INTERACTIONS BETWEEN COLICINOGENIC FACTORS AND FERTILITY FACTORS[1]

R. Nagel de Zwaig and D. N. Antón

Laboratorio de Genética I. Facultad de Ciencias Exactas y Naturales. Buenos Aires. Argentina

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Colicinogenic factors E_2, E_1, I, V and B are known to be transferred in conjugation among strains of Escherichia coli K12 as extrachromosomal particles (Nagel de Zwaig, Antón and Puig, 1962; Clowes, 1963; Nagel de Zwaig, 1963; Puig, 1963).

The present report is based on further observations on the behaviour of E. coli K12 strains colicinogenic for colicin V which suggest the existence of a relationship between colicinogenic factor V, colicinogenic factor I and the F factor.

EXPERIMENTAL

Transfer of colicinogenic factors V and I. Several strains of E. coli K12 originally classified as colicinogenic for colicin V (colV) (Nagel de Zwaig et al., 1962) were later found to synthesize, besides colicin V, another colicin. These observations applied also to the original strain E. coli 12-94, from P. Fredericq, from which the colV factor was transferred to these E. coli K12 strains.

By the employment of an indicator strain resistant to colicin V and sensitive to colicin I (V^rI^s) as well as by other criteria, such as size of the inhibition halo and sensitivity to temperature, this new colicin was classified as colicin I.

Study of the transfer of col+ factors between donor and recipient strains of E. coli K12 had indicated that while colV factor (from E. coli 12-94) was transferred with very high efficiency whatever the type of donor cell involved, colI factor (from E. coli CA 53) was al-

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ways transferred with very low frequency (Nagel de Zwaig et al., 1962).

Hence, crosses between strains F+ 112 colV+ colI+ str-s or HfrH colV+ colI+ str-s and the strain F- PA309 colV- colI- str-r were performed in order to determine the frequency of transfer of this colI factor derived from E. coli 12-94 and compare it with the frequency of transfer of colV factor (Table 1).

Table I

Transfer of colV and colI factors among E. coli K12 strains

Donors	Sexual type	Frequency of transmission of					
		colV	colI	thr+leu+	try+	his+	arg+
112	F+	-	-	$3x10^{-2}$	$1x10^{-2}$	-	$4x10^{-3}$
112 colV+colI+		1500	1600	$2x10^{-4}$	$3x10^{-4}$	-	0
C600	F-	-	-	-	0	0	0
C600 colV+colI+		1090	920	-	$8x10^{-4}$	$2x10^{-4}$	$3x10^{-4}$
HfrH	Hfr	-	-	38	6,3	3	0,1
HfrH colV+colI+		500	570	35	10	1,2	0,1

Strain PA309 colV- colI- V^rI^r thr- leu- his- try- arg- str-r was used as F- in crosses. Mating mixtures, in the proportion of 1 donor to 20 recipients, were sampled and plated on selective media at 90 min. after mixing the strains. An indicator strain V^rI^s was employed to determine the appearance of colI+ str-r cells. The frequency of chromosomal recombinants (thr+ leu+ str-r; try+ str-r, etc.) or transfer of col+ (col+ str-r) is expressed as the ratio of the number of recombinants or col+ str-r cells, respectively, to 100 initial bacteria of the donor parental strain.

Details of the conditions of mating, full reference of strains and media employed and detection of chromosomal recombinants and col+ str-r cells, are given in a previous paper (Nagel de Zwaig et al., 1962).

Symbols: thr, threonine; leu, leucine; his, histidine; arg, arginine; try, tryptophane; str, streptomycin; r, resistance; s, sensitivity.

As can be seen in Table 1, this colI is transferred from F+112 and HfrH strains with high efficiency and with frequencies similar for those measured for colV factor in the same crosses.

No segregation between colV and colI factors was observed among about 500 colicinogenic recombinants analyzed. This result suggests

that colV and colI factors are transferred in conjugation as closely linked markers.

These factors will be hereafter designated colV-colI.

Interference with chromosomal fertility. The presence of colV-colI factors in some donor strains of E. coli K12 strongly inhibits chromosomal fertility.

As shown in Table 1, strain F+ 112 (colV-colI)+ presents a marked decrease in chromosomal fertility as compared with the original F+ 112 strain, although it transfers colV-colI factors at high frequency.

