Interaction of a colicinogenic factor with a resistance factor and with the fertility factor F in *Escherichia coli* K-12*

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SUMMARY

The resistance (R)-factor R538-1*drd* of three F-like R-factors tested can cause the transfer of the non-transmissible ColV determinant from *E. coli* V. The col factor, once transferred to *E. coli* K-12 strains, was shown to be related to the fertility factor, F, on the basis of entry exclusion and plasmid incompatibility. The col factor was found to recombine with R538-1*drd*, yielding a transmissible plasmid comprised of the col determinant and the sex factor of the R-factor, but not the resistance genes. The recombinant plasmid was found to be incompatible with both R538-1*drd* and F, and excluded the entry of R538-1*drd* but not F.

1. INTRODUCTION

E. coli V was the first colicinogenic strain described (Gratia, 1925). Other colicinogenic strains were subsequently isolated and the colicins they produced were classified on the basis of their activity on sensitive and resistant indicator strains. The colicin of *E. coli* V was assigned to the V group (see reviews by Fredericq, 1957, 1963).

Genetical analysis of a number of colicinogenic strains has revealed that the determinants for colicin production are extrachromosomal. This can be clearly shown in cases where the determinant is borne on a plasmid, a colicinogenic (col) factor, which has sex factor activity and is transferred to recipient bacteria by conjugation. In cases where a col determinant itself is not transmissible, evidence that it is extrachromosomal can come from showing that it is efficiently transferred when conjugation is promoted by a second, transmissible plasmid without concomittant transfer of chromosomal markers. For example, the non-transmissible ColE factors are cotransferred with the fertility factor, F, or the transmissible ColI or ColV-K30 (see reviews by Meynell, Meynell & Datta, 1968; Novick, 1969).

In contrast to other ColV factors, the col determinant of *E. coli* V is not transmissible. Nor is it transferred when mating is promoted by ColI (MacFarren &

Clowes, 1967). In the present study, resistance (R) factors were tested for their ability to cause its transfer. The availability of mutant R-factors with increased conjugating ability (Meynell & Datta, 1967) facilitated this study. This paper describes the transfer of the col determinant by one R-factor, and also the interaction of the col factor with the fertility factor F.

2. MATERIALS AND METHODS

(i) *Media*

Nutrient broth was either Oxoid No. 2 or Difco Neopeptone broth supplemented with a beef–heart infusion. Difco Neopeptone agar was used for solid medium. Minimal medium was composed of TL salts (Tatum & Lederberg, 1947) without asparagine, supplemented with nutritional requirements as needed: thiamine, 0·1 mg/l.; amino acids, 50 mg/l.; sugars at 0·2 %. It was solidified with 1·5 % agar. MacConkey Lactose Medium (Difco) was used to indicate lactose fermentation. Antibiotics were used at the following concentrations for R⁺ strains: tetracycline HCl (TC), 10 mg/l.; chloromycetin (CM), kindly provided by Parke-Davis, 10 mg/l.; sulphadiazine (SU), 25 mg/l.; streptomycin sulphate (SM), 5 mg/l.; spectinomycin (SPC), a gift to Dr W. Maas from the Upjohn Company, 10 mg/l. To counterselect donors SM was used at 200 mg/l., or nalidixic acid (nal), provided by Sterling-Winthrop, at 50 mg/l.

(ii) *Bacterial strains*

E. coli V will be referred to by Fredericq's designation CA7, and its col factor ColV-CA7 (ColV1 of MacFarren & Clowes, 1967). The *E. coli* K-12 F⁻ strains used were PA309*nal*/V, a nalidixic acid-resistant, colicin V-CA7-resistant mutant of PA309 (*thi, thr, leu, trp, his, argH, strA, lac*), kindly provided by Dr Marylyn Cooke, and 712/V (*proA, trp, his, strA, lac*) resistant to colicin V-CA7, provided by Dr Elinor Meynell. A *recA* derivative of 712/V was isolated as a u.v. sensitive *his⁺* recombinant following a mating with KL16-99, a *recA* Hfr strain which transfers *recA* before *his⁺* (Low, 1968). The F*lac* donor was MA3020/V (*thi, pro, his, argE, lac⁻*/F' *lac⁺*), provided by Dr E. Dubnau. CA-77HN/V (*his, nal*) is an Hfr strain with a mode of chromosome transfer like Hfr Hayes. X478 (*leu, proC, purE, trp, lysA, metE, thi, lac*) was used as the recipient in the transductions. CA-77HN and X478 were provided by Dr JoAnne DeVries.

