Specific Piliation Directed by a Fertility Factor and a Resistance Factor of *Escherichia coli*

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SUMMARY

Production of pili directed either by the sex factor F or by the drug-resistance factor R 100 in *Escherichia coli* K 12 is regulated by a gene which acts by producing a repressor, and the mutant R 100–1 no longer produces this repressor. The specific pilus determined by R 100–1 resembled the F pilus morphologically, but differed in its affinity for F-specific RNA phages. Mutants of F and R 100–1 which had lost the ability to synthesize pili could each restore to the other the ability to produce pili on mixed infection of the same host.

INTRODUCTION

Filamentous appendages called pili (Brinton, 1965) or fimbriae (Duguid, Anderson & Campbell, 1966) are produced by most of the enterobacteriaceae under the control of chromosomal genes (Maccacaro, Colombo & Nardo, 1959; Brinton, Gemski, Falkow & Baron, 1961). However, a special kind of pilus is determined by the sex factor, F, which can be recognized because it specifically adsorbs the spherical particles of certain RNA phages, e.g. R 17 (Crawford & Gesteland, 1964), M 12 (Brinton, Gemski & Carnahan, 1964) and f 2 (Valentine & Strand, 1965). Brinton (1965) has suggested that the F pilus acts as the conjugation tube through which the chromosome of the male cell is transferred into female (F[–]) bacteria and whether or not this is the case, the presence of F pili is certainly essential to conjugation mediated by F.

Other extrachromosomal genetic elements such as Resistance Factors (R factors) responsible for contagious resistance to antibiotics in enteric bacteria (Ochiai, Yamanaka, Kimura & Sawada, 1959; Akiba, Koyama, Isshiki, Kimura & Fukushima, 1960) confer on F[–] bacteria the ability to conjugate, as well as to transfer chromosomal genes (Sugino & Hirota, 1962). To make it possible to observe the formation of specific pili, and thus to clarify the genetic relationship between pili and ability to conjugate, a strain of *Escherichia coli* K 12 was used which had none of the ordinary surface appendages like flagella or chromosomally determined pili. Certain R factors, termed *i*⁺, co-ordinately inhibit fertility, susceptibility to F specific phages and

formation of the specific f^+ antigen (Hirota, Nishimura, Ørskov & Ørskov, 1964) in F^+ bacteria, but mutants, designated i^- , have been isolated which lack this suppressive effect on F (Egawa & Hirota, 1962). As shown by Hirota, Fujii & Nishimura (1966), the i^- mutant R100-1, of one R factor, R100, can also restore full F function to bacteria carrying defective mutants of the F factor such as have been described by Cuzin (1962) and Nishimura (1964). No specific pili were present on F^+ or F^- bacteria when they carried R100, but pili which adsorbed F specific phage could be seen on F^+ bacteria, normal or defective, carrying R100-1. The i^- mutant, R100-1, in F^- bacteria brought about conjugation and gave chromosomal recombinants almost as frequently as F itself, suggesting that conjugation is regulated with R100 and that mutation in the i gene of the R factor derepresses its own conjugation function as well as that of F. Moreover, specific pili could now be seen on $F^-(R100-1)$ bacteria. Hirota *et al.* (1964) were able to show a serological relationship between an antigen determined by R100-1 and the F antigen, for an antiserum prepared against R100-1 in an F^- culture specifically agglutinated F^+ bacteria. Meynell & Datta (1966) have reported that in a series of R factors of independent origin, those that inhibited the functions of F themselves determined the production of a pilus sufficiently like the F pilus to adsorb F specific RNA phage. Experiments in Osaka demonstrated the presence of a pilus on $F^-(R100-1)$ bacteria, but no adsorption of phage could be detected. In view of the discrepancy between this result and the conclusion to be drawn from Hirota *et al.* (1964) and Meynell & Datta (1966), it was decided to re-examine the pili determined by R100-1 for adsorption of F specific phage.

METHODS

In Osaka

Bacteria. *Escherichia coli* K12 strain JE2217, used as host for F and R in most of the experiments, had lost type I pili and flagella by two independent mutations, being a recombinant resulting from a cross between w2802, a non-flagellate (*fla*⁻) mutant of Hfr Cavalli (Cavalli-Sforza, 1950), kindly provided by Dr J. Lederberg, Stanford University, California, and JE346 which is a *pil*⁻ mutant of JE344 unable to produce chromosomally determined pili. Strain JE344 was an F^- derivative obtained by acridine treatment of JE343 (Hirota, 1960). Inability to swarm on semi-solid agar (Stocker, Zinder & Lederberg, 1953) was taken to indicate absence of flagella, and failure to haemagglutinate chick red cells absence of type I pili (Duguid, Smith, Dempster & Edmunds, 1955). The absence of both flagella and type I pili in strain JE2217 was also confirmed by electron microscopy. Strain JE2442, a non-lactose fermenting (*lac*⁻) recombinant resulting from a cross between JE2217 and JE1428, Hfr Cavalli *lac*-52, was used as host for temperature-sensitive $F-lacT62$.

