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Characteristics of *E. coli* K12 strains carrying both an F prime and an R factor

By N. S. WILLETTS and D. J. FINNEGAN*
M.R.C. Molecular Genetics Unit, Department of Molecular Biology, University of Edinburgh, Scotland

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SUMMARY

The interrelationship between an F prime and an F-like or I-like R factor was studied in *Escherichia coli* K 12 strains carrying both. The donorability of such strains suggested that these plasmids were transferred independently by their own transfer systems. Plaque formation by several male- and female-specific phage was tested; the inhibition by F*lac* of several female-specific phage was not affected by an F-like R factor. Neither did an R factor affect the incompatibility between F*lac* and F*his*. However, the surface exclusion characteristic of F*lac* was lost in the presence of an F-like R factor. This is probably closely related to the simultaneous loss of donorability, the F-pilus, and the f^+ antigen: all may be the result of the inhibition by an R product of the formation or activity of a single F product.

1. INTRODUCTION

It has been known for some time that cells can carry both an F-prime factor and a transferable drug resistance factor (R), showing that there is no incompatibility between these two types of plasmid (Watanabe & Fukasawa, 1962). In such strains *fi*$^+$, or F-like, R factors seem to prevent the formation of both R-pili and F-pili, leading to poor donorability and resistance to male-specific phage (Watanabe & Fukasawa, 1962; Hirota *et al.* 1964; Nishimura *et al.* 1967). On the other hand, *fi*$^-$, or I-like R factors prevent the formation of their own pili, but not of F-pili. Mutants of both types of R factor are known, the 'depressed' mutants, which do not prevent pilus formation. We have examined in more detail the relationship between an F-prime factor and R factors of the various types mentioned above, in strains carrying both, with particular reference to properties of F which might be affected by the presence of an R factor.

2. MATERIALS AND METHODS

(*a*) *Bacterial strains*

The strains used are described in Table 1. Strains carrying the I-like R factors R64 and R64-11 ('repressed' and 'derepressed') were obtained from E. E. M. Moody, and strains carrying the F-like factors R 100 and R 100-1 ('repressed' and

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'derepressed') from Dr Y. Hirota via Dr E. Meynell. R 64 carries resistance to both streptomycin (20 μg/ml) and tetracycline (20 μg/ml). R 100 carries resistance to streptomycin (20 μg/ml), tetracycline (20 μg/ml), chloramphenicol ($\geqslant$ 100 ug/ml) and sulphonamide: in addition we found that it carried resistance to at least 100 μg/ml spectinomycin. Tetracycline was routinely used to select cells carrying either R factor.

Table 1. *Bacterial strains used**

Strain no.	F prime	R factor	His	Trp	Lys	Str	Spc	Lac
JC 3272	–	–	–	–	–	R	S	–
JC 3273	F*lac*†	–	–	–	–	R	S	+/–
JC 5462	–	–	–	+	+	R	R	–
JC 5465	–	–	–	–	+	S	R	–
JC 6535	F*his*‡	–	+/–	–	+	S	R	–
ED 15	–	R 64-11	–	–	–	R	S	–
ED 18	–	R 64	–	–	–	R	S	–
ED 21	–	R 100	–	–	–	R	S	–
ED 22	–	R 100-1	–	–	–	R	S	–
ED 24	–	–	+	+	+	S	R	–
ED 60	F*lac*	R 64	–	–	–	R	S	+/–
ED 64	F*lac*	R 64-11	–	–	–	R	S	+/–
ED 68	F*lac*	R 100	–	–	–	R	S	+/–
ED 72	F*lac*	R 100-1	–	–	–	R	S	+/–
ED 1668	F*lac*	R 100	–	–	+	S	R	+/–
KL 98	Hfr	–	+	+	+	S	S	+

The nomenclature used is that recommended by Demerec *et al.* (1966), Taylor & Trotter (1967), and Novick (1969).

* All Spc^S Str^R strains are derivatives of the *E. coli* K 12 strain, JC 3272, and all Spc^R Str^S strains are derivatives of the related strain JC 5455.

† A derivative of F 42 obtained from Dr J. Scaife.

‡ Isolated from Hfr AB 311 by T. Takano.

