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Isolation of High-Frequency Recombining Strains from *Escherichia coli* Containing the V Colicinogenic Factor

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The isolation and characterization of high-frequency recombining strains from different *Escherichia coli* host cells containing either the F factor or the Col V factor are described. The strains (with one exception) formed from three of the V^+ parents showed the same origin and polarity of transfer (*xyl-arg-pro-trp-his-mal*). The Hfr strains formed from the one remaining V^+ and the F^+ host cells showed a greater variety in their points of origin. In addition, several Hfr strains isolated from V^+ parents lost the ability to produce colicin V. F_v^+ segregants of these were isolated, and the F_v factors appeared to retain their preferential site for Hfr formation, but they lacked other propertes controlled by the Col V factor. Chromosomal integration of episomes and its relation to the fertility of F^+ and V^+ strains are discussed. Production of colicin V appeared to be uninfluenced by the state of the Col V factor within the cell.

Bacteria have been shown to contain extrachromosomal genetic elements called episomes or plasmids. Jacob and Wollman (14) listed characteristics such an element must possess to be an episome; among these was the ability to be integrated into the bacterial chromosome. The admission of colicinogenic factors to the episome group has been the subject of considerable discussion (7, 10, 20). However, as noted in a recent review (21), this distinction may be academic, and the episomal state may just be one point on a continuum of possible extrachromosomal states.

The Col V-K94 factor (this factor will subsequently be referred to as Col V, and strains producing colicin V, as V^+) possesses some of the properties of the F fertility factor (15, 16, 20). Fredericq (9, 10) isolated an Hfr Col B strain which he postulated arose from the integration of colicinogenic factor (Col B, V-K260) into the bacterial chromosome. This strain no longer produces colicin V, and it injects its chromosome with mannitol as a leading marker and streptomycin as a terminal marker. Fredericq (9, 10) also isolated an episomal element carrying the chromosomal genes *trp*$^+$, *cysB*$^+$, T_1^s, in addition to the B^+ and V^+ genes. Hickson, Roth, and Helinski (12) have shown this to be a circular deoxyribonucleic acid (DNA) molecule.

The present paper describes the isolation of Hfr strains of bacteria from different V^+ and F^+ parental strains. [The nomenclature of Demerec et al. (6) has been used throughout this paper. However the term Hfr will be used to describe the phenotypic state of high-frequency recombination, and Hfr^-, the loss of this state. Hfr_v refers to Hfr strains isolated from a V^+ parent.] The origins of the Hfr clones formed will be shown to be influenced by two factors; the original phenotype (V^+ or F^+) and some undefined chromosomal property of the parental strain. Chromosomal integration and the effect of Hfr_v formation on immunity and the production of colicin will be discussed.

MATERIALS AND METHODS

Organisms. The bacterial strains used in this study are described in Tables 1 and 2. The male specific phage MS2 was obtained from Houston Wade.

Media and culture methods. The media and most of the general methods have been described previously (15, 16). Recombination experiments for F^+ or Col V strains used a 1:1 ratio of donors to recipients for 180 min (15); Hfr and Hfr_v crosses used a 1:10 ratio of males to females for 120 min (16). Other techniques are described below.

Mutagenic treatment. Prior to isolation of Hfr or Hfr_v strains, a culture of *Escherichia coli* was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG)