(iii) *Resistance factors*

The derepressed R-factors were kindly provided by Dr Elinor Meynell. They are R136*drd* (TC, SU), R192*drd* (TC, CM, SM, SU) and R538-1*drd* (SU, CM, SM). R538-1*drd* also confers resistance to spectinomycin. These R-factors are F-like and confer sensitivity to MS-2.

Sensitivity to MS-2 was tested by placing a loopful of phage suspension (titred at 10^9 p.f.u./ml) on a lawn of bacteria, or by cross-streaking bacterial suspensions and phage suspensions and looking for the presence of lysis after incubation at 37 °C.

<div align="center">(iv) Other procedures</div>

Matings. Donor and recipient strains, grown in nutrient broth to a cell density of $2–5 \times 10^8$/ml, were mixed in a ratio of $1:1$ and incubated at 37 °C for 30 min. The mating mixture was gently shaken for F*lac* transfer; otherwise it was incubated standing. Samples of the mating mixture were agitated with a Vortex mixer to separate mating pairs prior to being diluted and plated on selective media. 'F⁻ phenocopies' were obtained by the procedure of Dubnau & Maas (1968).

Detection of colicinogeny. The methods described by Fredericq (1957) for detecting colicin production on plates were used, the sensitive indicator strain being J5*nal* (532) (*proA*, *metF*), resistant to CM, TC, and SU, provided by Dr Elinor Meynell.

Transductions. Preparation of transducing phage and transductions were performed by the methods described by McFall (1967).

<div align="center">3. RESULTS AND DISCUSSION</div>

<div align="center">(i) Cotransfer of Col with an R-factor</div>

Strain CA7 has no sex factor activity. Cultures contain no bacteria sensitive to male-specific phages such as MS-2 or Ifl, and no sex pili can be seen by electron microscopy. When the derepressed (*drd*) R-factors were introduced into CA7, the R⁺ progeny became MS-2 sensitive and donated the R-factors to an *E. coli* K-12 recipient. In the latter matings R⁺ transfer was selected by appropriate antibiotic and after 2 days the selective plates were overlayered with a lawn of indicator bacteria to determine whether any of the R⁺ recipients were also colicinogenic (col⁺). Approximately 2 % of the recipients of one R-factor, R538-1*drd*, were found to be col⁺. The frequency of cotransfer of col⁺ was high in comparison to the frequency of transfer of chromosomal markers by this same R-factor, which is of the order of 0·3 to 20 per 10^6 donors (Cooke & Meynell, 1969). The other R-factors were ineffective in transferring col⁺ although they were stably inherited in the col⁺ strain and were themselves efficiently transferred (Table 1). R538-1, the wild-type repressed R-factor from which R538-1 *drd* was derived, also cotransferred col⁺.

<div align="center">Table 1. Transfer of ColV-CA7 from various donors</div>

Donor	Recipient	Selection	Frequency R⁺ transfer/ donor	Col⁺/R⁺ recipient
CA7 (R136*drd*)	PA309*nal*/V	TC	2·0	0/10⁵
CA7 (R192*drd*)	PA309*nal*/V	CM	10·0	0/10⁵
CA7 (R538-1*drd*)	PA309*nal*/V	CM	10·0	2/10²
CA7 (R538-1)	PA309*nal*/V	CM	0·3	2/10²
PA309*nal*/V (538-1*drd*) (ColV-CA7)	712/V	CM	30·0	2/10²
712/V (538-1*drd*) (ColV-CA7)	PA309*nal*/V	CM	30·0	6/10²
712 *his⁺ recA*/V (538-1*drd*) (ColV-CA7)	PA309*nal*/V	CM	29·0	4/10³

Forty R$^+$ col$^+$ progeny of the K-12 recipient PA309nal/V were restreaked on the selective medium and single colonies were analysed. All had the antibiotic resistances and the MS-2 sensitivity associated with R538-1drd and were col$^+$. One was used as a donor with 712/V as a recipient, and the R$^+$ progeny of the mating were tested to determine the frequency of cotransfer of col. Again, 2 % of the recipient colonies tested were found to be also col$^+$, showing that the strain which received col$^+$ with the R-factor from CA7 did not always transfer col$^+$ with the R-factor. In the same way, with other R$^+$ col$^+$ donors col$^+$ was transferred to only a few per cent of the R$^+$ progeny. Col$^+$ was cotransferred with R by a $recA$ donor, but at a slightly lower frequency than by rec^+ donors (see Table 1).