Salmonella paratyphi B carrying F13 was kindly provided by Dr S. Iseki, Gunma University, Maebashi.

F factors. F8 and F13 have been described by Hirota & Sneath (1961). The temperature-sensitive mutant, $F-lacT62$ (Jacob, Brenner & Cuzin, 1963), was kindly provided by Dr F. Cuzin, Institut Pasteur, Paris. Infertile mutants of F8 were obtained by growing JE2217 (F8) in broth containing 10^{13} particles/ml. of phage M12 and selecting phage-resistant bacteria. One such mutant, F8-D15, *def-15*, was examined, together with mutants similarly obtained from $F-lacT62D$.

R factors. R100 is an R factor conferring resistance to streptomycin (Sm), chloramphenicol (Cm), tetracycline (Tc) and sulphonamide (Su), which appeared in a strain of *Shigella flexneri* 2b 222 isolated by Dr R. Nakaya and which has been observed to confer a low degree of fertility on F^- bacteria (Sugino & Hirota, 1962). Its i^- mutant R100-1 i^- was obtained by selecting, from cultures of Hfr Cavalli carrying R100, clones which retained the high fertility of the Hfr (Egawa & Hirota, 1962; Sugino & Hirota, 1962). R100-31, i^- , *def*-31 and R100-70, i^- , *def*-70 are mutants of R100-1 i^- which have lost their infectivity, and thus their ability to bring about conjugation.

A hybrid factor resulting from recombination between F13 and an incomplete R factor, R23 (Harada, Kameda, Suzuki & Mitsuhashi, 1964), was obtained from Dr K. Harada, Gunma University.

F specific phage. Phage M12 (Hofschneider, 1963) was kindly provided by Dr P. H. Hofschneider, Max-Planck Institut, München, Germany. Phage sensitivity was tested by the cross-brushing method (Lederberg, 1947), where a loopful of bacterial culture is streaked across a strip of nutrient agar plate previously inoculated with a loopful of phage.

Media. L-broth (Lennox, 1955) and Difco Brain Heart Infusion (BHI) broth were used, and the solid medium was BHI agar.

Electron microscopy. Bacterial cultures in L-broth were diluted 10-fold in fresh medium and grown for 3 hr at 37° without shaking. To test for adsorption of phage M12, the phage was added at multiplicities ranging from 10 to 100, together with 5.3×10^{-3} M-CaCl₂ (Loeb & Zinder, 1961) and the mixture was incubated for 10 min. before transferring a sample to the grid. To examine detached pili, confluent growth on BHI agar was harvested in Davis minimal medium (Davis & Mingioli, 1950), minus glucose, sedimented by low-speed centrifugation, and the supernatant examined. For shadowcast preparations, duplicate specimens for the electron microscope were prepared by placing drops of suspension on formvar-coated grids, and drawing off the excess. The grids were then rinsed twice with distilled water and allowed to dry in air, and then shadowcast with platinum-palladium alloy. Negative staining (Brenner & Horne, 1959) was carried out by mixing samples of suspension with phosphotungstate before transferring them to the grids.

In London

Bacteria. *Escherichia coli* K12 strains JE254 and JE255, which carried R100 and R100-1 respectively, were provided by Dr Y. Hirota. These bacteria were R^+ derivatives of strain W4354 *met*⁻, which is 58-161 cured of *F* by acridine treatment (Hirota, 1960). Other bacterial strains, all lines of *E. coli* K12, were J5-3 *pro*⁻*met*⁻, an acridine-cured derivative of an F^+ strain, and RC12 *thr*⁻*leu*⁻*thi*, which came from the F^- strain W1177. These strains are fully described in Meynell & Datta (1966). Strains 58-161 F^+ and HfrH were used as indicators for the phages.

F specific phages. Three RNA phages, MS2 (Davis, Strauss & Sinsheimer, 1961), μ 2 (Dettori, Maccacaro & Piccinin, 1961) and f2 (Loeb & Zinder, 1961), were tested.

