A STUDY OF THE F-LIKE GENETIC TRANSFER FACTOR pAP42

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UDC 579.252.52:579.254

KEY WORDS: plasmid; genetic transfer factor; transposon; transconjugant; plasmid incompatibility; genetic marking.

Genetic transfer factor pAP42 was first found in cells of a wild type enteropathogenic strain of *Escherichia coli* [7]. Later experiments showed that its molecular weight is $32.2 \cdot 10^6$ [1].

The object of the present investigation was to study the sensitivity of *E. coli* K-12 cells containing transfer factor pAP42 to F phages and also to determine the frequency of transmission of this test factor from some cells to others and to examine its relationship to plasmids of the F incompatibility groups.

EXPERIMENTAL METHOD

Variants of transfer factor pAP42 genetically marked by transposons Tn1 and Tn9 by methods described previously [4-6] were used. These variants will be referred to as pAP42::Tn1 and pAP42::Tn9. The sensitivity of bacteria containing transfer factor to F phages was determined by the agar layers method. The frequency of transmission of the transfer factor was studied in experiments by the standard method [3]. Compatibility (incompatibility) of the test transfer factor with reference plasmids of F incompatibility groups was studied with the aid of *E. coli* AP115, by the usual scheme [8].

EXPERIMENTAL RESULTS

Cells of the wild type strain of *E. coli* in which transfer factor pAP42 was found initially give a response consisting of a rise in the titer of phage MS2. However, it was impossible to determine the sensitivity of these bacteria to phage MS2 by the agar layers method. Conversely, introduction of this factor into *E. coli* K-12 cells led them to become sensitive to a number of F-specific phages, namely MS2, f1, fd, f2, Qβ, and M12; the phages formed plaques on the bacterial lawn in very high dilutions.

The frequency of transmission of transfer factor pAP42 was determined in crosses of *E. coli* AP106 trp his lac str rec A cells, containing one of its marked variants (pAP42::Tn1 or pAP42::Tn9) with *E. coli* AP115 met thi lac nal. Analysis of the results showed that the frequency of transmission of the test factor was $0.6 \cdot 10^0$-$4.0 \cdot 10^0$.

The results of experiments to study compatibility of transfer factor pAP42 with reference plasmids of incompatibility groups FI-FVIII are given in Table 1.

As Table 1 shows, in some cases there was marked surface exclusion of the introduced plasmids, but it was independent of the plasmid content of the isolated transconjugant colonies. Cells of the transconjugant colonies from all crosses each contained two plasmids — transfer factor pAP42 and one or other reference plasmid. The only exception were cases when the recipient plasmids were F$_0$lac plasmid (FV incompatibility group) and pAP42, whereas the introduced plasmids were pAP42 and Hly—P212 (FVI incompatibility group) respectively. In this case loss of one plasmid was observed (in a very small percentage of cases).

The results indicated neither compatibility nor incompatibility between the tested transfer factor and the reference plasmids. In order to determine the degree of stability of coexistence of transfer pAP42 with the reference plasmids, clonal tests were therefore carried out, in which the initial transconjugants were cultured in nutrient broth, plated out on

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 85-87, April, 1982. Original article submitted September 14, 1981.

0007-4888/82/9304-0491$07.50

TABLE 1. Compatibility of Transfer Factor pAP42::Tn9 with Plasmids of F Incompatibility Groups (in *E. coli* AP115)

