IDENTIFICATION OF COPY NUMBER MUTANTS OF GENETIC TRANSFER FACTOR pAP42

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Replication of bacterial plasmids is under genetic control, which is assumed to be negative [6]. In the case of individual R plasmids it has been shown that control extends simultaneously to replication and to incompatibility. In particular, it has been found that mutations changing the copy number of individual R plasmids, are accompanied by a change in the incompatibility of copy number mutants with the original R plasmids [4, 7, 8].

Genetic transfer factor pAP42 is an F-like drd-plasmid of E. coli, of average molecular dimensions.

Since it has been shown that plasmid pAP42 belongs to the incFIX incompatibility group [1], an attempt was made in the investigation described below to determine whether correlation exists between control of the copy number of this plasmid (the number of copies per bacterial cell chromosome) and control of its incompatibility.

EXPERIMENTAL METHOD

Transfer factor pAP42 :: Tn1, contained in E. coli was used. The copy number of the PAP42 :: Tn1 factor was determined by measuring β-lactamase activity of TEM type, determined by transposon Tn1 [9]. β-lactamase was determined quantitatively in cell-free extracts of E. coli by iodometric titration, by the method described in the USSR State Pharmacopoiea (Article 12 42-922 – 74), a modification of the macroiodometric method [5]. Copy number mutants of factor pAP42 :: Tn1 were induced with nitrosoguanidine, added to cultures of E. coli AP115 cells containing the test transfer factor, in a concentration of 100 μg/ml. The sensitivity of the bacteria to phage MS2 was determined by the agar layers method. The frequency of plasmid transfer was studied by the standard method [2]. Compatibility (incompatibility) of the mutant plasmid with reference plasmids of the F incompatibility groups was determined by the usual scheme [3].

EXPERIMENTAL RESULTS

The work began with determination of the number of copies of plasmid pAP42 :: Tn1 in E. coli C600 thr leu thi lac str^r. For this purpose activities of β-lactamase of E. coli C600 cells, containing plasmid pAP42 :: Tn1, and E. coli C600 cells containing plasmids with a known number of copies (plasmids RSF 2124 and RP4) were compared. Activity of β-lactamase was expressed in conventional VNIIA (All-Union Antibiotics Research Institute) units. The unit of activity was taken to be the smallest quantity of β-lactamase sufficient to inactivate 10^{-7} mole of benzylpenicillin per hour at 37°C in phosphate buffer (pH 6.8-7.0). The results of these experiments are given in Table 1.

It will be clear from Table 1 that activity of β-lactamase coded by plasmid RSF 2124 was 5.5 times higher than β-lactamase activity coded by plasmid pAP42 :: Tn1 which, in turn, was 1.3 times higher than β-lactamase activity coded by plasmid RP4. Since plasmid RSF 2124 exists in cells in 10-12 copies, and plasmid RP4 in one or two copies, it is easy to conclude that factor pAP42 :: Tn1 exists in about one or two copies per E. coli chromosome.

Determination of the number of copies of factor pAP42 :: Tn1 enabled experiments to induce copy number mutations of this plasmid to begin. As already stated, mutants with an increased number of copies were induced by treatment of AP115 met thi lac nal (pAP42 :: Tn1) cells with nitrosoguanidine. Among clonal cultures

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TABLE 1. Activity of β-Lactamase Coded by Plasmid in E. coli C600

Plasmid	β-Lactamase activity (in VNIIA units)	Number of plasmid copies
pAP42::Tn1	158,2	1—2
RSF2124	876,1	10—12
RP4	119,4	1—2

TABLE 2. Activity of β-Lactamase Coded by Plasmid pAP42 :: Tn1 cop 1 in Cells of Different Strains of E. coli

Strain of host bacteria	Plasmid	β-Lactamase activity (in VNIIA units)
C600	pAP42::Tn1 cop1	880,6
C600	RSF2124	864,2
AP115	pAP42::Tn1 cop1	179,8
C600	pAP42::Tn1	155

TABLE 3. Compatibility (incompatibility) of Plasmid pAP42 :: Tn1 cop 1 with Plasmids of F Groups (in E. coli AP115)

