INVESTIGATION OF COMPATIBILITY BETWEEN F-LIKE FACTORS OF GENETIC TRANSFER

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The study of the relationship of new F-like genetic transfer factors pAP22-4, pAP38, pAP39 and pAP41 to plasmids of the incompatibility F-groups has shown that transfer factor pAP38 is compatible with plasmids of all F-groups and is a representative of a new F-group of incompatibility, designated FVII/1, whereas the rest are characterized by compatibility with plasmids of some F-groups and by atypical incompatibility with plasmids of other F-groups. In particular, transfer factor pAP22-4 is partially incompatible with plasmids of groups FII, FIII and FIV, transfer factor pAP41 is partially incompatible with plasmids of groups FI and FIV, and transfer factor pAP39 is completely incompatible with plasmids of the FI and FIV groups [2].

Since each of the identified genetic transfer factors has an independent origin, their compatibility (incompatibility) with each other when introduced into *E. coli* was investigated and the results are given below.

EXPERIMENTAL METHOD

To carry out compatibility tests, the transfer factors to be studied, pAP22-4, pAP38, pAP39, and pAP41, were marked separately with transposons Tn1 and Tn9 (except factor pAP22-4), containing genes of resistance to ampicillin and chloramphenicol, respectively. The method of marking with transposon Tn1 was described previously [1, 2]. Marking with transposon Tn9 was carried out by a similar method, using cells of *E. coli* KS864 (the strain was obtained from G. B. Smirnov's laboratory, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), containing a temperature-sensitive mutant of plasmid RP1 with this transposon, as donor of the Tn9 transposon. Plasmids labeled with transposons were designated by symbols pAP22-4::Tn1, pAP38::Tn1, pAP38::Tn9, pAP39::Tn1, pAP39::In9:-pAP41::In1:pAP41::Tn9.

Compatibility (incompatibility) of the transfer factors was investigated by conjugation introduction of one or other transfer factor, labeled with transposon Tn9 from *E. coli* AP106 into cells of *E. coli* strain AP115, containing one of the transfer factors to be studied, labeled with transposon Tn1, as resident plasmid, and vice versa, with a subsequent study of cells of transconjugant colonies, for the presence of one or both plasmids in them. Each of the transfer factors for study was thus tested as introduced plasmid and as resident plasmid. Whenever necessary, clones of cells which were tested for plasmid content after culture and seeding on medium with different antibiotics (clonal test) and for the character of transfer of the plasmid contained in them in further crosses with suitable recipient cells, were selected from transconjugant colonies.

EXPERIMENTAL RESULTS

To determine compatibility (incompatibility) of the transfer factors with each other, 1800 transconjugant colonies, isolated from 18 direct and reciprocal crosses (100 of each) were selected and studied for their plasmid content; with this number it was possible to study the behavior of each transfer factor as introduced plasmid and as resident plasmid.

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TABLE 1. Compatibility (Incompatibility) of Transfer Factors pAP22-4, pAP38, pAP39, and pAP41 in *E. coli* AP115

Plasmid		Selective marker	Frequency of transfer (per donor cell)	Index of surface exclusion	Number of colonies (in %) cells of which contain		
introduced	resident				introduced plasmid	resident plasmid	both plasmids
pAP38::Tn9	pAP22-4::Tnl	Lm	$1,6 \cdot 10^{-3}$	40	100	95	95
pAP38::Tn9		Lm	$6,5 \cdot 10^{-2}$		100		
pAP22-4::Tnl	pAP38::Tn9	Ap	$3,7 \cdot 10^{-2}$	15,4	100	100	100
pAP22-4::Tnl		Ap	$5,7 \cdot 10^{-1}$		100		
pAP38::Tn9	pAP39::Tnl	Lm	$5,2 \cdot 10^{-2}$	1,2	100	100	100
pAP39::Tnl	pAP38::Tn9	Ap	$4,8 \cdot 10^{-4}$	2,2	100	100	100
pAP39::Tnl		Ap	$1,1 \cdot 10^{-3}$		100		
pAP38::Tn9	pAP41::Tnl	Lm	$5 \cdot 10^{-2}$	1,3	100	100	100
pAP41::Tnl	pAP38::Tn9	Ap	$1,9 \cdot 10^{-3}$	2,2	100	100	100
pAP41::Tnl		Ap	$4,3 \cdot 10^{-3}$		100		
pAP39::Tn9	pAP22-4::Tnl	Lm	$1 \cdot 10^{-4}$	3,5	100	100	100
pAP39::Tn9		Lm	$3,5 \cdot 10^{-4}$		100		
pAP22-4::Tnl	pAP39::Tn9	Ap	$8,2 \cdot 10^{-1}$	1,2	100	100	100
pAP22-4::Tnl		Ap	$1 \cdot 10^{0}$		100		
pAP39::Tn9	pAP38::Tnl	Lm	$3 \cdot 10^{-3}$	0,11	100	100	100
pAP38::Tnl	pAP39::Tn9	Ap	$4,7 \cdot 10^{-2}$	1,1	100	100	100
pAP38::Tnl		Ap	$5,2 \cdot 10^{-2}$		100		
pAP39::Tn9	pAP41::Tnl	Lm	$6 \cdot 10^{-5}$	5,8	100	100	100
pAP41::Tnl	pAP39::Tn9	Ap	$7 \cdot 10^{-3}$	1,4	100	100	100
pAP41::Tnl		Ap	$1 \cdot 10^{-2}$		100		
pAP41::Tn9	pAP22-4::Tnl	Lm	$5,3 \cdot 10^{-3}$	4,7	100	95	95
pAP41::Tn9		Lm	$2,5 \cdot 10^{-2}$		100		
pAP22-4::Tnl	pAP41::Tn9	Ap	$8,9 \cdot 10^{-1}$	1,1	100	100	100
pAP22-4::Tnl		Ap	$1 \cdot 10^{0}$		100		
pAP41::Tn9	pAP38::Tnl	Lm	$1,2 \cdot 10^{-2}$	2,0	100	100	100
pAP38::Tnl	pAP41::Tn9	Ap	$4,5 \cdot 10^{-2}$	1,1	100	100	100
pAP38::Tnl		Ap	$5,2 \cdot 10^{-2}$		100		
pAP41::Tn9	pAP39::Tnl	Lm	$1 \cdot 10^{-2}$	2,5	100	0	0
pAP39::Tnl	pAP41::Tn9	Ap	$9 \cdot 10^{-6}$	333	100	97,5	97,5
pAP39::Tnl		Ap	$3 \cdot 10^{-3}$		100		