TABLE 1. *List of strains*

Strain	Characteristics	Source
C600	thr⁻ leu⁻ thi⁻ lac⁻, Vʳ, Elʳ Azʳ	Kahn and Helinski, 1964
C600 (Col V)	same as C600 except (Col V)	Kahn and Helinski, 1964
C600 F⁺	same as C600 except F⁺	Kahn and Helinski, 1964
C600 F_v^+	same as C600 except F_v^+	This paper
C600 (Col V-PK2)	same as C600 except contains Col V from PK2	This paper
YS40	*his⁻ pro⁻ strʳ*	D. Helinski
A327	(F-lac)	R. Herman
HfrH (Col V)h[a]	*thi⁻* Elʳ Vʳ *Azʳ*	Kahn and Helinski, 1965
PA309[b]	*thr⁻ leu⁻ thi⁻ trp⁻ his⁻ arg⁻ lac⁻ gal⁻ mtl⁻ strʳ* Tlʳ T5ʳ Vʳ Elʳ	R. Krisch
AB2495	*thr⁻ leu⁻ thi⁻ trp⁻ pro⁻ his⁻ arg⁻ thy⁻ lac⁻ gal⁻ ara⁻ xyl⁻ mtl⁻ strʳ* T6ʳ Vʳ	R. P. Boyce
AB1157	*thr⁻ leu⁻ thi⁻ pro⁻ his⁻ arg⁻ lac⁻ gal⁻ ara⁻ xyl⁻ mtl⁻ strʳ* T6ʳ Elʳ Vʳ	R. P. Boyce
AB2463	same as 1157 but *rec⁻*	R. P. Boyce
AB1450	*thi⁻ ilv⁻ arg⁻ met⁻ his⁻ lac⁻ gal⁻ xyl⁻ mal⁻ strʳ* T6ʳ λʳ Vʳ	R. Herman
AB444	*thi⁻ purF aroC argA mal⁻ xyl⁻ mtl⁻ lac⁻ strʳ* T6ʳ Vʳ	A. L. Taylor
AB1359	*thi⁻ proA argA his-4 aroD xyl⁻ mtl⁻ strʳ* T6ʳ Vʳ	A. L. Taylor
W1485 F⁺		D. Helinski
W1485 (Col V)	Vˢ or Vʳ	This paper
χ402 F⁺	*met⁻*	L. Caro
χ402 (Col V)	*met⁻* Vˢ or Vʳ	This paper
X7026	*thi⁻* (pro B lac)⁻ X111—deletion (*see* 1)	E. Signer
X7026 (Col V)	As X7026; also Vˢ or Vʳ	This paper
X7026 F⁺	Vʳ, as X7026	This paper
X7026 (F-lac)	Vʳ, as X7026	This paper
PK2464	same as AB2463 but F lac⁺	F-lac infection from A327
PK201	same as C600 (Col V)	V⁺ segregant of PK2
PK301	same as C600 (F_v)	F_v^+ segregant of PK3
PK202	same as C600	This paper
PK203	same as C600	This paper
PK204	same as C600	This paper
PK205	same as C600	This paper
PK211	same as C600	Acridine orange curing of PK201 This paper
PK211 (Col V)	same as C600	Infection of PK211 with Col V from χ 402 (Col V) This paper
PK1201	same as χ402	V⁻, Hfr_v^+ segregant of PK12
PK801	same as C600	This paper
PK802	same as C600	This paper
PK401, PK1301	same as PK4, PK13, but *spcʳ* (resistant to 300 μg/ml of spectinomycin sulfate)	NTG treatment of PK4 and PK13

[a] HfrH V⁺ F_v^+(h) in reference 16.

[b] Unless otherwise specified, strains used in crosses with colinogenic donors were first made resistant to the colicins.

using the following procedure (E. Signer, *personal communication*). A fresh overnight culture was diluted 1:10 and grown (aerated, 37 C) for 4 hr. Samples (10 ml) were centrifuged, washed once, and suspended in 0.5 ml of 0.1 M acetate buffer, *p*H 5.0. Then 0.1 ml of NTG (4 mg/ml) freshly prepared in the same buffer was added. The culture was incubated for 2 hr standing at 37 C. An 8-ml amount of broth was added, and the culture was centrifuged and resuspended in 10 ml of broth. Portions (0.5 ml) of this culture were then added to several 10-ml tubes of broth. These were grown at 30 or 37 C overnight and then were used to isolate Hfr or Hfr_v strains. Hfr strains arising in separate tubes or with distinct polarities (*see* Results) were considered to be of independent origin.

TABLE 2. *List of Hfr and Hfr$_v$[a] strains isolated*

Strain	Parent	Origin[b]	Direction[c]	*V*
PK1	C600 (Col V)	*xyl*	C	+
PK2	C600 (Col V)	*aroC*	CC	+
PK3	C600 (Col V)	*xyl*	C	−
PK4	C600 (Col V)	*xyl*	C	+
PK5	C600 (Col V)	*xyl*	C	−
PK6	C600 (Col V)	*xyl*	C	+
PK8	C600 (Col V)	*xyl*	C	+
PK10	χ402 (Col V)	*xyl*	C	+
PK11	χ402 (Col V)	*xyl*	C	+
PK12	χ402 (Col V)	*xyl*	C	+
PK13	χ402 (Col V)	*xyl*	C	+
PK14	W1485 (Col V)	*xyl*	C	+
PK15	W1485 (Col V)	*xyl*	C	+
PK16	X7026 (Col V)	*aroC*	CC	+
PK17	X7026 (Col V)	*xyl*	C	+
PK18	X7026 (Col V)	*ser*	CC	+
PK19	X7026V^r (Col V)	*his*	C	+
PK20	X7026V^r (Col V)	*aroC* (?)	C	+
PK21	C600 F$^+$	*ara*	C	−
PK22	C600 F$^+$	*trp*	CC	−
PK23	C600 F$^+$	*ara*	CC	−
PK24	C600 F$^+$	*arg*	CC	−
PK25	C600 F$^+$	*thy*	CC	−
PK26	C600 F$^+$	*xyl*	C	−
PK27	W1485 F$^+$	*his*	CC	−
PK28	W1485 F$^+$	*his*	CC	−
PK29	W1485 F$^+$	*ara*	C	−
PK30	χ402 F$^+$	*his*	CC	−
PK31	χ402 F$^+$	*pro*	CC	−
PK35	χ402 F$^+$	*his*	CC	−
PK32	C600 F$_v^+$	*xyl*	C	−
PK33	C600 F$_v^+$	*xyl*	C	−
PK34	C600 F$_v^+$	*pro*	CC	−

[a] Hfr strains formed from a V$^+$ parent.

