COMPATIBILITY OF F-LIKE FACTOR OF pAP43 GENETIC TRANSFER WITH F-GROUP INCOMPATIBILITY PLASMIDS

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The pAP43 genetic transfer factor has been identified in cells of a serologically typed strain of *Escherichia coli* isolated from man. With a molecular weight of 57.7 megadaltons, it is an F-like plasmid and is designated Fi^+.

To determine the character of binding of pAP43 genetic transfer factor with other F-like plasmids, in the investigation described below the compatibility of this factor with plasmids of all seven F incompatibility groups in *E. coli* was investigated [2, 3, 5].

EXPERIMENTAL METHOD

Genetic marking of the pAP43 factor was carried out by means of transposon Tn1 by the method described previously [1, 2]. Experiments to determine the F incompatibility group to which the test factor belonged were carried out in accordance with Datta's scheme [4].

EXPERIMENTAL RESULTS

Altogether 18 direct and back-crosses of *E. coli* were carried out in which the pAP43:Tn1 genetic transfer factor, marked with transposon Tn1, was introduced from *E. coli* AP106 cells into *E. coli* AP115 cells, containing as resident plasmid one of the reference plasmids of each of the seven F incompatibility groups, and vice versa. This enabled the behavior of the test factor to be studied as introduced plasmid and as resident plasmid. To obtain information on surface exclusion and spontaneous elimination of the plasmid crosses were carried out in which the test transfer factor and each of

TABLE 1. Compatibility of Transfer Factor pAP43 with F-Group Incompatibility Plasmid (in *E. coli* AP115)

Plasmid		Selective marker	Frequency of transfer (per donor cell)	Surface exclusion index	Number of colonies (in percent) whose cells contain		
introduced	resident				introduced plasmid	resident plasmid	both plasmids
pAP43::Tn1	R386 (FI)	Ap	$5{,}7\cdot10^{-3}$	1,1	100	100	100
pAP43::Tn1		Ap	$6{,}4\cdot10^{-3}$		100		
R386 (FI)	pAP43::Tn1	Tc	$8\cdot10^{-4}$	77	100	100	100
R386 (FI)		Tc	$6{,}6\cdot10^{-2}$		100		
pAP43::Tn1	R1—19 (EII)	Ap	$7\cdot10^{-3}$	0,91	100	100	100
R1—19 (FII)	pAP43::Tn1	Km	$1\cdot10^{0}$	1	100	100	100
R1—19 (FII)		Km	$1{,}1\cdot10^{0}$		100		
pAP43::Tn1	colBR3 (FIII)	Ap	$1\cdot10^{-2}$	0,64	100	100	100
colBR3 (FIII)	pAP43::Tn1	Lm	$2{,}1\cdot10^{-2}$	2,0	100	100	100
colBR3 (FIII)		Lm	$4{,}3\cdot10^{-2}$		100		
pAP43::Tn1	R124 (FIV)	Ap	$1{,}3\cdot10^{-3}$	4,9	100	100	100
R124 (FIY)	pAP43::Tn1	Tc	$1{,}8\cdot10^{-3}$	2,7	100	100	100
R124 (FIY)		Tc	$4{,}9\cdot10^{-3}$		100		
pAP43::Tn1	F_0lac (FY)	Ap	$4{,}7\cdot10^{-3}$	1,3	100	100	100
F_0lac(FY)	pAP43::Tn1	Lac	$1{,}4\cdot10^{-5}$	3,8	100	100	100
F_0lac (FY)		Lac	$5{,}4\cdot10^{-5}$		100		
pAP43::Tn1	Hly—P212 (FYI)	Ap	$7{,}3\cdot10^{-3}$	0,87	100	100	100
Hly—P212 (FVI)	pAP43::Tn1	Hly	$1{,}8\cdot10^{-1}$	1,7	100	100	100
Hly—P212 (FYI)		Hly	$3{,}1\cdot10^{-1}$		100		
pAP43::Tn1	pAP38::Tn9 (FYII)	Ap	$5\cdot10^{-4}$	12,8	100	100	100
pAP38::Tn9 (FYII)	pAP43::Tn1	Lm	$1{,}2\cdot10^{-3}$	44	100	100	100
pAP38::Tn9 (FYII)		Lm	$5{,}3\cdot10^{-2}$		100		

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TABLE 2. Clonal Test for Compatibility (incompatibility) of Transfer Factor pAP43 with F-Group Plasmid

Plasmid		Selective marker	Transconjugant clones (serial no.)	Number of colonies (in percent) whose cells contain		
introduced	resident			introduced plasmid	resident plasmid	both plasmids
pAP43::Tn1	R386 (FI)	Ap	1	100	100	100
R386 (FI)	pAP43::Tn1	Tc	1	100	100	100
pAP43::Tn1	R1—19 (FII)	Ap	1	100	100	100
R1—19 (FII)	pAP43::Tn1	Km	1	100	100	100
pAP43::Tn1	colBR3 (FIII)	Ap	1	100	100	100
colBR3 (FIII)	pAP43::Tn1	Lm	1	100	100	100
pAP43::Tn1	R124 (FIY)	Ap	1	100	100	100
R124 (FIY)	pAP43::Tn1	Tc	1	100	100	100
pAP43::Tn1	F_0lac (FY)	Ap	1	100	100	100
F_0lac (FY)	pAP43::Tn1	Lac	1	100	100	100
pAP43::Tn1	Hly—P212 (FYI)	Ap	1	100	100	100
Hly—P212 (FYI)	pAP43::Tn1	Hly	1	100	100	100
pAP43::Tn1	pAP38::Tn9 (FYII)	Ap	1	100	100	100
pAP38::Tn9 (FYII)	pAP43::Tn1	Lm	1	100	100	100