Plasmid		Selective marker	Frequency of transfer to one donor cell	Surface exclusion index	Number of colonies (in %) whose cells contain		
introduced	resident				introduced plasmid	resident plasmid	both plasmids
pAP42::Tn1cop1	R386 (FI)	Ap	$2{,}9\cdot10^{-1}$	4,5	100	100	100
pAP42::Tn1cop1		Ap	$13\cdot10^{-1}$		100		
R386 (FI)	pAP42::Tn1cop1	Tc	$7{,}6\cdot10^{-2}$	1,0	100	100	100
R386 (FI)		Tc	$7{,}9\cdot10^{-2}$		100		
pAP42::Tn1cop1	R1-19 (FII)	Ap	$2{,}4\cdot10^{-1}$	5,4	100	100	100
R1-19 (FII)	pAP42::Tn1cop1	Km	$4{,}5\cdot10^{-1}$	1,0	100	100	100
R1-19 (FII)		Km	$4{,}5\cdot10^{-1}$		100		
pAP42::Tn1cop1	ColBR3 (FIII)	Ap	$8{,}3\cdot10^{-1}$	1,6	100	100	100
ColBR3 (FIII)	pAP42::Tn1cop1	Lm	$6{,}7\cdot10^{-3}$	1,8	100	100	100
ColBR3 (FIII)		Lm	$1{,}2\cdot10^{-2}$		100		
pAP42::Tn1cop1		Ap	$8{,}3\cdot10^{-2}$	15,7	100	100	100
R124 (FIV)	pAP42::Tn1cop1	Tc	$3{,}9\cdot10^{-3}$	1,2	100	100	100
R124 (FIV)		Tc	$4{,}6\cdot10^{-3}$	11	100		
pAP42::Tn1cop1	Folac (FV)	Ap	$4{,}5\cdot10^{-1}$	2,9	100	100	100
Folac (FV)	pAP42::Tn1cop1	Lac	$3{,}5\cdot10^{-5}$	1,9	95	100	95
Folac (FV)		Lac	$6{,}7\cdot10^{-5}$		100		
pAP42::Tn1cop1	Hly-P212 (FVI)	Ap	$15\cdot10^{-1}$	0,9	100	95	95
Hly-P212 (FVI)	pAP42::Tn1cop1	Hly	$2{,}3\cdot10^{-3}$	1,2	100	100	100
Hly-P212 (FVI)		Hly	$2{,}7\cdot10^{-3}$		100		
pAP42::Tn1cop1	pAP38::Tn9 (FVII)	Ap	$21\cdot10^{-1}$	0,6	100	100	100
pAP38::Tn9 (FVII)	pAP42::Tn1cop1	Lm	$4{,}7\cdot10^{-2}$	1,2	100	100	100
pAP38::Tn9 (FVII)		Lm	$5{,}7\cdot10^{-2}$		100		
pAP42::Tn1cop1	pAP43::Tn9 (FVIII)	Ap	$10\cdot10^{-1}$	1,3	100	100	100
pAP43::Tn9 (FVIII)	pAP42::Tn1cop1	Lm	$2{,}3\cdot10^{-3}$	1,0	100	100	100
pAP43::Tn9 (FVIII)		Lm	$2{,}4\cdot10^{-3}$		100		
pAP42::Tn1cop1	pAP42::Tn9 (FIX)	Ap	$2{,}0\cdot10^{-1}$	6,5	100	0	0
pAP42::Tn9 (FIX)	pAP42::Tn1cop1	Lm	$5{,}9\cdot10^{-3}$	220	100	35	35
pAP42::Tn9 (FIX)		Lm	$13\cdot10^{-1}$		100		

grown from single colonies of bacteria, treated with mutagen, one clone of cells resistant to ampicillin in a concentration of 8000 μg/ml was found, whereas cells containing the original pAP42 :: Tn1 factor, were resistant to this antibiotic in concentrations not exceeding 2000 μg/ml. Since cells of the selected resistant clone preserved their sensitivity to phage MS2, it was postulated that the considerable increase in resistance of these cells to ampicillin was due to mutation of the plasmid pAP42 :: Tn1 which they contained, accompanied by an increase in the number of copies of the latter. This mutant plasmid was designated pAP42 :: Tn1 cop 1.