Plasmid		Selective marker	Frequency of transfer per donor cell	Surface exclusion index	No. of colonies of transconjugants (in %) whose cells contain		
introduced	resident				introduced plasmid	resident plasmid	both plasmids
pAP42::Tn9	R386 (FI)	Lm	$4{,}4\cdot10^{-1}$	9,1	100	100	100
pAP42::Tn9		Lm	$4{,}0\cdot10^{0}$		100		
R386 (FI)	pAP42::Tn9	Tc	$2{,}3\cdot10^{-3}$	15,6	100	100	100
R386 (FI)		Tc	$3{,}6\cdot10^{-2}$		100		
pAP42::Tn9	RI—19(FII)	Lm	$6{,}6\cdot10^{-1}$	6,1	100	100	100
RI—19 (FII)	pAP42::Tn9	Km	$6{,}1\cdot10^{-1}$	0,6	100	100	100
RI—19 (FII)		Km	$4{,}1\cdot10^{-1}$		100		
pAP42::Tn9	Col BR3 (F III)	Lm	$1{,}3\cdot10^{-1}$	30,7	100	100	100
Col BR 3 (F III)	pAP42::Tn9	Sm	$2{,}8\cdot10^{-2}$	14,2	100	100	100
Col BP 3 (F III)		Sm	$4{,}0\cdot10^{-2}$		100		
pAP42::Tn 9	R124 (F IV)	Lm	$3{,}1\cdot10^{-1}$	12,9	100	100	100
R124 (F IV)	pAP42::Tn9	Tc	$9{,}1\cdot10^{-4}$	1,1	100	100	100
R124 (F IV)		Tc	$1{,}1\cdot10^{-3}$		100		
pAP42::Tn9	F_0lac (FV)	Lm	$9{,}0\cdot10^{-1}$	4,4	100	65	65
F_0lac (FV)	pAP42::Tn 9	Lac	$9{,}0\cdot10^{-5}$	0,04	100	100	100
F_0lac (FV)		Lac	$3{,}7\cdot10^{-6}$		100	100	100
pAP42:Tn9	Hly—P212 (FVI)	Lm	$10{,}0\cdot10^{-1}$	4	100	100	100
Hly—P212 (F VI)	pAP42::Tn 9	Hly	$2{,}6\cdot10^{-5}$	54	100	95	95
Hly—P212 (FVI)		Hly	$1{,}4\cdot10^{-2}$		100		
pAP42::Tn 9	pAP38::Tn (F VII)	Lm	$2{,}0\cdot10^{0}$	2	100	100	100
pAP38::Tn I (F VII)	pAP42::Tn 9	Ap	$1{,}3\cdot10^{-3}$	0,7	100	100	100
pAP38::Tn I (F VII)		Ap	$9{,}5\cdot10^{-4}$		100		
pAP42::Tn 9	pAP43:Tn I(F VIII)	Lm	$3{,}0\cdot10^{-1}$	13,7	100	100	100
pAP43::Tn 1 (F VIII)	pAP42::Tn9	Ap	$1{,}4\cdot10^{-2}$	0,5	100	100	100
pAP43::Tn I (F VIII)		Ap	$7{,}5\cdot10^{-3}$		100		

TABLE 2. Genetic Transfer of Plasmids from Diplasmid *E. coli* AP115 Donors to *E. coli* AP106