It appeared that the col determinant in the R$^+$ col$^+$ strains was not integrated in the R-factor, but rather was borne on a separate plasmid which was transferred when mating was promoted by the R-factor. This plasmid will be referred to as ColV-CA7. Further studies showed that although it is non-transmissible it is related to the fertility factor, F.

Table 2. *Frequency of F*lac *transfer per F*lac *donor to colicinogenic and non-colicinogenic recipients**

	Recipient†	Exponential phase culture	Phenocopy culture
A	712/V	20	40
	712/V (538-1drd)	37	55
	712/V (538-1drd) (ColV-CA7)	0·5	47
B	PA309nal/V	10	19
	PA309nal/V (538-1drd) (ColV-CA7)	0	7
	PA309nal/V (ColV-CA7)-1	0	14
	PA309nal/V (ColV-CA7)-2	0	14
	PA309nal/V (ColV-CA7)-3	0	13

* Frequency based on results with 200–300 donors.

† The F$^-$ strain PA309nal/V itself was not a good recipient of Flac, but this was apparently due to a chromosomal mutation which made its growth sensitive to galactose, a product of lactose hydrolysis (Yarmolinsky *et al.* 1959). When this strain was plated on a minimal medium containing galactose its growth was inhibited, but some resistant colonies grew out which were found to be good recipients of Flac. Therefore, galactose-insensitive derivatives of the PA309 strains cited in this table were used.

(ii) *The relationship of colV-CA7 to F*

In matings with an Flac donor, 712/V and 712/V (538-1drd) were good recipients, giving rise to stable Flac lines. 712/V (538-1drd) (ColV-CA7), however, was a poor recipient of Flac. One possibility was that Flac was not entering the col$^+$ recipient under the standard mating conditions, a phenomenon known as 'entry exclusion'. It has been shown that F is excluded from recipients which themselves harbour an F-factor (Lederberg, Cavalli & Lederberg, 1952), and this appeared to be the case with the recipients harbouring the col factor. When the col$^+$ strain used as recipient was pregrown for mating under conditions which convert F-carrying cells into good recipients ('F$^-$ phenocopies') its recipient ability was increased 100-fold (Table 2A).

The lac$^+$ progeny which do result from the mating with the R$^+$ col$^+$ recipient are unstable. In one experiment 50 lac$^+$ colonies were restreaked on MacConkey lactose plates, and over two-thirds of them yielded a high proportion of lac$^-$ segregants. When lac$^+$ daughter colonies from each of these colonies were tested for col, almost all were found to be col$^-$. The lac$^-$ segregants, on the other hand, were col$^+$. The lac$^+$ colonies which did not yield lac$^-$ segregants were invariably col$^-$ (see Table 3). Thus, the instability in the inheritance of F*lac* appeared to be due to its inability to replicate along with the col factor leading to the segregation of one or the other plasmid – a phenomenon known as 'plasmid incompatibility'. The R-factor, on the other hand, was stably inherited by both the lac$^+$ col$^-$ segregants and by the lac$^-$ col$^+$ segregants.

Five colonies did yield lac$^+$ col$^+$ derivatives, but on further testing those of two colonies were found to be unstable. The derivatives of the remaining three were stable, but did not transfer lac$^+$ to recipient bacteria. The *lac*$^+$ gene in these strains was shown to be integrated in the chromosome. In transductions with phage P1, *lac*$^+$ was cotransduced with *proC*$^+$ at frequencies of 14–20 %. Thus, no strains were found which carried F*lac* with ColV-CA7. ColV-CA7 was also incompatible with F when this was integrated in the chromosome of an Hfr strain, CA-77HN/V. After mating in F$^-$ phenocopy with 712/V (538-1*drd*) (ColV-CA7), no *col*$^+$ Hfr colonies could be detected among many thousands that had received the R factor.

Table 3. *Segregation patterns of lac$^+$ progeny from mating an F*lac *donor with 712/V (538-1*drd) (*ColV-CA7*)

Types of segregants*			No. of lac$^+$ progeny yielding these types
lac	col	R	
+	−	+	31
−	+	+	
+	−	+	5
−	+	+	
+	+	+	
+	−	+	14

* Three to five lac$^+$ and one to two lac$^-$ segregants were analysed for col and for R.

