EXPERIMENTAL GENETICS

INCOMPATIBILITY AND REPLICATION OF PLASMIDS

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Plasmids which belong to several incompatibility groups at the same time, i.e., which possess atypical incompatibility [2], are characterized by replication which is under concordant control, because of their identical basic replicons, whereas compatible plasmids are characterized by replication under discordant control, i.e., they have different basic replicons [3].

In a study of compatibility (incompatibility) of F-like plasmid pAP42, which is a plasmid of the genetic transfer factor type, we found that it belonged to one incompatibility group, namely the Inc FIX group. This suggested that it contains one replicon in its genome. However, it was later discovered that this plasmid possesses two replicons. The aim of this investigation was accordingly to study the basic properties (molecular weight, copy number, Fin-activity, ability to carry out their own transfer, stability) of the replicons of plasmid pAP42 and to establish the role of each of its replicons in the incompatibility of this plasmid. The results are described below.

EXPERIMENTAL METHOD

Plasmid pAP42 and its transposon-containing variants pAP42::Tn1 and pAP42::Tn9, and also reference plasmid F'lac were used. All plasmids were maintained in *E. coli* AP115 cells.

Plasmid DNA was isolated by the method in [7], using centrifugation in a CsCl density gradient. DNA restriction was carried out by enzyme HindIII. Restriction fragments were dispersed in 0.8% agarose gel by horizontal slab electrophoresis, and their dimensions were obtained by using plasmid DNA of phage λ as the reference DNA [4].

Replicons of plasmid pAP42::Tn1 were constructed by partial HindIII-hydrolysis of its DNA, ligation of the fragments thus obtained, and transformation of *E. coli* HB101 cells by the standard method [6].

The copy number of replicons of plasmid pAP42::Tn1 was determined by measuring activity of β-lactamase of the TEM type, determined by transposon Tn1 [9]. Quantitative determination of β-lactamase was carried out in cell-free extracts of *E. coli* by iodometric titration, as described in the USSR State Pharmacopoiea (FS 1242-922-74), which is a modification of the macroiodometric method [8]. The highest level of resistance of the bacterial cells to ampicillin was determined on nutrient agar with antibiotic, in a concentration (μg/ml) yielding growth of 100% of cells.

The compatibility (incompatibility) of the replicons with reference-plasmids of the F-groups of incompatibility was determined by the usual method [5]. The sensitivity of the bacteria to phage was determined by the agar layers method. The frequency of plasmid transfer was studied by the standard method.

EXPERIMENTAL RESULTS

As was mentioned in the introduction, the work began with preparation of replicons (mini-plasmids) of plasmid pAP42::Tn1, for which purpose we used partial hydrolysis by restriction endonuclease HindIII of its DNA, ligation of the restriction fragments by ligase T4, and transformation of the HB101 cells by ligated mixtures of DNA.

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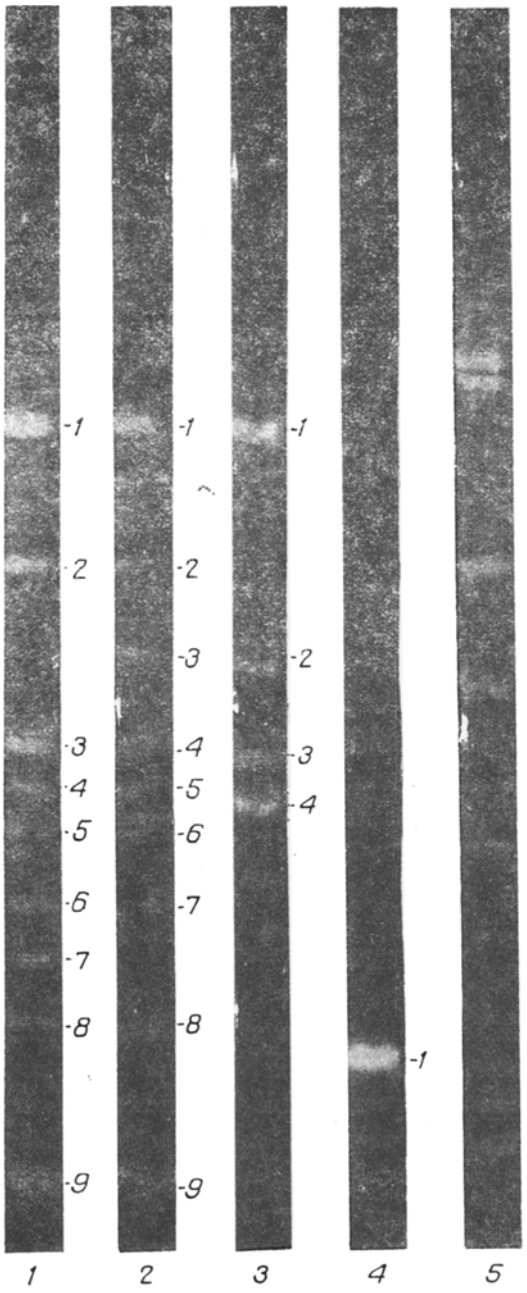