To confirm the hypothesis of the mutant nature of plasmid pAP42 :: Tn1 cop 1 in resistant cells of the isolated clone, and also to discover how this plasmid behaves in E. coli of strains not exposed to the action of the mutagen, it was introduced into E. coli C600 cells, after which the latter were tested for β-lactamase activity and compared with E. coli C600 (RSF 2124), AP115 (pPA42:: Tn cop 1) and C600 (pAP42:: Tn 1). The results of these experiments are given in Table 2.

It will be clear from Table 2 that E. coli C600 cells containing plasmid pAP42 :: Tn1 cop 1 possessed β-lactamase activity 5.7 times greater than the β-lactamase activity of E. coli C600 containing plasmid pAP42 :: Tn1, and equal to that of E. coli C600 (RSF 2124). When these data are analyzed it can be concluded that plasmid pAP42 :: Tn1 cop 1 is in fact a copy number of transfer factor pAP42 :: Tn1, and that the number of copies of the mutant plasmid is 10-12 copies per chromosome (like plasmid RSF 2124).

It is also clear from Table 2 that β-lactamase activity of E. coli C600 (pAP42 :: Tn1 cop 1) is higher than the β-lactamase activity of E. coli AP115 (pAP42 :: Tn1 cop 1). Assuming that this is related to some special features of the host cells, in additional experiments we transferred plasmid pAP42 :: Tn1 cop 1 back from C600 cells to plasmid-free AP 115 cells and analyzed the β-lactamase activity of the latter. The experiments showed that β-lactamase activity determined by plasmid pAP42 :: Tn1 cop 1 is reduced when this plasmid is "returned" to E. coli AP115. These data thus indicate the role of the host cell in expression of the β-lactamase gene.

To discover whether correlation exists between control of the copy number and control of incompatibility, in the next experiments compatibility of mutant plasmid pAP42 :: Tn1 cop 1 with the original plasmid pAP 42 (pAP42 :: 9), and also with reference plasmids of all incompatibility groups of F-like plasmids, was studied. The results of these experiments are given in Table 3.

It will be clear from Table 3 that introduction of normal factor pAP42 :: Tn 9 into recipient cells containing pAP42 :: Tn1 cop 1 as resident plasmid (direct crossing) is accompanied by considerable surface exclusion, and also by considerable elimination (65%) of the resident plasmid from the transconjugant cells. Conversely, introduction of mutant pAP42 :: Tn1 cop 1 into bacterial cells containing as resident plasmid pAP42 :: Tn 9 (back crossing), is accompanied by complete supplanting of the latter. The very small loss (5%) of one of the plasmids was observed in cases when the introduced plasmid was Folac (incFV) or the resident plasmid was Hly-P212 (incFVI). As regards the relations of factor pAP42 :: Tn1 cop 1 to reference plasmids of groups incFI, FII, FIII, FIV, FVII, and FVIII are concerned, they were completely compatible with these plasmids.

To obtain final proof that mutant factor pAP42 :: Tn1 cop 1 is incompatible with wild-type factor pAP42 :: Tn 9, but at the same time compatible with reference plasmids of incFV and incFVI groups, clonal analysis of the transconjugants are carried out. The results of clonal analysis (culture of cells of 1-5 colonies of transconjugants containing both plasmids, in nutrient broth followed by seeding on nutrient agar and determination of the plasmid contents of cells of 20 colonies) showed that transconjugants obtained in direct and back crosses, in which the relationship of plasmid pAP42 :: Tn1 cop 1 to plasmid pAP42 :: Tn 9 was determined, lost their resident plasmid almost completely (100%). This indicates that these plasmids are in fact completely incompatible. Conversely, cells of two of the five clones of transconjugants tested, containing introduced plasmid Folac and resident plasmid pAP42 :: Tn1 cop 1, after culture, lost plasmid Folac (35 and 10% of the total number of cells respectively). Cells of all clones containing plasmids pAP42 :: Tn1 cop 1 and Hly-P212, preserved these plasmids. This indicates compability of plasmid pAP42 :: Tn1 cop 1 with plasmids Folac and Hly-P212.

The results can be summed up in the conclusion that plasmid pAP42 is subject to mutations accompanied by a change in copy number. The identified mutants pAP42 :: Tn1 cop 1 preserves its incompatibility with original plasmids pAP42 and its compatibility with plasmids of other inc-groups of F-like plasmids. In other words, copy number mutation of factor pAP42 is probably located in a genetic region which is not involved in the control of its compatibility (incompatibility) with other plasmids.

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