[b] Proximal marker injected of those tested.

[c] C, clockwise; CC, counterclockwise; as in Matney et al. (18) and Fig. 1 and 2 of this paper.

Isolation of Hfr or Hfr$_v$ strains. The method of indirect selection by replica plating was used (14). Donors were always counterselected with streptomycin, and different auxotropic characters of the recipients AB1157, PA309, or AB2495 were used. The position of the marker in relation to the origin of the strain selected did not seem to be of much importance. For example many of the Hfr$_v$ strains (Fig. 1) with an origin of injection near *xyl* were isolated as donors of *trp*, *gal*, or *his*—markers a good distance away on the genetic map (22). All Hfr and Hfr$_v$ strains were characterized by interrupted-mating experiments (14).

Assay of colicin production. Fresh overnight cultures of the strains to be tested were diluted 1:10 into 20 ml of nutrient broth (15; ENB) in a 250-ml flask and grown at 37 C in a rotary-shaker water bath. After 1 hr, mitomycin C was added to a final concentration of 1 μg/ml. The cultures were grown for an additional 4 hr and then centrifuged; the pellet was suspended in 0.1 volume of distilled water. Several drops of $CHCl_3$ were added, and the resuspended pellet was agitated and then serially diluted to test for colicin activity. The number of colicin units per milliliter was defined as the highest dilution giving a clear zone of inhibition of growth of the indicator bacteria (11). The supernatant fluid was also tested.

Immunity. To test strains for immunity to the killing activity of colicin V, a V$^+$ strain was streaked on an ENB plate and, after several days of incubation, the strains to be tested were streaked on the same plate, perpendicular to the original streak. A V-resistant strain, a V$^+$ strain, and a V-sensitive strain were added to each plate as controls. The plates were examined after 4 hr and again after overnight incubation. A strain resistant to colicin V showed no zone of inhibition at either time; a strain sensitive to colicin V showed the same inhibition zone at both times, and a strain giving an immune response showed a clear inhibition zone at 4 hr and either no zone or a very reduced one after overnight incubation.

Test of recombinants for unselected markers. Colonies recombinant for a particular marker were inoculated into NaCl solution and then streaked, several to a plate, on the same selective plate. After 1 or 2 days of growth they were tested for other genetic markers (usually production of colicin V) by replica-plating. When they were also to be tested for MS2 sensitivity, single colonies were picked from the streak, inoculated into 1 ml of ENB, and grown overnight prior to testing.

RESULTS

Isolation of Hfr strains from V$^+$ and F$^+$ donors. Table 2 lists the origins, directions of injection, and parental derivation of the Hfr strains isolated. Their position on the bacterial chromosome in relation to known markers (22) is shown in Fig. 1 for those isolated from V$^+$ strains and in Fig. 2 for those isolated from F$^+$ strains. Strains were considered to be of independent origin if they grew in different tubes after NTG treatment or if they showed different sites of transfer initiation. For example, each of the groups PK1 and PK2; PK17 and PK18; and PK23, PK24, PK25, and PK26 were isolated from one tube. However, PK8 was one of three clones with identical origin and polarity isolated from an individual tube. Since the independent origin of these three was in doubt, two were not tabulated here (PK801 and PK802 of Table 1 and Fig. 3). This procedure clearly tends to emphasize differences and minimize similarities.

Although no quantitative work on this point was done, it appeared to be much more difficult to isolate Hfr strains from untreated cells, and none were obtained without preliminary NTG treatment. PK6 was isolated by the fluctation technique used by Broda (4), and the others were isolated by replica-plating.

It should be noted that strains PK17, PK18, PK19, and PK20 were isolated from the same

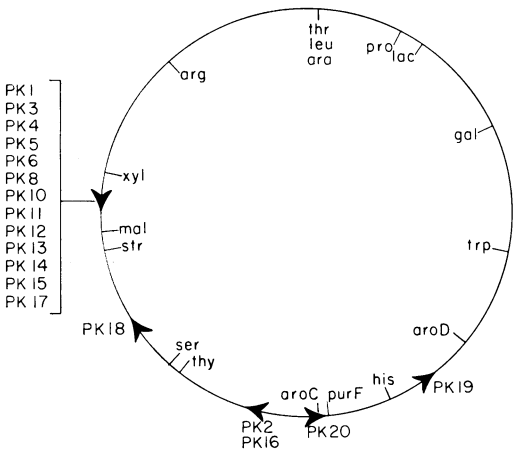

FIG. 1. *Linkage map showing the point of origin of chromosome transfer for the Hfr$_v$ strains isolated from several E. coli K-12 (Col V) strains.*