Sensitivity of a bacterial strain to lysis was tested both by spotting a drop of high-titre phage preparation on the surface of a nutrient agar plate spread with a loopful of broth culture, and by assaying dilutions of the phage preparation for plaques in a soft agar overlay. The presence of any phage-sensitive bacteria in cultures was detected by incubating mixtures of bacteria with excess phage, and plating for infective centres

(phage-infected bacteria) on the sensitive indicator strain, HfrH, after passing the mixture through antiphage serum to inactivate residual free phage. The media and experimental techniques are given fully in Datta, Lawn & Meynell (1966).

RESULTS

In Osaka

Strains JE2217 and JE2443, which produced neither flagella nor type I pili, were examined in the electron microscope after infection with the different F and R factors.

The results are shown in Table 1 and Plates 1 and 2, and can be summarized as follows.

Specific piliation directed by the F factor. F⁻R⁻ bacteria showed no pili (Table 1, lines 1 and 2; Pl. 1, fig. 1). Infection with F8 led to the formation of a few pili per bacterium, to which the F specific phage M12 could attach (Table 1, lines 3 and 4; Pl. 1, figs 2, 3 and Pl. 2, fig. 4). These pili were generally longer than type I pili, and some measured 20 μ or more. Addition of anti-f⁺ serum to the cultures causes agglutination of the pili; this is a specific effect of the antibody and does not occur with normal serum, showing that the F pilus constitutes at least part of the specific f⁺ antigen (Ishibashi, 1967).

Salmonella paratyphi B carrying F13 could be seen to produce similar pili.

Detached pili in the supernatant of centrifuged broth cultures showed an outer diameter of about 100 Å and were often aggregated. They were still capable of adsorbing particles of phage M12, and the protein coats of the attached phage particles were empty, in contrast to what has been reported for phage f2 (Valentine & Strand, 1965).

Inhibition of F piliation by R factors. The wild type *i*⁺ R factor, R100, co-ordinately inhibits the functions of F, so that an F⁺(R100) culture loses its sensitivity to lysis by F specific phage and agglutinability by F specific antiserum, and conjugates at much reduced frequency (Hirota *et al.* 1964). Such F⁺(R100) bacteria could not be seen to produce any F pili (Table 1, line 8). When, however, instead of R100, its *i*⁻ mutant, R100-1, was used, which does not suppress the function of F, F pili were formed to which phage M12 could readily be seen to adsorb (Table 1, line 9). Thus, all the several effects of the *i*⁺ character of R100 appear to result from inhibition of formation of the F pilus.

Piliation directed by R factors. No pili could be detected on bacteria infected with the wild-type R factor, R100 (Table 1, line 5), and no detached pili could be found in the supernatant of centrifuged broth cultures. The *i*⁻ mutant, R100-1, brings about conjugation and transfer of drug resistance at much higher frequency than R100 (Table 1, line 6) and with R100-1, bacteria bearing pili could readily be seen (Pl. 2, fig. 5). The pili on F⁻(R100-1) bacteria were morphologically distinguishable from both flagella and type I pili, and disappeared when the R factor was eliminated by acridine treatment. They evidently differed from F pili, however, for there was no adsorption of phage M12. The frequency of piliation in F⁻(R100-1) cultures was about one tenth of that in F⁺ cultures, and a larger proportion of the pili produced were very short (0.1-1.0 μ).

Piliation directed by a hybrid of F and an R factor. When R factors are transduced by salmonella phages, they are generally transferred only in part, and the transductants