Fig. 1. Electrophoretic fractionation of restriction products of plasmid pAP42 and its replicons by enzyme HindIII. 1) pAP42, 2) pAP42::Tn1, 3) rep 1, 4) rep 2, 5) λ.

Since it was expected that cloned fragments of DNA of the original plasmid, capable of spontaneous replication (replicons) must be present in the transformants, the transformants obtained were studied for sensitivity to phage MS2, resistance to ampicillin and streptomycin, and also ability of the plasmid fragments contained in them to transfer themselves. As a result of these experiments, transformants with the phenotype $Tra^{-}MS2^{r}Ap^{r}Sm^{r}$ were selected for further work, and from them the plasma DNAs were isolated and subjected to HindIII-restriction analysis. In control experiments DNA of plasmid pAP42 and pAP42::Tn1 was analyzed in a similar way.

The results of the experiments to study the molecular properties of the presumed replicon structures are illustrated in Fig. 1 and Table 1.

TABLE 1. Molecular Weights of Restriction HindIII Fragments of Plasmid pAP42::Tn1 and its Replicons

Plasmids	Restriction fragments and molecular weights (in megadaltons)									Total mass
	f1	f2	f3	f4	f5	f6	f7	f8	f9	
pAP42:Tn1	12,5	6,4	4,7	3,7	3,4	3,0	2,4	1,6	0,9	38,5
rep1	12,5	4,7	3,7	3,4	—	—	—	—	—	24,3
rep2	1,4	—	—	—	—	—	—	—	—	1,4
pAP42	12,5	6,4	3,7	3,4	3,0	2,4	2,0	1,6	0,9	35,8

TABLE 2. Activity of β-Lactamase Coded by Plasmids in *E. coli* HB101

Plasmid	Activity of β-lactamase (in AU Ari units)	Number of plasmid copies
pAP42::Tn1	1 023	1—2
rep1	1 107	1—2
rep2	26 046	30—40

It will be clear from Fig. 1 and Table 1 that two replicons, designated rep 1 and rep 2, were cloned in the experiments described above. Replicon rep 1 is a structure composed of four HindIII-fragments with total molecular weight of 24.3 megadaltons (MD). Conversely, replicon rep 2 consists of only one HindIII-fragment with mol. wt. of 1.4 MD.

In subsequent experiments the number of copies of replicons rep 1 and rep 2 in the *E. coli* HB101 cells was determined. To determine the copy number, activity of β-lactamase of *E. coli* HB101 cells containing replicon rep 1 or rep 2, and of *E. coli* HB101 cells containing the original plasmid pAP42::Tn1, which exists in the cells in an amount equal to one or two copies per chromosome [1], was compared. The β-lactamase activity was expressed in conventional All-Union Antibiotics Research Institute units, the unit of activity being taken to be the smallest quantity of β-lactamase sufficient to inactivate 10^{-7} M benzylpenicillin per hour at 37°C in phosphate buffer (pH 6.8-7.0).

The results obtained in these experiments are given in Table 2.