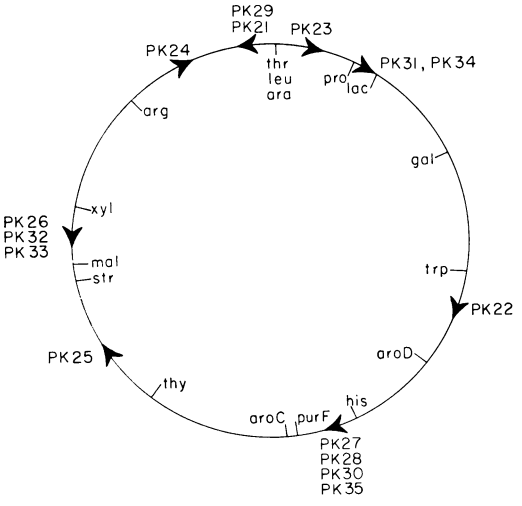

FIG. 2. *Linkage map showing the point of origin of chromosome transfer for the Hfr strains isolated from several F$^+$ E. coli K-12 strains.*

parental strains, X7026 (Col V) or X7026V^r (Col V). PK32, PK33, and PK34 were derived from C600 infected with the F$_v$ factor from PK301.

Properties of Hfr strains isolated. From Table 2, it can be seen that some of the Hfr$_v$ strains isolated have lost the ability to produce colicin V. It was also found that the Hfr$_v$ strains exhibited varying degrees of stability, with some tending to segregate into V$^+$ Hfr$^-$, V$^-$ Hfr, and V$^+$ Hfr lines. Unstable strains often did not show reduced recombination frequency, but they always showed a great increase in the ability to transfer the Col V factor. Figure 3 illustrates these properties for PK8 and PK801 and PK802 isolated at the same time. Although originally these three were considered to be separate strains because of their different properties, Fig. 3 illustrates how PK8 and PK802 could have arisen from PK801 in the course of growth of the NTG-treated culture.

In most of the crosses with Hfr or Hfr$_v$ donors, recombination frequencies after 120 min, for the proximal markers, were close to 100%, computed from the initial donor cell titer. Transfer of Col V from the stable Hfr$_v$ strains was at least a factor of 10 below this. For HfrH (Col V)h, the rate of transfer of Col V was more than a factor of 10 higher than the number of recombinants formed for the most proximal markers. Transfer of Col V from this strain began immediately and leveled off approximately 20 min after the start of the cross.

Mapping of the fertility factor or the Col V factor as an unselected marker. Table 3 shows transfer of colicinogeny among recombinants for several markers from crosses utilizing different Hfr strains as donors.

Separation of fertility and colicinogenic activity and the chromosomal integration of the genes for colicin V production. The derivation of strains in which the genes controlling the production of colicin V appear to be separated from those determining the fertility properties of the cell (Hfr$_v$ or F$_v^+$) is shown in Fig. 4. After transferring an F-lac factor to PK2 (step I), two types of new clones are isolated. PK202 produces colicin V, does not ferment lactose, is resistant to phage MS2, and is unable to recombine or to transfer the Col V factor. PK203 produces colicin V, ferments lactose, is sensitive to MS2, and transfers the lac property at a high level and the ability to produce colicin V at a very low level. As shown in Fig. 4, it is concluded that PK202 has no cytoplasmic fertility factor and PK203 has a cytoplasmic F-lac factor. This is supported by recombination data. After infection with an F factor (step II yielding PK205), it regains MS2 sensitivity and the ability to recombine at the level of an F$^+$ cell, but it transfers the Col V factor at a very low level (i.e., at the level of transfer of chromosomal markers). It appeared that, in the competition between the infecting F-lac factor and the V colicinogenic factor (15, 16), strains losing their Col V-associated fertility properties but retaining the ability to produce colicin V could arise. The location of the genetic determinant of colicin V production appears to be chromosomal in these strains, as will be seen below. The Col V genes of these strains, PK202 and PK203, still retain some ability to compete with an infecting F factor, as step II in both cases produces V$^+$F$^-$ and F$^+$V$^-$ clones.