Five different isolations of F+ 112 strain made (colV-colI)+ by mixed culture with Hfr P4x (colV-colI)+ were found to behave similarly.

An interference between colV-colI factors and the F factor became also apparent in strains Hfr P10 and Hfr AT IIIA. During isolation of clones (colV-colI)+ two types of bacteria were detected: 1) those retaining their Hfr property but having lost colV-colI factors. 2) those remaining (colV-colI)+ while presenting a drastic reduction in the transmission of chromosomal markers.

This type of interference was not manifested in strains HfrH (Table 1) and HfrP4x (Nagel de Zwaig et al., 1962).

Transfer among F-strains. It was observed that all F-cells acquiring colV-colI factors became sensitive to phage MS2, which is known to plate only on donor cells. In view of this fact, the transfer of colV-colI between F- strains was investigated.

Crosses between F- C600 thr- leu- thi- str-s (colV-colI)+, which received its colV-colI factors from an Hfr P4x (colV-colI)+ strain, and F- PA309 (colV-colI)- V^rI^r, were performed. It was determined in these crosses that colV-colI could be transferred from an F- strain with frequencies comparable to those measured for F+ or Hfr donor strains (Table 1). Morover, the presence of colV-colI factors in the F- cell also appeared to confer to it a chromosomal fertility, which though low, was not evidenced in the control cross col- x col- (Table 1).

ColV-colI factors and F-gal factor. To facilitate the study of the fate of the F factor in donor cells becoming (colV-colI)+, these factors were transferred to strain F' W3103 F-gal. Three different types of

colonies (colV-colI)+ derived from this strain were isolated:

1) gal+ (colV-colI)+ colonies, which, unlike the F-gal original strain, presented a very low chromosomal fertility (10^{-4} - 10^{-5}%) and did not transfer any longer the gal+ character during conjugation.

2) gal+ (colV-colI)+ colonies, which were shown to transfer the chromosome with the frequency characteristic of an Hfr strain, with thr, leu as proximal markers and gal as a distal one.

3) gal-(colV-colI)+ colonies, which presented the low chromosomal fertility characteristic of F-(colV-colI)+ cells (10^{-4}%).

All these three types of colonies transferred colV-colI factors at high frequency (about 1000%). In contrast to the behaviour of the original F-gal strain, neither of the two types of gal+(colV-colI)+ colonies studied segregated the gal- character.

These results suggest that the F- gal particle in the autonomous state does not coexist in the same cell with colV-colI factors. When picking (colV-colI)+ cells, therefore, one would be automatically selecting those which became gal- by loss of the F-gal particle (colonies of type 3) or those with the gal+ character integrated in the chromosome (colonies of types 1 or 2).

The mechanism of formation of these types of colonies is being investigated.

DISCUSSION

Results reported above show that the presence of colicinogenic factors V-I in some donor strains of E. coli K12 reduces markedly the frequency of recombinant formation, although these colicinogenic factors are being transferred very efficiently from these same cells. Morover, it was also observed that the presence of colV-colI factors in an F- cell confers to it the ability to conjugate, enabling their efficient passage to other F- cells.

Two different hypothesis accounting for the observed facts can be formulated: 1) ColV-colI factors interfere with the expression of the F factor in donor cells. Epistasis on the F factor might be complete or at least affecting the functions related to the transfer of the chromosome. 2) ColV-colI factors interfere with the presence of the F factor in donor cells.

Results with F-gal strains made (colV-colI)+ suggest that exclusion between both types of factor occurs.

One may also ask whether the F-like properties displayed by colV-colI factors are carried in their own genomes, as appears to be the case with colI or colB (Ozeki, Stocker and Smith, 1963) or by an F factor closely associated with them, as described by Fredericq (1963) with colV and colB factors. It should be pointed out that these colV-colI factors might not be identical to the colV or colI factors studied by different authors (Ozeki et al., 1963; Fredericq, 1963; Nagel de Zwaig et al., 1962).

Similar results to those reported here were presented by Kahn and Helinski (1964) for colV factor. Cases of interactions with F factors had also been reported for colB (Puig, 1963) and resistance transfer factors (Watanabe and Fukasawa, 1962).

Studies tending to elucidate the relationship between colV, colI and the fertility factor, as well as the nature of the interactions observed, are in progress.

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