(*b*) *Phage strains*

The male-specific phages f1, f2, and Qβ were obtained from Dr M. Achtman. The female-specific phages ϕ_I, W 31, and T 3 were obtained from Dr S. Glover, and ϕ_{II} from Dr M. Achtman. The I-specific phage If1 was obtained from Dr E. Meynell.

(*c*) *Media*

The complex medium used was Luria (L) broth containing 10 g Bacto-tryptone, 5 g Difco yeast extract, and 10 g NaCl/l., adjusted to pH 7. A minimal medium based upon M 9 was used, containing 7 g Na_2HPO_4, 3 g KH_2PO_4, 0·5 g NaCl, 1 g NH_4Cl, and 250 mg $MgSO_4.7H_2O$ per litre. Sugars were added to a final concentration of 0·2 %, vitamin B 1 to 0·2 μg/ml, L-amino acids to 20 μg/ml, streptomycin to 200 μg/ml, spectinomycin (a gift of the Upjohn Company, Kalamazoo, Michigan) to 100 μg/ml, and tetracycline to 20 μg/ml. For solid media 1·5 % Davis agar was added. LC top agar contained 10 g Bacto-tryptone, 5 g Difco yeast extract, 5 g NaCl, and 7 g agar per litre. After this had been autoclaved $CaCl_2$ was added to a final concentration of 5 mM. Oxoid plates contained 25 g Oxoid No. 2 nutrient broth and 15 g agar per litre.

(*d*) *Mating conditions*

In general, 30 min uninterrupted crosses in broth were performed at 37 °C. 0·5 ml aliquots of the donor cultures were mixed with 4·5 ml of the recipient cultures, both at about 2×10^8 cells/ml in exponential phase, in a 100 ml conical flask. However, to determine recipient ability, equal volumes (2·5 ml) of the exponential donor and recipient cultures were mixed, to reduce discrepancies due to R^- or F^- segregants. These crosses were allowed to proceed for 30 min when JC 6535 was used as donor and 60 min when KL 98 was used. In all cases the cultures used for matings were checked to ensure that > 99 % of the cells carried the appropriate plasmids.

After all matings appropriate dilutions were plated in 2·5 ml molten 0·7 % water agar containing 0·1 ml L broth to avoid 'step-down' conditions (Gross, 1963).

Plate matings were conducted by the technique of Clark & Margulies (1965).

In the text, squared brackets indicate the contraselecting markers.

(*e*) *Phage techniques*

Phage stocks were prepared by a confluent plate lysis technique, sterilized by chloroform or by millipore filtration, and stored in the refrigerator. Except for If1 (Meynell & Lawn, 1968) plaque assays were conducted by mixing 0·1 ml of a phage stock dilution containing about 2×10^3 p.f.u./ml, 0·2 ml of a standing overnight broth culture of the indicator strain, and 2·5 ml LC top agar and pouring this mixture on to an L plate. For If1, 0·2 ml of an exponential culture of the indicator strain was added to LC top agar containing 0·4 % agar and this mixture poured onto Oxoid plates. Again the cultures were checked where appropriate to ensure that > 99 % of the cells still carried the F*lac* and/or the R factor.

(*f*) *Curing*

An inoculum of about 100 cells from an overnight broth culture was added to 2 ml L broth (pH 7·8) containing 50 μg/ml purified acridine orange (AO). The tube was covered with aluminium foil to exclude light, and was shaken for about 20 h at 37°.

3. RESULTS

(*a*) *Growth and stability*

The presence of the plasmids R64, R64-11 and F*lac* did not affect the growth rate of the cells. However, R100 increased the growth rate whereas R100-1 decreased it substantially, both for reasons not at present understood (Table 2).

The strains carrying F*lac*, an R factor, or both were generally stable. During the course of this work several hundred clones of each strain derived from overnight broth cultures were tested, and almost no R^- or F^- segregants were observed. In all cases the F*lac* could be cured by AO treatment, albeit at the rather low frequency generally found for this particular cell line. The R factor was never observed to be

cured; i.e. the frequency of curing was less than 1 %. This indicated that the F*lac* and the R factor were present as separate entities retaining their separate responses to acridine orange.