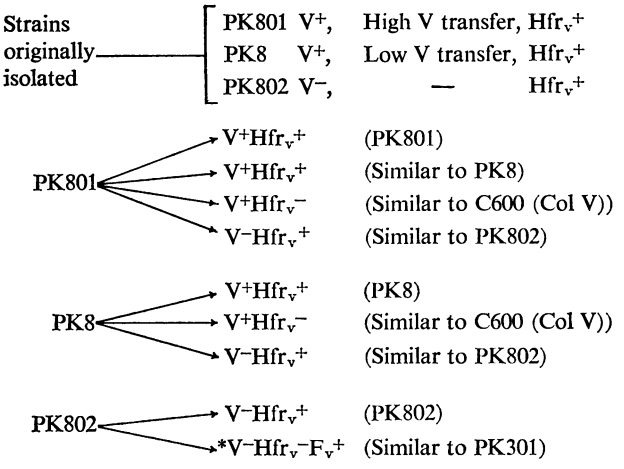

FIG. 3. *Derivation of V+ and V− Hfr$_v$ strains. Step indicated with asterisk was not demonstrated with this strain.*

TABLE 3. *Transfer of colicinogeny to recombinants selected for listed markers: Hfr and Hfr$_v$ donors*

Hfr	Recombinant[a] for	No. of recombinants		
		Colicinogenic	Hfr[b]	HfrV− or V+Hfr−
PK19	*purF, thy, arg*			—
	trp, his	0 (150)[c]	—	—
	aroD	2 (70)	2 (2)[c]	0
PK13 or PK1301	*xyl, arg*			
	pro, trp	0 (94)	—	—
	his	1 (30)	0 (16)	1 V+Hfr−
	thy	1 (53)	1 (16)	0
	spc[d]	14 (60)	7 (14)	1 V+Hfr−
PK4 or PK401	*arg, pro*			
	trp, his	0 (120)	—	—
	thy	1 (30)	0 (25)	1 V+Hfr−
	spc	20 (60)	11 (28)	6 V+Hfr−
PK20	*purF, aroC*			
	thy	0 (120)	—	—
	trp	1 (30)	1 (1)	0
	his	3 (60)	3 (3)	0
HfrH (Col V)h	*thr leu*	31 (40)	—	—
	pro	30 (40)	—	—
	thy	21 (27)	—	—
	arg	22 (28)	—	—

[a] Different recipients with the appropriate auxotrophic characteristics (Table 1) were used. The results have been pooled to simplify tabulation. Recombinants were picked after 120 min of mating. To detect spectinomycin-resistant (*spc*) recombinants, the cross was agitated at 120 min to separate mating pairs, diluted 1:10, and grown at 37 C for 3 hr to allow for segregation and detection of spc^r colonies.

[b] Usually all V+ recombinants were tested for MS2 sensitivity and the ability to promote recombination. An additional set of noncolicinogenic recombinants was also usually tested. In all cases, only V+ recombinants exhibited sensitivity to phage MS2.

[c] Numbers in parentheses show the number tested.

[d] The *spc* locus is at about 62.5 min (*thr* at 0 min), just below *str* at 64 min (22).

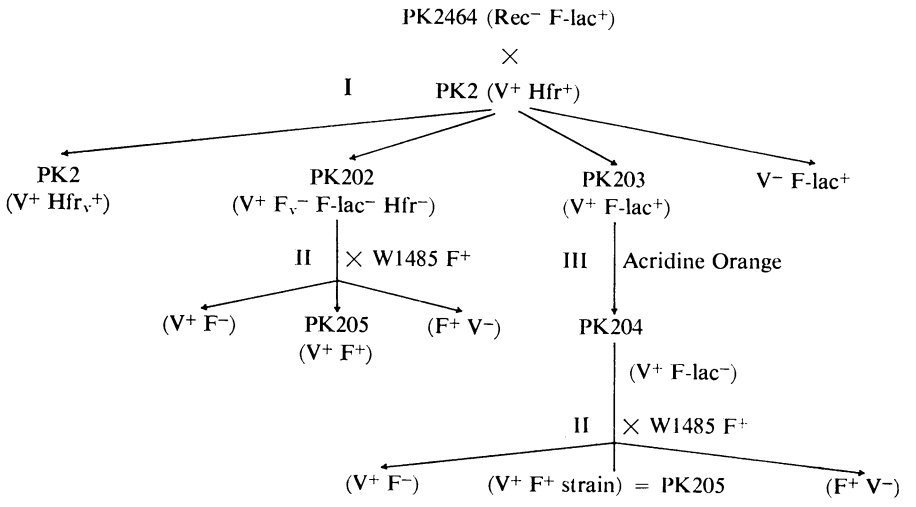

FIG. 4. *Derivation of strains from F-lac infection of PK2.*

PK205 was then used as the donor in crosses with various recipients, and recombinants for different markers were picked and tested for the ability to produce colicin V in order to see whether the V⁺ genes appeared linked to any chromosomal genes (Table 4).

Production of colicin V corresponding to different states of the Col V factor. Production of colicin from C600 V⁺, PK2, PK201, PK202, and PK205 was tested several times. As shown in Table 1 and Fig. 4, these strains represent all presently known possible states of the Col V factor. There were no consistent differences in the production of colicin V from these strains. All strains showed the same enhancement of activity with the addition of mitomycin C (approximately a factor of two), and the distribution of colicin activity between supernatant fluid and pellet was also the same (all activity resided in the pellet).

