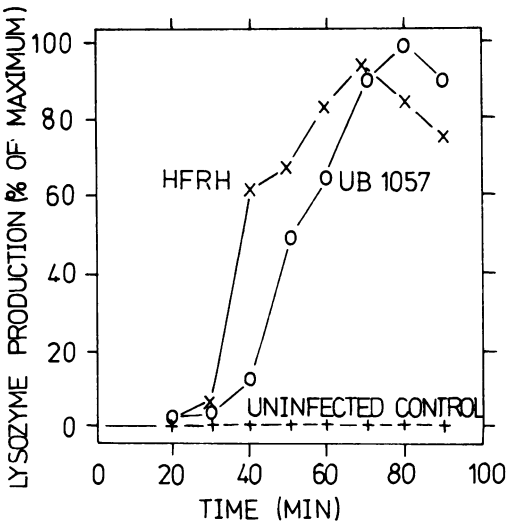

FIG. 7. *Assay for lysozyme production from HfrH and UB1057 at varying times after MS2 infection.*

tomycin resistance. Mutants which were chromosomally resistant to a high concentration of streptomycin were selected from both strains by plating 10-fold concentrates of fully grown overnight nutrient broth cultures onto nutrient agar containing 200 μg of streptomycin per ml. Table 2 shows that the isolation of resistant mutants in UB1057 derivatives was correlated with the phenotypic suppression of the FPA resistance, as has been found in *P. aeruginosa* (34), and that this was accompanied by reversion of the MS2 plaque morphology to that normally seen on HfrH. In contrast, streptomycin-resistant derivatives of HfrH showed no changes in this respect.

Effect of Su. 3$^+$. The introduction of the amber suppressor, *Su. 3$^+$*, into HfrH was shown to result in a change of MS2 plaque morphology to that normally seen on the FPA-resistant mutant, although, as expected, all the derivatives tested remained sensitive to FPA (Table 3). On the other hand, *Su. 3$^+$* had no effect on the phenotype of derivatives of UB1057.

DISCUSSION

Fluorophenylalanine is a bacteriostatic agent which exerts its inhibitory effect by randomly replacing phenylalanine in newly synthesized protein (5, 20, 21, 27), owing to the ability of phenylalanyl tRNA to utilize FPA (26). Resistance to FPA has been shown to involve an alteration in an aminoacyl RNA synthetase in *E. coli* (9), and one explanation for the observation that more than half of the FPA-resistant mutants isolated in *P. aeruginosa* showed pleiotropic alteration in host-controlled modification (HCM) (8, 13) could be that alteration in the tRNA aminoacyl synthetase resulted in a lack of specificity. On the other hand, the finding that the expression of FPA resistance could be suppressed by a mutation to streptomycin resistance in both *P. aeruginosa* (34) and in our strains makes it more likely that the mutation to FPA resistance is an informational suppressor of the type well-documented elsewhere (6, 10, 11, 15, 16, 24), and this notion is further supported by our evidence on the action of the amber suppressor *Su. 3$^+$* in our strains.

Genetic studies of a number of the small male-specific RNA phages similar to MS2 have revealed three cistrons (12, 14, 22, 33). The *A* cistron codes for a maturation (A) protein which

TABLE 2. *Effect of mutation to streptomycin resistance on HfrH and UB1057*

Determination	HfrH	UB1057	HfrH streptomycin^R	UB1057 Streptomycin^R
Growth on nutrient agar	+[a]	+	+	+
Growth on FPA agar	−	+	−	−
Growth on streptomycin agar	−	−	+	+
MS2 plaque type	As seen on HfrH	As seen on UB1057	As seen on HfrH	As seen on HfrH

[a] +, Growth; −, no growth. Six isolates of each strain were tested.

TABLE 3. *Effect of introduction of Su. 3$^+$ into HfrH and UB1057*

Determination	HfrH	HfrH ϕ80 *p. Su. 3$^+$*	UB1057	UB1057 ϕ80 *p. Su. 3$^+$*
Growth on nutrient agar	+[a]	+	+	+
Growth on FPA agar	−	−	+	+
MS2 plaque type	As seen on HfrH	As seen on UB1057	As seen on UB1057	As seen on UB1057

[a] +, Growth; −, no growth. Six isolates of each strain were tested.

is a minor constituent of the phage coat (2, 25, 28, 30) and which appears to be essential for adsorption and eclipse (23). The *B* cistron encodes the main coat protein, and the *C* cistron specifies the viral RNA synthetase (17, 32). None of the three known genes is directly related to cell lysis, which is the mechanism of release of progeny phage (12, 14, 18), although it has been suggested that phage f2 coat protein may be the lytic agent (35). Whether this is so, or whether the phage coat protein is an inducer of some host-specified lytic process, we can conclude that a lysozyme is produced at peak levels at a fixed point in the lytic cycle and that the FPA^r mutation results in a delay in the onset of this peak of production.

Our hypothesis to explain the observed changes in plaque morphology and phage propagation accompanying mutation to FPA resistance is that there is an inhibition of early lysis in the mutant strain which allows the production of more progeny phage per cell, resulting in the higher mean value and greater homogeneity of burst size which we observed in the one-step growth and single-cell burst experiments. Whether this effect is due solely to the delayed lysozyme production, or whether it is a general manifestation of informational suppression in this strain, we do not yet know.

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