Table 2. *Growth rates and curing characteristics*

Strain	F prime	R factor	Mean generation time (min)*	% Curing by 50μg/ml AO†	
				R factor	F*lac*
JC 3272	.	.	27	.	.
JC 3273	F*lac*	.	27	.	20
ED 15	.	R 64-11	27	< 1	.
ED 18	.	R 64	27	< 1	.
ED 21	.	R 100	21	< 1	.
ED 22	.	R 100-1	36	< 1	.
ED 60	F*lac*	R 64	27	< 1	30
ED 64	F*lac*	R 64-11	30	< 1	45
ED 68	F*lac*	R 100	21	< 1	65
ED 72	F*lac*	R 100-1	40	< 1	50

* This was measured during exponential growth at 37 °C in L broth.

† The technique used for curing is described in Materials and Methods. After curing, 100 single clones were tested for the Lac$^+$ and Tet^R characters.

Table 3. *Donorability*

Donor strain	F prime	R factor	Lac$^+$ conjugants per 100 donor cells	% coinheriting Tet^R	Tet^R conjugants per 100 donor cells	% coinheriting Lac$^+$
JC 3273	F*lac*	.	145	.	.	.
ED 18	.	R 64	.	.	0·12	.
ED 15	.	R 64-11	.	.	8·1	.
ED 21	.	R 100	.	.	0·31	.
ED 22	.	R 100-1	.	.	99	.
ED 60	F*lac*	R 64	155	< 2	4·6	98
ED 64	F*lac*	R 64-11	147	8	22	81
ED 68	F*lac*	R 100	0·7	16	0·6	3
ED 72	F*lac*	R 100-1	130	92	140	68

Crosses were performed as described in Materials and Methods, using JC 5465 as recipient selecting Lac$^+$[Spc^R] or Tet^R[Spc^R] progeny. For donor strains carrying R 100 or R 100-1, ED 24 was used as recipient and Lac$^+$[His$^+$Trp$^+$Lys$^+$] or Tet^R[His$^+$Trp$^+$Lys$^+$] progeny were selected. 50–100 clones were patched and tested for their ability to transfer *tet*$^+$ or *lac*$^+$ to JC 5462 and for the Lac$^+$Tet^R phenotype.

(*b*) *Donorability*

The donorabilities of strains carrying both an F*lac* episome and an R factor were measured. Progeny which had received either the F*lac* or the R factor were examined for coinheritance of the other plasmid (Table 3). R 100-1 was transferred at about the same frequency as F*lac*, but R 61-11 was transferred at a twenty-fold lower rate. As expected, only R 100 inhibited the transfer of F*lac*.

If transfer of the R factor and F factor were totally independent events, then in the mating mixture used (1:10, donor:recipient) the frequency of cells receiving both R and F factors would be the product of their separate frequencies of transfer. For example, when ED 72 was used as donor, approximately 13 % and 14 % of the recipient population received F*lac* and R 100-1 respectively (Table 3), so that on this hypothesis 1·8 % should have received both. In fact, about 11 % received both; this suggests that the formation of a mating pair often results in the transfer of both plasmids separately to the same cell, and this seemed to be true in all cases where the donor strain carried both F*lac* and an R factor. Although the frequencies of transfer of R 64 and R 64-11 were increased in the presence of F*lac*, these were still well below the transfer frequency of F*lac* itself, indicating that they could not be transferred efficiently by the F transfer system.

Table 4. *Strains carrying R100 deletions*

Strain	F prime	R factor	Male specific phage sensitivity*	Conjugants per 100 donor cells†	
				Lac^+	Tet^R
ED 110	F*lac*	EDRF 3	S	160	0·3
ED 111	F*lac*	EDRF 4	S	42	60
ED 112	F*lac*	EDRF 0‡	R	0·06	0·14
ED 118	.	EDRF 3	R	.	$< 10^{-5}$
ED 119	.	EDRF 4	R	.	$< 10^{-5}$
ED 120	.	EDRF 0‡	R	.	0·13

* Sensitivity to the male-specific phage f 1, f 2, Qβ and M 12, determined by a spot test.