The transmissible ColV factors (ColV-K30, ColV-K94) do not stably coexist with F (Nagel de Zwaig & Anton, 1964; Kahn & Helinski, 1964, 1965; MacFarren & Clowes, 1967), but entry exclusion of F by these ColV factors does not occur (MacFarren & Clowes, 1967). For this reason it was of interest to know whether exclusion observed in the R$^+$ ColV-CA7$^+$ recipients was due to col factor alone, or to a combined effect of the col factor and the R-factor. It was possible to test this in a K-12 strain by isolating from a mating between CA7 (538-1*drd*) and PA309*nal*/V on antibiotic-free plates some col$^+$ recipients which were not R$^+$. These did not carry any of the antibiotic resistances associated with the R-factor and were resistant to MS-2, showing that they did not carry the sex-factor portion of the R-factor.

The results of matings using three independent col$^+$ R$^-$ recipients are given in Table 2B. They show that phenocopy conditions are necessary for efficient F*lac* transfer to the col$^+$ recipient whether or not it is R$^+$. Thus, the col factor itself appears to be responsible for the exclusion of F*lac*.

(iii) *Isolation of transmissible ColV-CA7 plasmids*

When the mating mixture of strains CA7 (538-1*drd*) and PA309*nal*/V was plated on antibiotic-free medium and the col$^+$ progeny tested for the presence or absence of R, in addition to the R$^+$ and R$^-$ types, a third type was found. This was anti-biotic-sensitive but still lysed by MS-2, showing that the sex factor, or RTF, was carried without the antibiotic-resistance genes. Of 63 col$^+$ progeny recovered from one mating, 40 were col$^+$ R$^+$, 16 were col$^+$ R$^-$ and 7 were col$^+$MS-2-sensitive. The col$^+$MS-2-sensitive phenotype will be referred to as V$^+$.

Five V$^+$ isolates were used as donors, and unlike the original CA7 (538-1*drd*) donor each transferred col$^+$ at a frequency of 20–30 %. The transfer frequency is as high as the transfer frequency of CM-resistance by R538-1*drd* donors. The progeny of one V$^+$ donor were analysed. Eleven col$^+$ and 75 col$^-$ colonies were tested for sensitivity to MS-2. All of the col$^+$ but none of the col$^-$ were found to be sensitive to the phage, showing linkage of the col determinant with the RTF.

To test the possibility that the col determinant was joined to the RTF, several V$^+$ strains were tested as recipients of the complete R-factor, R538-1*drd*. If the superinfecting R-factor and the resident RTF were incompatible they should se-gregate from one another. If col$^+$ also segregated, this would suggest that it was attached to the RTF.

Three V$^+$ strains were tested. In the exponential phase of growth they were poor recipients of the R-factor. The frequency of transfer of the R-factor could be increased over 100-fold, to 2–4 %, with the V$^+$ strains in phenocopy. Entry exclu-sion of the R-factor did not occur with a col$^+$ MS-2-resistant recipient. The frequency of transfer of R was 30 % to an exponentially growing population.

To test for incompatibility between the plasmids, R$^+$ col$^+$ progeny from the three V$^+$ recipients were tested for stable inheritance of both R and col. Four CM-resistant col$^+$ colonies from each of the recipients were subcultured for 4–5 genera-tions in nutrient broth to allow segregation to occur. The broth cultures were streaked out on nutrient agar plates and five colonies from each culture were tested for col$^+$ and CM-r. The results, given in Table 4, showed that col$^+$ and CM-resistance usually segregated from one another. The few col$^+$CM-resistant progeny from these matings proved to be unstable on subculture, yielding col$^-$ or CM-sensitive segregants. On the other hand, segregation of col$^+$ and CM-resistance was only rarely observed with the col$^+$MS-2-resistant recipient (Table 4).

The transmissible col factor in the V$^+$ strains will be referred to as ColV-CA7T. Its relationship to F was studied. Four V$^+$ strains were tested as F*lac* recipients. Unlike recipients which carry ColV-CA7 alone or with the R-factor, those carrying ColV-CA7T did not exclude F*lac*. In the exponential phase of growth the frequency of transfer of F*lac* to V$^+$ recipients was 10–30 %, comparable

with that to an F⁻ recipient. However, when the mating mixture was plated on MacConkey lactose medium, almost all of the lac⁺ colonies with the V⁺ recipient were variegated, indicating incompatibility between F*lac* and ColV-CA7T. When four lac⁺ offspring from one mating were analysed, they yielded mainly lac⁺col⁻ and lac⁻col⁺ colonies. The minority of cell lines which were stably lac⁺col⁺ were shown to carry the *lac*⁺ gene integrated in the bacterial chromosome, co-transducible with *proC* by phage P1. Thus, the recombinant plasmid ColV-CA7T carries the F-incompatibility function of ColV-CA7 in addition to the col determinant. The properties of the recombinant plasmid are summarized in Table 5.