It will be clear from Table 2 that the β-lactamase activity of cells containing replicon rep 1 is virtually equal to the β-lactamase activity of HB101 cells containing plasmid pAP42::Tn1. Hence it was concluded that replicon rep 1 has the same copy number as the original plasmid. Conversely, activity of β-lactamase coded by replicon rep 2 was 25 times greater than the β-lactamase activity coded by the original plasmid pAP42::Tn1. Since plasmid pAP42::Tn1 exists in the cells in one or two copies, it can be considered that replicon rep 2 is of the multiple copy type, and exists in the cells in 30-40 copies per chromosome.

In experiments with the aim of determining the level of resistance of cells containing different replicons to ampicillin, the highest level of resistance (2500 μg/ml) was observed for cells containing replicon rep 2. The level of resistance of these cells was 5 times higher than that of cells containing both the original plasmid and the replicon rep 1 (500 μg/ml). Clearly the increase in the copy number in the case of replicon rep 2 was accompanied by an increased gene dose effect.

The study of the Fin-activity of the constructed replicons, i.e., their ability to inhibit F-determined pilus-formation and transfer of plasmid F during conjugation, showed that it is largely a feature of replicon rep 1, for its presence in HB101 cells containing plasmid F'lac, completely abolishes their sensitivity to phage MS2 (inhibits F-pilus formation) and reduces the frequency of transmission of plasmid F'lac from cells of biplasmid donors into recipient (AP115) cells more than 11-fold compared with cells containing only one F'lac plasmid. Conversely, Fin-activity of replicon rep 2 is at a very low level. In particular, its presence in biplasmid cells reduces their sensitivity to phage MS2 by only 2-4 times, whereas their transfer frequency of plasmid F'lac is virtually unchanged. According to the results of control experiments, the original plasmid pAP42::Tn1 is able to inhibit the transmission frequency of plasmid F'lac by 25 times, and to reduce the phage-sensitivity of the biplasmid cells by half.

Identified replicons rep 1 and rep 2 were unable to transfer themselves from some cells to others, but they are maintained with stability in HB101 cells, as was shown by a study of their spontaneous elimination from cells of 100 arbitrarily chosen cloned cultures in the course of a year at different time intervals.

In the concluding experiments we studied the compatibility (incompatibility) of replicons rep 1 and rep 2 with reference plasmids of all 10 F-groups of incompatibility, including group Inc FIX, to which plasmid pAP42 belongs. The results of these experiments are given in Table 3.

TABLE 3. Compatibility (incompatibility) of Replicons Rep 1 and Rep 2 with Reference Plasmids of F-Groups of Incompatibility

Plasmids introduced		Index of compatibility with resident plasmids (in %)		
reference plasmid	incompatibility group	rep1	rep2	pAP42::Tn1
R386	FI	100	61	100
R1—19	FII	100	64	100
R3	FIII	100	71	100
R124	FIV	100	63	100
F°lac	FV	100	40	95
Hly—P212	FVI	100	100	95
pAP38::Tn9	FVII	81	100	100
pAP43::Tn9	FVIII	40	20	100
pAP42::Tn9	FIX	0	100	0
pAP18—1	FXI	100	100	100

It will be clear from Table 3 that replicon rep 1 is fully compatible with reference plasmids of seven incompatibility groups (FI, FII, FIII, FIV, FV, FVI, and FXI), it exhibits partial incompatibility with reference-plasmid group Inc FVIII, and complete incompatibility with reference-plasmid group Inc FIX, i.e., with the original plasmid pAP42. Conversely replicon rep 2 was found to be fully compatible with the reference-plasmid group Inc FIX, i.e., with the original plasmid, and also with reference plasmids of groups Inc FVI, FVII, and FXI, but incompatible with the reference-plasmid of group Inc FVIII. Replicon rep 2 exhibits weak partial incompatibility with plasmids of the remaining Inc groups.

It can be concluded from the discussion of the experimental results described in this paper that identified basic replicons rep 1 and rep 2 of plasmid pAP42 belong to different incompatibility groups (Inc FIX and Inc FVIII), which characterize partial incompatibility with reference-plasmids of other Inc F groups simultaneously. These results point directly to a connection between incompatibility of plasmids and their replication. As regards the atypical incompatibility arising simultaneously in the replicons, its nature requires special investigation.

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