† A standard cross was performed as described in Materials and Methods using JC 3272 as recipient and selecting $Lac^+[Str^R]$ and $Tet^R[Str^R]$ progeny.

‡ EDRF 0 = R 100.

One unexpected phenomenon was the appearance amongst the Lac^+Tet^R progeny sired by ED 68 ((R 100)(F*lac*)) of clones transferring F*lac* at a high frequency. About 10 % of the Lac^+Tet^R progeny showed this characteristic, and two clones, ED 110 and ED 111 were purified for further study, together with ED 112, a strain representative of the 90 % of the progeny showing normal poor donorability. Both ED 110 and ED 111 were sensitive to male-specific phage and transferred *lac*$^+$ to JC 3272 at a high frequency, whereas ED 112 was resistant to male specific phage and transferred *lac*$^+$ at the expected low frequency (Table 4). JC 3272 derivatives which had received the F*lac* episome but not the R factor from these strains, were purified and R 100 derivatives made. These were resistant to male specific phage and transferred *lac*$^+$ at a low frequency, indicating that the F*lac* factors were not mutants whose donorability was unaffected by R 100.

ED 110, ED 111 and ED 112 were treated with acridine orange to remove the F*lac* factor, leaving the R factor. The donorabilities and male-specific phage sensitivities of these strains, ED 118, ED 119 and ED 120 respectively, were determined. The R factors in ED 118 and ED 119 (designated EDRF 3 and EDRF 4) seemed to

be completely transfer deficient compared to R100, and male-specific phage resistant (Table 4). Derivatives of these strains obtained by infection with wild-type F*lac* regained the properties of the original strains EC110 and ED111.

EDRF3 and EDRF4 seem, then, to have lost simultaneously both transfer ability and the ability to prevent the transfer of F*lac*. They may therefore be deletion mutants of R100 which were selected in the original mating because of their inability to prevent the transfer of F*lac*. They were found to retain resistance to tetracycline, chloramphenicol, streptomycin and spectinomycin. Complementation analysis to discover which transfer genes had been lost (N. S. Willetts, unpublished experiments) confirmed that both EDRF3 and EDRF4 were deletion mutants, and showed that EDRF3 had suffered the longer deletion. This larger deletion might cover a gene the product of which is necessary for R transfer, but whose F analogue does not exist or cannot be used. This could explain why EDRF3 is transferred at a low frequency by ED110, whereas EDRF4 is transferred at a high frequency by ED111 (Table 4).

Table 5. *Phage resistance patterns*

Strain	f 1	f 2	Qβ	M 12	If 1	ϕ_I	ϕ_{II}	W 31	T 3
JC 3272	0	0	0	0	0	100	100	100	100
JC 3273	100	100	100	100	0	20	2	8	12
ED 18	0	0	0	0	0	1	0	3	0
ED 15	0	0	0	0	100	2	0	0	1
ED 21	0	0	0	0	0	80	120	170	110
ED 22	30	12	5	1	0	170	130	140	90
ED 60	80	75	90	90	0	0	0	0	0
ED 64	110	70	85	105	65	1	0	0	0
ED 68	0	0	0	0	0	14	4	12	8
ED 72	40	70	60	70	0	10	4	13	7

Each strain was used as indicator as described in Materials and Methods, plating about 200 phage, and the plaque count expressed as a percentage of that observed using JC 3273 (for male-specific phage), JC 3272 (for female-specific phage) or ED 15 (for If 1).

(*c*) *Phage resistance*

The efficiencies of plating of a variety of male-specific phages were determined (Table 5). As expected, the strain carrying F*lac* was sensitive to these phage and that carrying R100-1 was partially sensitive, whereas strains carrying R100, R64, or R64-11 were resistant, as was the strain carrying both R100 and F*lac*. In cells carrying both F*lac* or R64-11 plaque formation was not affected. The simultaneous presence of R100-1 with F*lac* somewhat reduced plaque formation perhaps via abortive adsorption of some phage to the R100-1 pilus or as a result of the poorer growth characteristics of this strain.

Hfr cells carrying a derepressed R64 have been shown to produce both I-like and F-like pili (Meynell, Meynell & Datta, 1968), and in the present strains the I-specific phage If1 was adsorbed by the R64-11 pilus and gave plaques with similar efficiency whether or not F*lac* was simultaneously present.