Table 1. Piliation directed by *F* and *R* factors

TABLE 1. *Phage infection of E. coli* and *phage* factors

Line	Host bacteria	Infected with:		Phage M 12	Conjugal fertility	No. of bacteria observed	Adsorption of M 12 on pili	% Bacteria with pili numbering:							No. of unattached pili	Detached pili in the supernatant of mass culture	
		F	R					0	1	2	3	4	5	6			7
1	JE2217	—	—	r	—	344	—	100	0	0	0	0	0	0	0	0	—
2	JE2443	—	—	r	—	157	—	100	0	0	0	0	0	0	0	0	—
3	JE2217	F 8	—	s	++	224	+	34.4	28.6	23.2	8.0	2.7	2.7	0.4	0	0	+
4	JE2443	F 8	—	s	++	132	+	32.6	34.1	15.9	9.1	3.0	3.0	0.8	1.5	0	+
5	JE2217	—	R 100	r	+	262	—	100	0	0	0	0	0	0	0	0	—
6	JE2217	—	R 100-1	r	+	401	+	93.3	6.2	0.2	0.2	0	0	0	0	0	+
7	JE2443	F 13 × R 23 hybrid	—	s	+	104	+	41.3	29.8	17.3	6.7	1.9	1.0	1.0	1.0	0	+
8	JE2217	F 8	R 100	r	+	311	—	100	0	0	0	0	0	0	0	0	—
9	JE2217	F 8	R 100-1	s	++	130	+	46.9	23.8	18.5	7.7	1.5	0	1.5	0	0	++
10	JE2217	F 8-D 15	—	r	—	219	+	96.8	2.3	0.9	0	0	0	0	0	0	+
11	JE2443	F-T 62D	—	r	+	191	—	100	0	0	0	0	0	0	0	0	—
12	JE2217	—	R 100-31	r	—	225	—	100	0	0	0	0	0	0	0	0	—
13	JE2217	—	R 100-70	r	—	232	—	100	0	0	0	0	0	0	0	0	—
14	JE2217	F 8-D 15	R 100-1	s	++	71	+	63.4	23.9	5.6	4.2	2.8	0	0	0	0	+
15	JE2443	F-T 62D	R 100-1	s	++	105	+	81.0	13.3	3.8	1.9	0	0	0	0	0	+
16	JE2217	F 8-D 15	R 100-31	s	++	79	+	83.5	11.4	5.1	0	0	0	0	0	0	+
17	JE2217	F 8-D 15	R 100-70	s	++	141	+	29.1	34.0	18.4	8.5	5.6	1.4	2.8	0	0	+

fail to receive some of the drug resistances as well as the ability to pass on the R factor by conjugation (Watanabe & Fukasawa, 1961). Such an incomplete R factor, R23, is part of a complete R factor, R10 (Tc Cm Sm Su), conferring only its tetracycline resistance. A hybrid factor resulting from recombination between F13 and R23 was obtained by Harada *et al.* (1964), and shown to give sensitivity to F specific phage. Strain JE2443 carrying this hybrid factor produced pili to which phage M12 could be seen to adsorb with the same efficiency as to F pili (Table 1, line 7).

Defective mutants of F and R factors. The reduced fertility mutant F8-D15 was found to give only an occasional piliated bacterium, to which phage M12 adsorbed (Table 1, line 10). Defective mutants similarly derived from the temperature-sensitive F-*lacT62* factor gave, at 30°, under optimal conditions for reproduction of the F-*lacT62* factor very low frequencies of F-*lac* transfer (*c.* 10^{-5} per donor) and no chromosomal recombinants nor piliated bacteria could be found (Table 1, line 11). Nor could piliated bacteria be seen with R100-31 or R100-70, mutants of R100-1 which showed reduced frequency of drug-resistance transfer, and were taken to be less effective in promoting conjugation (Table 1, lines 12 and 13). Thus, mutants of F or R factors defective in conjugal activity are also defective in piliation.

Restoration of defective F piliation by R100-1. When bacteria carrying the defective F factor, F8-D15, were also infected with R100-1, this restored their ability to transfer the *gal* gene of F8 and to give chromosomal recombinants; in addition, the numbers of piliated bacteria increased to nearly the level found with F8 itself (Table 1, line 14). The pili present on F8-D15 (R100-1) bacteria resembled typical F pili in readily adsorbing phage M12. Similar results were obtained with R100-1 and the defective mutants of F-*lacT62* (Table 1, line 15).

Complementation evidently occurred between defective F factors and defective R factors for bacteria carrying both F8-D15 and one or other of R100-31 and R100-70, produced pili like typical F pili (Table 1, lines 16 and 17). This is in accord with the previous observation that Hfr strains that had lost fertility and phage sensitivity through a mutation in the F factor (Lederberg & Lederberg, 1956) regained both properties when they were infected with R100-1 itself or with one of its defective mutants (Nishimura, 1964; Hirota *et al.* 1966).

In London

Presence of F phage sensitive bacteria in cultures of bacteria carrying R100 and R100-1. When phage MS2 was added at 5×10^9 particles/ml. to cultures of JE254 (R100) which had been freshly grown to a bacterial concentration of 2×10^8 /ml., and 8 min. were allowed for adsorption before free phage was neutralized by addition of antiserum, the numbers of plaques produced on the indicator strain, HfrH, suggested that about 1 in 1000 of the bacteria had been infected. The conclusion that phage-sensitive bacteria were present in this strain was supported by finding that further incubation of the cultures for about 2 hr led to a considerable increase of phage.