Table 4. *Analysis of R⁺ progeny of col⁺ recipients**

Recipient type

	col⁺MS-2 sens.				col⁺MS-2 sens.				col⁺MS-2 sens.				col⁺MS-2 resis.			
R	+	+	−	−	+	+	−	−	+	+	−	−	+	+	−	−
col	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
	0	0	5	0	0	4	1	0	0	4	1	0	5	0	0	0
	0	0	5	0	0	4	1	0	0	3	2	0	5	0	0	0
	0	3	2	0	4	0	1	0	0	5	0	0	4	1	0	0
	1	1	3	0	1	0	4	0	0	5	0	0	5	0	0	0

* Four R⁺col⁺ progeny of each recipient were subcultured in broth, then streaked on nutrient agar plates. Five colonies from each were then scored for R and for col. (See text.)

Table 5. *Properties of antibiotic-sensitive progeny of CA7(538-1drd) and PA309nal/V**

Type	Lysis by MS-2	Transmissible	Exclusion of F*lac*	Incompatibility with F*lac*	Exclusion of R538-1*drd*	Incompatibility with R538-1*drd*
ColV-CA7	No (16)	No (16)	Yes (3)	Yes (3)	No (3)	No (3)
ColV-CA7T	Yes (7)	Yes (5)	No (4)	Yes (4)	Yes (4)	Yes (4)

* The number given in parentheses is the total number of independent isolates tested for each property.

The observation that neither R538-1*drd* nor F*lac* replicate with ColV-CA7T is difficult to reconcile with the 'maintenance site' hypothesis of plasmid incompatibility. In terms of this hypothesis, incompatible plasmids are those which would occupy the same cellular attachment site for replication (see Novick, 1969). ColV-CA7T, in this case, would occupy both the site for F-replication and the site for R-replication. However, if ColV-CA7T could occupy the F-site and the R-site, it might be expected to be compatible with both F and R, replicating in the F-site when R superinfected and vice versa.

Incompatibility is not always explained by the maintenance-site hypothesis. In Hfr-F⁺ incompatibility the F-factor integrated in the chromosome of the Hfr cell presumably does not require a maintenance site for replication, yet it prevents superinfection by F. Possibly other mechanisms are involved in plasmid incompat-

ibility, such as plasmid-specific repressors of replication (Dubnau & Maas, 1968), which might account for the behaviour of ColV-CA7T. The recombinant plasmid, in this theory, would have retained sensitivity to both the R and the F (or ColV) control mechanisms.

Recombination between the col factor and the R-factor appears to occur frequently. When colonies of $R^+ col^+$ strains are screened, between 0·5 and 5·0 % are V^+ as judged by the loss of antibiotic sensitivity, while still being col^+ and MS-2 sensitive. When equivalent numbers of colonies of $R^+ col^-$ strains were screened for antibiotic sensitivity (600–700) none were found to be sensitive. This indicated that the R-factor itself is quite stable and suggested that the loss of resistance genes in the $R^+ col^+$ strain was due to recombination between the plasmids.

In summary, the results presented here demonstrate specific interactions between ColV-CA7 and R538-1*drd*, as shown by cotransfer and recombination. The two phenomena may be related and may depend on the ability of the two plasmids to become associated in the host cell. Possibly there are regions of homology in the structure of the two plasmids which permit association. However, the ability to recombine stably is probably not a requirement for cotransfer since ColEl was not reported to recombine with the transmissible plasmids which cotransfer it (Smith, Ozeki & Stocker, 1963; Kahn & Helinski, 1964; Nagel de Zwaig & Puig, 1964; MacFarren & Clowes, 1967). Also the non-transmissible antibiotic resistance determinant K does not recombine with F, which cotransfers it (Anderson, Mayhew & Grindley 1969). It is likely that the interactions between the transmissible and non-transmissible plasmids vary with the different pairs of plasmids observed.

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