Four female-specific phage (ϕ_I, ϕ_{II}, W 31, and T 3) were tested for the efficiency of plaque formation. R 64 and R 64-11 prevented plaque formation completely while R 100 and R 100-1, unlike F*lac*, did not lead to any reduction either in plaque formation or in plaque size (these two parameters always being associated). This absence of any effect of R 100 and R 100-1 on these phages allows study of the effect of the R factors on the inhibition of female-specific phage by F*lac*.

These results, with ED 68 and ED 72, were of particular interest as the efficiencies of plating were the same as when JC 3273, carrying F*lac* alone, was used as an indicator, showing that R 100 did not affect the F function which inhibits the growth of any of the female-specific phage tested.

Table 6. *Relative recipient abilities*

Strain	$His^+[Str^R]$ recombinants with KL 98	Surface exclusion index*	$His^+[Str^R]$ progeny with JC 6535
JC 3272	100†	1	100‡
JC 3273	0·3	300	0·2
ED 18	60	2	81
ED 15	95	1	46
ED 21	30	3	70
ED 22	25	4	47
ED 60	0·2	500	0·2
ED 64	0·5	200	0·1
ED 68	17	6	1·8
ED 72	2·1	50	0·6

Crosses were peformed as described in Materials and Methods. Selection was made for Tet^R and Lac^+ when the recipient possessed these properties. The figures given represent the average from several experiments with each strain.

* Surface exclusion index = $His^+[Str^R]$ recombinants with JC 3272/$His^+[Str^R]$ recombinants with that strain, in crosses with KL 98.

† 100% = 4·5 $His^+[Str^R]$ recombinants per 100 cells of the limiting parent.

‡ 100% = 280 $His^+[Str^R]$ conjugants per 100 cells of the limiting parent.

(*d*) *Surface exclusion*

Cells carrying an F prime such as F*lac* are poor recipients in matings with an Hfr strain, giving a reduced yield of recombinants. This property of surface exclusion was measured in matings with the Hfr strain KL 98, selecting $His^+[Str^R]$ progeny (Table 6).

Neither R 64 nor R 64-11 alone affected surface exclusion whereas R 100 and R 100-1 gave a slight increase and F*lac* gave a 300-fold increase. Similarly the surface exclusion due to F*lac* was not affected by the simultaneous presence of R 64 or R 64-11. However, the presence of R 100 reduced the level of surface exclusion by F*lac* about 50-fold, whereas R 100-1 reduced it by only about fivefold, suggesting that surface exclusion is subject to control by the R factor. The small difference found between ED 68 and JC 3272 may be correlated with the low but appreciable level of transfer of F*lac* by ED 68, and the decreased surface exclusion of ED 72 compared to JC 3273 may be explained by replacement of part of the

F-coded surface-excluding components by equivalent but non-excluding R components.

As a further demonstration of the effect of R100 upon surface exclusion by F*lac*, the recipient ability of an HFT culture of an (R100)(F*lac*) strain was measured; such a culture is known to show good donorability (Watanabe, 1963). The inoculum for this was made by mixing 10^4 cells/ml of ED1668 with 10^5 cells/ml of JC3273 in L broth, and incubating them for 12 h. Four per cent of the cells in this culture were $Lac^+Tet^RStr^R$ (i.e. R100 derivatives of JC3273), which compared well with the 5% calculated from a measurement of the transfer of R100 by this culture assuming full 'derepression' to the level of transfer found with R100-1. All the $His^+Lac^+Tet^R[Str^R]$ progeny in the cross with KL98 must therefore have been derived from cells carrying a phenotypically 'derepressed' R factor. On this basis, the surface exclusion index of such cells was 40 which compares well with the figure of 50 previously found for ED72 carrying the *genetically* 'derepressed' R factor.

(*e*) *Incompatibility*

It is well known that autonomous F prime factors cannot coexist in the same cell (Scaife & Gross, 1962; de Haan & Stouthamer, 1963). We therefore attempted to introduce an F*his* episome into the cells carrying both F*lac* and an R factor to see whether the latter had any effect on the incompatibility expected between F*lac* and F*his* (Table 6).