When JE255 (R100-1) was tested in the same way for phage-sensitive bacteria, a much larger number of infective centres was obtained, corresponding to 2-10% of the bacteria in different experiments, and the increase of phage after incubation of the cultures was proportionately greater. It was not possible to estimate the precise proportions of bacteria which could be infected, for cultures of JE255 in liquid medium undergo spontaneous aggregation into clumps (Hirota *et al.* 1964); thus an individual

plaque could just as well arise from a clump of several infected bacteria as from a single one. Broth cultures inoculated from morphologically smooth colonies agglutinated as markedly as cultures of bacteria producing rough colonies. The aggregation in broth was evidently directly due to the pilus, for pili are poorly formed on solid medium, and broth cultures of JE254 (R 100), which contained very few piliated bacteria, were evenly dispersed.

From these results, it appeared that the pilus determined by the R factor, R 100, did have some affinity for phage MS2. Electron-microscopic examination of mixtures of JE255 bacteria and phage MS2, kindly performed by Dr A. M. Lawn, Lister Institute of Preventive Medicine, showed in addition to type I pili, spherical particles of the phage attached to the specific pili. Seventy per cent of the bacteria bore pili with particles of phage MS2 attached, but fewer phage particles were attached to each of these pili than in the usual preparations of F^+ bacteria (A. M. Lawn, personal communication).

Table 2. *Relative efficiencies of plating of male-specific RNA phages MS2, μ 2 and f2 on JE 255(R100-1)*

Phage stocks were prepared by picking single plaques on either 58-161 F^+ or JE255 and growing the phage on the same strain in broth. The efficiencies of plating are with reference to the numbers of plaques produced on 58-161 F^+ and are average values from several experiments.

Phage	Single plaque isolate last grown on	Efficiency of plating on JE255
MS 2	58-161 F^+	2×10^{-8}
	JE255	2×10^{-9}
μ 2	58-161 F^+	1×10^{-9}
	JE255	2×10^{-9}
	58-161 F^{+*}	2×10^{-9}
f 2	58-161 F^+	8×10^{-9}
	JE255	1×10^{-1}

* Plaque produced by stock previously grown on JE255.

The numbers of phage MS2 infective centres obtained with strain JE255 (R 100-1) suggested that the proportion of infectable bacteria might be high enough to allow the phage to lyse cultures macroscopically on solid medium, and, on testing, drops of undiluted phage stock produced patches of clearing, and dilutions gave plaques. Strain JE255 was less sensitive, however, than F^+ indicator strains like 58-161 F^+ or HfrH, for the clearing with undiluted phage was less complete and individual plaques were more turbid and heterogeneous in size. The efficiency of plating was considerably lower than on F^+ bacteria and most of the plaques were extremely small. Table 2 shows the results of testing the three *F* specific RNA phages, MS2, μ 2 and f2, in parallel on 58-161 F^+ and JE255. It can be seen that the efficiency of plating on JE255 differed for each phage; the values remained approximately the same in repeated titrations and reproducible titres were obtained with the phage stocks, thus excluding the possibility that the differences between the phages were due to chance fluctuations resulting from occasional failure to observe and count the smallest plaques.

Heterogeneity in plaque size, such as was observed here, is often indicative of inefficient adsorption. The rates of adsorption to JE255 and 58-161 F^+ were directly

compared using preparations of the three phages made on JE255. Phage was mixed for 10 min. at 37° with bacteria freshly grown to a concentration of 5×10^8 /ml. in broth, and the supernatant was assayed after centrifugation. With strain 58-161 F⁺, 77 % of phage MS2 (added at 1.5×10^7 /ml.), 76 % of phage μ 2 (added at 4×10^7 /ml.) and 90 % of phage f2 (added at 2.6×10^7 /ml) were absorbed; with JE255, adsorption was too poor for a decrease in free phage to be detected with any of the three phages.

Strain JE255 is a derivative of 58-161 cured of its F factor by acridine treatment. In case the particular kind of pilus, able to adsorb phages MS2, μ 2 and f2, determined in this strain was due to some peculiarity of the strain itself, R100-1 was tested in two other lines of *E. coli* K12, strain RC12 and strain J5-3, and these were both lysed macroscopically by phage MS2.