Fertility of F⁺, V⁺, and F$_v$⁺ strains. Kahn and Helinski (15) found a quantitative difference for the production of recombinants with V⁺ or F⁺ donors. This result was reinvestigated to see whether a relationship existed for each episome between the number of recombinants for certain markers and the direction of the majority of Hfr strains produced (Table 5A). In all cases, the data for V⁺ strains were compared to the same strain with an F factor to eliminate interstrain differences (3). Table 5B shows recombination data for C600 or strains derived from C600 containing fertility factors with different histories. C600 F$_v$⁺ contains the fertility factor from PK301, an Hfr⁻ revertant of PK3. PK201 is an Hfr⁻, V⁺ revertant of PK2. PK211 was obtained by curing the Col V factor from PK201 with acridine orange and reinfection with Col V from χ402 (Col V) to obtain PK211 (Col V). C600 (Col V-PK2) was obtained by infection of C600 with the Col V factor of PK201.

TABLE 4. *Transfer of colicinogeny to recombinants selected for listed markers: PK205 donor*[a]

Recipient	Recombinant for	No. of recombinants		
		Total tested	Colicinogenic	
			No.	Per cent
AB2495	*arg*	29	0	0
	pro	30	0	0
	trp	69	1	1.5
	his	71	5	7
	thy	70	18	26
AB444	*arg*	60	0	0
	aroC	90	27	30
	purF	85	6	7
AB2531	*ser*	60	9	15
	his	59	1	1.7
	purF	30	4	13

[a] Recombinants were also tested for MS2 sensitivity, and this property did not appear linked to the ability to produce colicin V. In all cases (with one exception), the ability of the recombinant to produce colicin V was not transferred at high frequency (i.e., it appeared to have a chromosomal location). With C600 (Col V) or PK201 as donor, transfer of Col V linked to MS2 sensitivity under identical conditions was 100% to all recombinants tested (*arg*, *pro*, *his*).

Immunity of the V colicinogenic strains. To test the linkage of immunity or partial resistance to the same colicin produced by a cell (8, 20) and

TABLE 5. *Recombination of F$^+$, V$^+$ and F$_V$$^+$ strains*

Part	Donor	Episome	*arg*		*pro*[b]		*his*	
			No.[a]	Per cent F$^+$	No.	Per cent F$^+$	No.	Per cent F$^+$
A	C600	(Col V)	134	44	91	27.5	15.1	5.1
		F$^+$	304	100	330	100	297	100
	W1485	(Col V)	175	64	74	16.5	5.3	1.9
		F$^+$	275	100	449	100	284	100
	χ402	(Col V)	263	282	263	100	63	21
		F$^+$	93	100	261	100	298	100
	X7026	(Col V)	96	83	49	71	4.7	5.0
		F$^+$	115	100	69	100	94	100
		F-lac$^+$	138	120	203	335	92	98
B	C600	F$^+$	110	100	68	100	130	100
	C600	(Col V)	101	92	39	57	5.9	4.5
	C600	(F$_V$$^+$)	155	150	60	88	5.6	4.3
	C600	(Col V-PK2)	150	136	32.5	48	3.0	2.3
	PK201	(Col V-PK2)	131	120	52	76	49	38
	PK211	(Col V)	109	99	41	60	49	38

[a] A number of 100 would be equivalent to a recombination rate of approximately 10^{-4} per initial donor cell.

[b] *thr leu* for X7026 strains.

colicin production, Hfr$_V$ strains PK10 to PK18 inclusive were made from parental strains which were initially sensitive to colicin V. However, strains receiving colicin V do not exhibit very good immunity (8) but remain partially sensitive to the colicin they produce. This is particularly apparent when the cells are grown on an ENB plate where production of colicin V is very efficient. Thus, in the course of isolation of the Hfr$_V$ strains, many became resistant. PK10, PK12, PK14, and PK15, did show an immune reaction rather than a resistant reaction (*see* Materials and Methods). PK1201, a V$^-$Hfr$_V$ derivative of PK12, was completely sensitive. When the F$_V$ factors from several isolates of PK3 which had lost their Hfr fertility (i.e., similar to PK301) were transfered to a sensitive strain, none of the recipients showed an immune reaction. It should also be noted that, whereas three of the original Hfr$_V$ strains isolated from a V-resistant parental strain were V$^-$, no V$^-$Hfr$_V$ were directly isolated from a V-sensitive parent. However, after subculture with spreading cells on minimal plates to lessen production of colicin V, some V$^-$Hfr segregants, like PK1201, were isolated. Thus, although the data are somewhat limited, it is concluded that immunity to action of colicin V and production of colicin V are not usually separated with the methods presented here.