As was to be expected from the stable coexistence of F*lac* and the various R factors, and the lack of surface exclusion between F and R, none of the R factors alone affected the ability of the cells to form F*his* derivatives. When the F*lac* derivative, JC3273, was used as recipient, however, a reduced number of His^+Lac^+ progeny was found, although the frequency with which they were obtained was similar to that found when the Hfr KL98 was used as donor. This suggested that surface exclusion was responsible for the major part of this reduction and that once the F*his* had succeeded in entering the cell, it had a high chance of giving rise to a His^+Lac^+ clone. This was in general also true when a strain carrying both F*lac* and an R factor was used as recipient. Experiments using the recipients in stationary phase to reduce entry exclusion gave analogous results.

These His^+Lac^+ clones seemed to carry both F*his* and F*lac* at first, since all transferred *lac*$^+$ to a $Rec^-Lac^-His^-Spc^R$ recipient, and most transferred *his*$^+$. The F*lac* and F*his* may have been present in the autonomous state, or unstably integrated into the chromosome. In an attempt to distinguish between these possibilities one clone from each cross, transferring both *lac*$^+$ and *his*$^+$, was purified and tested for curability and ability to transfer the chromosomal markers *aroD* and *pro*. The results suggested that these clones were unstable and that during purification they frequently gave rise to His^+ strains haploid for *his* but carrying F*lac* in the integrated state. These events are probably similar to those found by Dubnau & Maas (1968) in the case of an F*lac* episome inherited by an Hfr strain. The presence of any of the R factors had no additional effect and further analysis of these clones was therefore discontinued.

Since the phenomenon of incompatibility was obscured by frequent integration of the F primes into the chromosome, some of these crosses with JC6535 were repeated using RecA$^-$ analogues of JC3272, ED21, ED22, ED68, and ED72 No stable His$^+$Lac$^+$ progeny were found, confirming that integration of the F primes into the chromosome by recombination was responsible for the His$^+$Lac$^+$ progeny found in the Rec$^+$ strains above. Neither R100 nor R100-1 allowed F*lac* and F*his* to coexist in the autonomous state.

4. DISCUSSION

Although not definitive, the independent curing and transfer of F*lac* and an R factor present in the same cell, suggest that they coexist as separate replicons. Spatial separation inside the cell might explain why neither R64 nor R64-11 could be transferred very efficiently by the F transfer system when this was present in the same cell. Cells carrying both R64-11 and F*lac* formed both types of pili, as evidenced by their sensitivity to both F-specific and I-specific phages, and the two replicons may be closely associated with the transfer structures to which they give rise. This may also be true for F-like R factors, since the 'derepressed' transfer-deficient R100 mutant EDRF3 was also transferred very poorly in the presence of F*lac*.

Of the F encoded properties tested for repression (other than transfer and pilus production), female-specific phage inhibition, surface exclusion and incompatibility were not affected by R64, and only surface exclusion was affected by R100. Hirota *et al.* (1964) have shown previously that R100 has no effect on the resistance of a male strain to another female-specific phage *tau*.

The surface exclusion experiments showed that either production by F*lac* of the substance responsible for surface exclusion, or its action, was prevented by R100 but not by R100-1 nor by an HFT culture of R100. This loss of surface exclusion could have been due to the known loss of the F-pilus itself. However, many transfer-deficient mutants which are resistant to male-specific phage and have therefore presumably lost the F-pilus, still show surface exclusion (N. S. Willetts & M. Achtman, unpublished experiments). The F-pilus is therefore not responsible for this property. However, one class of transfer-deficient point mutants which are male-specific phage resistant has simultaneously lost the surface exclusion property. The product of the gene in which these strains are mutant is therefore necessary both for surface exclusion and for F-pilus formation.

Hence loss of donorability, the F-pilus, the f$^+$ antigen (Hirota *et al.* 1964) and surface exclusion, by a cell carrying both F*lac* and R100, may be the result of inhibition by an R100 product of the formation or activity of the single F gene product. This F product might be a surface component necessary for synthesis of the F-pilus structure and itself responsible for surface exclusion, or a protein controlling the activity of the several F functions affected.

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