DISCUSSION

The *i*⁺ R factor, R100, which suppresses conjugation in F⁺ bacteria by producing a repressor of F function (Egawa & Hirota, 1962) prevents the formation of F pili on F⁺(R100) bacteria. However, with the *i*⁻ mutant, R100-1, F⁺ bacteria continue to conjugate normally and can be seen to produce pili apparently identical with those of F⁺R⁻ bacteria. Using bacterial strains without other surface appendages such as flagella or type I pili, it has been possible to observe that F⁻(R100-1) bacteria produce pili which are morphologically like F pili but which can be distinguished from them by a lesser affinity for F specific RNA phages. With strains JE2217 and JE2443 used in Osaka, no adsorption of phage M12 to these pili could be detected. With strains JE255, RC12 and J5-3, examined in London, some attachment of phages MS2, μ 2 and f2 could be demonstrated, but it was markedly less than with an F pilus.

The *i*⁺ to *i*⁻ mutation leading from R100 to R100-1 is associated both with a loss of inhibition of F function and with the appearance of pilated bacteria in F⁻(R) cultures. Since pili are produced by F⁻ bacteria when they carry R100-1 but not when they carry R100, it may be concluded that the cytoplasmic repressor produced by the *i* gene of R100 (Egawa & Hirota, 1962; Hirota *et al.* 1964) suppresses its own piliation as well as that of F. These regulatory changes may be compared with the operon model for enzyme synthesis in *E. coli* (Jacob & Monod, 1961), R100-1 being a constitutive mutant R factor unable to synthesize the cytoplasmic repressor of R and F mediated piliation. Meynell & Datta (1966) reported that those R factors which determine the production of a pilus resembling the F pilus are the same as those whose own repressor of function also repressed the function of F. Minor differences between the pili determined by these R factors and by F would not have been detected in their experiments, but in the present case it has been possible to discern a difference in structure between R100-1 pili and F pili as reflected in their different affinities for F specific phages. At the same time, using both F⁺ and (R100-1)⁺ bacteria as indicator strains, it was possible to detect differences between each of the three F specific phages, MS2, μ 2 and f2.

The interactions between F and R100 in pilus production are set out in Table 3. Wild-type F produces pili constitutively, while R100-1 is a constitutive mutant of R100 failing to synthesize repressor. F⁺(R100-1) bacteria produce F pili, while F⁻(R100-1) bacteria produce R100 pili, which, although serologically related (Hirota *et al.* 1964), differ somewhat in structure from F pili. The structural genes determining pilus production in F and in R100 may be thought of as allelic, for they are subject to

the same control of function and their products, although not identical, are alike in kind. Defective mutants of F and of R100-1 unable to determine the production of pili have evidently mutated in genes controlling an organelle producing pili specific for F or R100; pilus production is restored to the cell when a normal F or R100-1 factor is introduced, showing that the absence of pili is due to a mutation which is recessive to the wild type, as opposed to production of repressor.

Table 3. Control of pili formation by F and R factors

Genotypes of F and R	Pili in established cultures	Pili on newly infected bacteria	Type of pilus
F(<i>i</i> ⁻)	Present	.	F
Ri ⁺ /F(<i>i</i> ⁻)	Absent	.	.
Ri ⁻ F(<i>i</i> ⁻)	Present	.	F
Ri ⁺	Absent	Present*	R
Ri ⁻	Present	.	R
Ri ⁺ /Ri ⁻	Absent	.	.
Ri ⁻ /F(<i>i</i> ⁻) <i>def</i>	Present	.	F
Ri ⁻ <i>def</i> /F(<i>i</i> ⁻) <i>def</i>	Present	.	F

* Datta, Lawn & Meynell (1966).

It is also clear that genetic alterations resulting in loss or acquisition of F or R pili are strictly correlated with loss or gain of ability to transfer the chromosome by conjugation. Thus, the presence of pili, determined by F or by R factors, is essential for conjugation, whether or not the pilus itself acts as the tube through which the donor chromosome is transferred to the recipient bacterium as postulated by Brinton (1965).

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EXPLANATION OF PLATES

PLATE 1

Electron micrographs by platinum-palladium shadow-casting technique (× 20,000).

Fig. 1. JE 2217 (F⁻*pil*⁻*fla*⁻).

Fig. 2. JE 2217 carrying F8.

Fig. 3. Phage M12 adsorbed to JE 2217 (F8).

PLATE 2

Fig. 4. Electron micrograph of detached F pili with absorbed phage M12: phosphotungstate negative staining technique (× 100,000).

Fig. 5. Electron micrograph of JE 2217 carrying R100-1: platinum-palladium shadow-casting technique (× 20,000).