Irreversibility of the loss of colicin V activity. In all cases, the loss of the ability to produce colicin V appeared irreversible. Although the transition Hfr$_V$V$^+$→Hfr$_V$V$^-$ occurred for many of the Hfr$_V$ strains, the reverse, Hfr$_V$V$^-$→Hfr$_V$V$^+$, did not. Strains PK3, PK5, and PK802 were treated with NTG and then plated to see whether V$^+$ revertants were present. Approximately 15,000 colonies of each were examined and no V$^+$ clones were found. PK1201, an Hfr$_V$V$^-$ strain from a strain initially sensitive to colicin V, could be more thoroughly tested on this point. A test culture of PK1201 containing C600 (Col V) at a ratio of 1 V$^+$ cell to 10^8 cells of PK1201 was grown over a period of several days. After many generations of growth, diluting fully grown cultures into fresh media and regrowing, colicin V-producing cells could be detected on plates. However PK1201, untreated or treated with NTG and grown in the same manner, never yielded any colicinogenic revertants (i.e., less than 10^{-8}).

DISCUSSION

The data presented here are in agreement with previous work showing that Hfr strains do not arise with their origins distributed randomly about the chromosome (4, 18). This is particularly apparent for the Hfr$_V$ strains, as shown in Fig. 1, where most of those isolated inject *xyl* as the initial marker and *mal* as the terminal marker. It should also be noted that Fredericq's Hfr ColB strain may have its origin in the same region. From Table 2 and Fig. 1 and 2, it can be seen

that there are at least two factors influencing the origin of an Hfr: (i) the type of episome, Col V or F, and (ii) the host cell chromosome. The Hfr_v strains PK1 to 15, inclusive, derived from three different parental strains, with one exception (PK2) have apparently identical origins and polarity. [C600 is of the *thr*$^-$ *leu*$^-$ *thi*$^-$ branch of *E. coli* K-12; χ402 is derived from 58-161; and W1485 is another one of Lederberg's K-12 strains (17).] PK16-20 are derived from X7026 V^r V^+ or X7026 V^+, and each one shows a different origin. For F^+ strains, although some clusters are seen, the range of origins isolated from the same parental strains is much wider. It should be noted that PK32 and PK33 are isolated from an F_v^+ strain, and their origin may be indicative of their history. Following Campbell's model (5) for the integration of an episome into the chromosome, it can be said that the Col V factor shows homology with the region of the bacterial chromosome between *xyl* and *mal*. For some reason, this homology is absent in X7026. The integration of the F_v factor (a Col V factor which only retains its fertlity properties and no longer allows the cell to produce colicin V or to be immune to the action of colicin V) also seems to show the same homology. It should be noted again that the selection process and criteria of independent isolation used tend to minimize similarities. For example, six additional Hfr_v and two Hfr (F_v) strains with the same origin (*xyl*) were discarded because their independent derivation was in doubt.

It has been proposed (14) that the fertility of F^+ strains is due to small numbers of Hfr clones formed in the culture. If this is so, recombination from a V^+ strain should reflect any bias in Hfr formation. As shown in Table 5A, this appears to be so, with the exception of X7026 V^+. Parenthetically, it should be noted that Beckwith et al. (2) found certain favored sites for the integration of a temperature-sensitive F-lac factor in X7026. Of the Hfr strains isolated, 20% had an origin and direction of injection *pyr C-trp-his-str*-etc. No Hfr strains with this origin were found in this work, and this bias is also not reflected in the recombination data for X7026 V^r (F-lac). Table 5B shows additional data pertinent to the question of the dual role of chromosome and episome in determining fertility and the origin of Hfr strains. Among the Hfr_v strains formed from C600 (Col V), χ402 (Col V), and W1485 (Col V), there is only one with a unique origin and direction of transfer, PK2. With the isolation of a V^+ segregant from this strain, PK201, additional strains with either the chromosome or the episome from PK201 could be obtained and their fertility examined. The data in Table 5B demonstrate that the chromosome of PK201 determines the type of fertility; PK201 and PK211 (with the chromosome of PK201 and a normal V factor) show atypical Col V-directed fertility, whereas C600 (Col V-PK2) with the Col V factor from PK2 (actually PK201) shows the usual Col V-directed recombination frequencies (high for *arg* and considerably lower for *his*). Two Hfr_v strains were also isolated from NTG-treated PK201 and had the apparent origin of PK2. It is not concluded that Hfr_v clones are completely responsible for the recombinants produced from a V^+ donor, but that the two processes show a similar bias.