TABLE 3. Genetic Transfer of Plasmids from Diplasmid *E. coli* AP115 Donors into *E. coli* AP106

Plasmids	Selective marker	Frequency of transfer	Analysis of nonselective markers of transconjugants		
			marker	number of transconjugants studied	number of transconjugants containing marker
AP115(R386)(oAP43::Tn1)xAP106	Ap	$3\cdot10^{-2}$		20	9
	Tc	$1\cdot10^{-2}$		20	20
AP115(pAP43::Tn1)(R386)xAP106	Ap	$2\cdot10^{-3}$		20	15
	Tc	$9\cdot10^{-4}$		20	19
AP115(R1—19)(pAP43::Tn1)xAP106	Ap	$4\cdot10^{-2}$		20	0
	Km	$8{,}4\cdot10^{-4}$		20	3
AP115(pAP43::Tn1)(R1—19)xAP106	Ap	$1{,}4\cdot10^{-2}$		20	2
	Km	$1{,}3\cdot10^{-2}$		20	1
AP115(colBR3)(pAP43::Tn1)xAP106	Ap	$1{,}5\cdot10^{-2}$		20	14
	Lm	$1\cdot10^{-2}$		20	12
AP115(pAP43::Tn1)(colBR3)xAP106	Ap	$7\cdot10^{-3}$		20	14
	Lm	$4\cdot10^{-3}$		20	17
AP115(R124)(pAP43::Tn1)xAP106	Ap	$2\cdot10^{-2}$		20	4
	Tc	$1\cdot10^{-2}$		20	19
AP115(pAP43::Tn1)(R124)xAP106	Ap	$9{,}3\cdot10^{-3}$		20	5
	Tc	$3{,}4\cdot10^{-3}$	Ap	20	20
AP115(F_0lac)(aAP43::Tn1)x106	Ap	$1{,}3\cdot10^{-3}$	Lac	20	0
	Lac	$9\cdot10^{-7}$	Ap	20	20
AP115(pAP43::Tn1)(F_0lac)xAP106	Ap	$4{,}6\cdot10^{-3}$	Lac	20	0
	Lac	$1{,}5\cdot10^{-4}$	Ap	20	18
AP115(Hly—P212)(pAP43::Tn1)xAP106	Ap	$1{,}8\cdot10^{-3}$	Hly	20	0
	Hly	$2\cdot10^{-3}$	Ap	20	5
AP115(pAP43::Tn1)(Hly—P212)xAP106	Ap	$9{,}0\cdot10^{-3}$	Hly	20	0
	Hly	$1{,}4\cdot10^{-3}$	Ap	20	6
AP115(pAP38::Tn9)(pAP43::Tn1)xAP106	Ap	$1{,}8\cdot10^{-2}$	Lm	20	3
	Lm	$1\cdot10^{-3}$	Ap	20	2
AP115(pAP43::Tn1)(pAP38::Tn9)xAP106	Ap	$7{,}7\cdot10^{-3}$	Lm	20	0
	Lm	$4{,}1\cdot10^{-3}$	Ap	20	1

the reference plasmids were introduced separately into recipient *E. coli* AP115 cells free from plasmid.

The results of these experiments (Table 1) showed that the frequencies of introduction of plasmid into cells already containing one of the plasmids did not differ significantly from the frequencies of introduction of plasmid into plasmid-free cells.

Surface exclusion of introduced plasmid either did not take place or was observed but with a low frequency. The only exceptions were cases when transfer factor pAP43 was the resident plasmid in back-crosses and reference plasmids of F groups I and VII were introduced plasmids.

To study the plasmid content of transconjugants, 20 transconjugant colonies were selected from each cross. As Table 1 shows, they all contained two plasmids each – the test transfer factor and one of the reference plasmids of each F incompatibility group.

The presence of both plasmids (introduced and resident) in transconjugants from all crosses (in the absence of loss of plasmid in the control crosses) was presumptive evidence of compatibility of the transfer factor with plasmids of all F incompatibility groups.

To test this hypothesis clonal tests were used and the character of transfer effected by diplasmid transconjugants was studied (Table 2).

In the clonal tests, one plasmid transconjugant was taken arbitrarily from each cross, transferred to nutrient broth, and seedlings were grown for 18 h at 37°C. The clonal cultures thus obtained were then seeded on nutrient agar to obtain isolated colonies, the cells of which were tested for the presence of plasmid – both introduced and resident (20 colonies of each clone). As Table 2 shows, according to the results of the clonal tests pAP43 transfer factor exhibits complete compatibility with all F-group incompatibility reference plasmids.

The experiments to study the character of transfer effected by diplasmid transconjugants involved their crossing as donors with *E. coli* AP106 recipient cells; separate transfer of introduced and resident plasmids was studied in these crosses. As the results of these experiments showed (Table 3), pAP43 transfer factor and plasmids belonging to different F incompatibility groups were transmitted with different frequencies, indicating separate transfer of the plasmids. The results of analysis of nonselective markers of daughter transconjugants given in Table 3 also indicate separate transfer of the test plasmids.

The results as a whole are evidence that pAP43 transfer factor is compatible with all seven F-group incompatibility reference plasmids. In other words, it is a representative of an independent F incompatibility group which, after description of group FVII [2], must be designated group FVIII.

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