Cross	Selective marker	Frequency of transfer	Analysis of unselective transconjugant markers		
			marker	no. of transconjugants studied	no. of transconjugants studied containing marker
AP115 (R386) (pAP42::Tn9)×AP106	Lm	$2{,}0\cdot10^{-3}$	Tc	20	15
	Tc	$1{,}7\cdot10^{-1}$	Lm	20	0
AP115(pAP42::Tn9) (R386)×AP106	Lm	$6{,}6\cdot10^{-2}$	Tc	20	8
	Tc	$5{,}4\cdot10^{-2}$	Lm	20	9
AP115 (R1—19) (pAP42::Tn9)×AP106	Lm	$9{,}0\cdot10^{-4}$	Km	20	3
	Km	$1{,}1\cdot10^{-3}$	Lm	20	4
AP115 (pAP42::Tn9) (R1—19)×AP106	Lm	$5{,}0\cdot10^{-3}$	Km	20	1
	Km	$7{,}7\cdot10^{-3}$	Lm	20	5
AP115 (ColBR3) (pAP42::Tn9)×AP106	Lm	$1{,}6\cdot10^{-2}$	Col	20	17
	Col	$4{,}2\cdot10^{-2}$	Lm	20	4
AP115 (pAP42::Tn9) (ColBR3)×AP106	Lm	$8{,}0\cdot10^{2}$	Col	20	7
	Col	$3{,}7\cdot10^{-2}$	Lm	20	8
AP115 (R124) (pAP42::Tn9)×AP106	Lm	$2{,}3\cdot10^{-1}$	Tc	20	1
	Tc	$1{,}9\cdot10^{-3}$	Lm	20	13
AP115 (pAP42::Tn9) (R124)×AP106	Lm	$1{,}8\cdot10^{-2}$	Tc	20	0
	Tc	$3{,}2\cdot10^{-4}$	Lm	20	8
AP115(F_0lac) (pAP42::Tn9)×AP106	Lm	$3{,}4\cdot10^{-5}$	Lac	20	0
	Lac	$5{,}0\cdot10^{-6}$	Lm	10	2
AP115 (pAP42::Tn9) (F_0lac)×AP106	Lm	$4{,}7\cdot10^{-1}$	Lac	20	0
	Lac	$1{,}0\cdot10^{-4}$	Lm	20	20
AP115 (Hly—P212) (pAP42::Tn 9)×AP106	Lm	$8{,}0\cdot10^{-3}$	Hly	20	0
	Hly	$8{,}4\cdot10^{-3}$	Lm	20	0
AP115 (pAP42::Tn9) (Hly—P212)×AP106	Lm	$7{,}1\cdot10^{-4}$	Hly	20	0
	Hly	$3{,}7\cdot10^{-4}$	Lm	20	3
AP115 (pAP38::Tn1) (pAP42::Tn9)×AP106	Lm	$7{,}1\cdot10^{-2}$	Ap	20	0
	Ap	$5{,}0\cdot10^{-2}$	Lm	20	11
AP115(pAP42::Tn9) (pAP38::Tn1)×AP106	Lm	$5{,}2\cdot10^{-2}$	Ap	20	1
	Ap	$1{,}9\cdot10^{-2}$	Lm	20	16
AP115 (pAP43::TnI) (pAP42::Tn9)×AP106	Lm	$5{,}6\cdot10^{-2}$	Ap	20	0
	Ap	$3{,}0\cdot10^{-3}$	Lm	20	4
AP115(pAP42::Tn9) (pAP43::TnI)×AP106	Lm	$7{,}7\cdot10^{-3}$	Ap	20	0
	Ap	$5{,}8\cdot10^{-4}$	Lm	20	6

nutrient agar, and their plasmid content was analyzed in 20 colonies (clones) originating from each transconjugant.

The clonal tests showed that cells of the clonal cultures of all transconjugants could each maintain under stable conditions a pair of plasmids — transfer factor pAP42 and one or other reference plasmid (of each F incompatibility group). The very slight loss of one of the plasmids after culture of the bacteria containing them was noted in cases when the introduced plasmid was F$_0$lac (5% of the cells lost the F$_0$lac plasmid) and Hly—P212 (5-10% of cells lost the transfer factor pAP42).

In the final experiments the character of transfer of plasmids from diplasmid transconjugant cells to recipient bacteria was studied.

The data in Table 2 show that transmission of each of the two plasmids studied (transfer factor pAP42 and one or other reference plasmid) takes place at different frequencies in nearly every case. This indicates separate (independent) transfer of the plasmids, i.e., absence of recombination between the plasmids. Analysis of unselective markers of the transconjugants confirmed this character of the transfer.

The results on phage sensitivity of *E. coli* K-12 cells containing transfer factor pAP42 and the frequency of transmission of the latter from some *E. coli* K-12 cells to other such cells, undoubtedly indicate that the test factor is a F-like plasmid, derepressed for the conjugativeness function (a drd mutant). To generalize the results of the experiments on determination of the relationship between transfer factor pAP42 and other F-like plasmids it can be concluded that this factor is fully compatible with reference plasmids of the F incompatibility groups studied. The existence of groups FI-FVI has recently found additional confirmatory arguments [9]. Groups FVII and FVIII were established in our own laboratory [2, 5]. Consequently, factor pAP42 is a number of a new incompatibility group of F-like plasmids — group FIX.

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