The isolation of V^- Hfr_v clones is of considerable interest. A possible explanation is insertion of the fertility factor into the chromosome by a crossover occurring within the episomal gene for the production of colicin V. An irreversible loss of activity of a chromosomal gene has been observed accompanying the chromosomal integration of an F-lac factor (2, 20). Similarly, the transition $V^- \rightarrow V^+$ was not observed, even though several V^- Hfr_v clones were treated with NTG in attempts to induce the reversion. In addition to the V^- strains listed in Table 2, Hfr V^- clones were also isolated from PK6, PK8, PK11, PK12, and PK15. When several Hfr_v^- clones were isolated from PK3 (e.g., PK301) and tested for ability to promote recombination for different markers (Table 5B), the result was similar to that expected for recombination from a V^+, rather than an F^+, strain. This bias was also reflected in the Hfr strains formed from C600 F_v^+: PK32, PK33, and PK34. Two of the three have the usual origin found for an Hfr_v strain.

The state of the Col V factor in all the Hfr_v strains is not yet completely understood. Transfer of the Col V factor to recipients is often only a factor of 10 below the recombination frequencies for the earliest markers, which is higher than that found for distal markers. This is transfer of a cytoplasmic Col V factor, unlinked to chromosomal genes. Picking recombinants for different markers in the Hfr ColB strain, Fredericq (9, 10) found a gradient of linked transmission of colicinogeny and the Hfr property ranging from 0 for *met* to 0.1 for *his* and 0.7 for *str*. Table 3 shows that transfer of the Col V factor from the Hfr_v strains to recombinants is considerably lower and is only observed with distal markers. An association of colicinogeny and the Hfr property with the most distal marker transferred was observed. The V^+ recombinant strains tested usually exhibited the same origin and polarity of recombination as their parental strain. The one exception appears to be PK20, shown in Fig. 1 injecting *purF* as its last marker. However, linkage of colicinogeny and Hfr recombining ability was

found only with *his* and *trp* recombinants. In addition, these recombinants transferred *purF* as a proximal marker. It is possible that PK20 actually injects *purF* or some part of it as its first marker, although the aro C recombinants appear to occur with a frequency almost 10^3 times greater. In general, the Col V factor was transferred at very low frequency from the V$^+$ Hfr recombinants and at a high frequency from the V$^+$ Hfr$^-$ recombinants. The latter probably arise from the low transfer of a Col V factor independently of the chromosome discussed previously. Data on the transfer of the Col V factor from Hfr H(Col V)h (16) are also given in Table 3. Here, transfer of the Col V factor to recombinants for different markers is high (approximately 75%) and also random. In this strain, fertility is determined by an integrated F factor, and the Col V factor has an independent (cytoplasmic?) position in the cell. From the data presented in this table, it is clear that an analogous system does not exist in the Hfr$_v$ strains.

The results presented here demonstrate some integration of the V colicinogenic factor along with its associated fertility properties into the bacterial chromosome. It is not clear that this is a stable integration occurring in all Hfr$_v$ strains according to the Campbell model (5). The isolation of V$^-$ clones, retaining Hfr$_v$ fertility from many of the Hfr$_v$ strains is not easily explained by this model. It is possible that at least some of the Hfr$_v$ strains represent an intermediate state of association between episome and chromsome. However, their fertility is as high as that of a standard Hfr, so they are not to be classified as intermediate donors, such as the strains containing F-lac factors.

For one strain, PK202 and its F$^+$ derivative PK205, chromosomal integration of part of the Col V factor can be shown (Table 4 and Results). In this case, the cytoplasmic F or F-lac factor (in PK203) are easily removed after growth in acridine orange (13, 15), whereas the ability of the cell to produce colicin V is unaffected. Furthermore, the V$^+$ property cannot be transferred unless a fertility factor is present, and the transfer then observed is at the same level as the recombination of chromosomal genes. From Table 4 and Fig. 1, it is apparent that the genes controlling colicin V production have a chromosomal location between *thy* and *aroC*, the postulated origin for the parental Hfr$_v$ strain PK2. This reinforces the hypothesis that the Hfr$_v$ strains represent at least a state intermediate to chromosomal integration.

It was also observed that the production of colicin V was not affected by the state of the colicinogenic factor. This is analogous to observations on the production of β-galactosidase (2), which was invariant upon transposition of the F-lac factor to a variety of chromosomal locations.

Although two major properties of the Col V factor, its fertility properties and its ability to produce colicin V, have been separated as reported in this paper and elsewhere (16, 19), the state of the genes governing the immunity property is not clear. From the work reported here, it can be stated that separation of immunity to the action of colicin V and the ability to produce colicin V has not been demonstrated. However, the number of cases examined to date is small, and this investigation will be continued.

A major point of this paper is the dual effect of the episome and the chromosome (in X7026 and PK2) in determining the origin of an Hfr strain. Whether or not the formation of Hfr$_v$ strains proceeds according to the model of Campbell (5), their origins are probably determined by regions of genetic homology between chromosome and episome. It will be of interest to investigate the site of Hfr origins formed by other colicinogenic factors with fertility properties.

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