Analysis of the results of these experiments (Table 1) showed that all 100 transconjugant colonies from crosses in which the relationship between transfer factors was shown to be pAP38::Tn9 to pAP39::Tnl and vice versa, pAP39::Tn9 to pAP22-4::Tnl and vice versa, pAP39::Tn9 to pAP38::Tnl and vice versa, pAP39::Tn9 to pAP41::Tnl and vice versa, and also paP41::Tn9 to pAP38::Tnl and vice versa, consisted of cells containing both an introcuced plasmid and a resident plasmid. Conversely, among transconjugant colonies isolated from crosses in which the relationship of transfer factors pAP38::Tn9 and pAP41::Tn9 to pAP22-4::Tnl were analyzed, cells which had lost the resident plasmid were observed in a small proportion of cases (5%) although all transconjugants from back-crosses each contained two plasmids. Finally, cells of all 100 colonies from crosses in which the introduced plasmid was transfer factor pAP41::Tn9 and the resident plasmid was transfer factor pAP39::Tnl contained only an introduced plasmid, although all transconjugants from the back-cross contained two plasmids each (introduced and resident).

The presence of both transfer factors (introduced and resident) in the transconjugants in the case of the combinations mentioned above indicated, conjecturally, that these transfer factors are compatible with each other, whereas partial or total loss of resident transfer factor by the transconjugants in other combinations could be evidence of partial or complete incompatibility of the two plasmids. To test these hypotheses, a few colonies the cells of which each contained two plasmids were selected from each cross (except that in which the introduced transfer factor was pAP41::Tn9 and the resident pAP39::Tnl), the colonies were seeded in nutrient broth, cultured for 18 h, after which seedings were taken from the clonal cultures thus obtained on nutrient agar in order to obtain isolate colonies, which were then tested for the presence of transfer factors — introduced and resident. The results of these experiments are given in Table 2. As Table 2 shows, cultivation of clonal cultures of transconjugants each containing a pair of transfer factors, preliminarily estimated to be compatible, was accompanied by stable preservation of both these plasmids by the cells. Additional experiments to study the character of transfer from these diplasmid factors confirmed this hypothesis, for they showed that transfer factors are transmitted separately to recipient cells. In the case of transconjugant clones containing transfer factor pAP38::Tn9 as introduced plasmid and transfer factor pAP22-4::Tnl as resident plasmid, the cells of most clones

TABLE 2. Clonal Test for Compatibility (Incompatibility) of Transfer Factors pAP22-4, pAP38, pAP39, and pAP41 with Each Other

Plasmid		Selective marker	Transconjugant clones	Number of colonies (in %) cells of which contain		
introduced	resident			introduced plasmid	resident plasmid	both plasmids
pAP38::Tn9	pAP22-4::Tn1	Lm	1	100	95	95
			2	95	95	90
			3	100	100	100
			4	100	100	100
			5	100	100	100
pAP22-4::Tn1	pAP38::Tn9	Ap	1	100	100	100
pAP38::Tn9	pAP39::Tn1	Lm	1	100	100	100
pAP39::Tn1	pAP38:Tn9	Ap	1	100	100	100
pAP38::Tn9	pAP41::Tn1	Lm	1	100	100	100
pAP41::Tn1	pAP38::Tn9	Ap	1	100	100	100
pAP39::Tn9	pAP22-4::Tn1	Lm	1	100	100	100
pAP22-4::Tn1	pAP39::Tn9	Ap	1	100	100	100
pAP39::Tn9	pAP38::Tn1	Lm	1	100	100	100
pAP38::Tn1	pAP39::Tn9	Ap	1	100	100	100
pAP39::Tn9	pAP41::Tn1	Lm	1	100	100	100
pAP41::Tn1	pAP39::Tn9	Ap	1	100	100	100
pAP41::Tn9	pAP22-4::Tn1	Lm	1	100	100	100
			2	100	100	100
			3	100	100	100
			4	100	100	100
			5	100	100	100
pAP22-4::Tn1	pAP41::Tn9	Ap	1	100	100	100
pAP41::Tn9	pAP38::Tn1	Lm	1	100	100	100
pAP38::Tn1	pAP41::Tn9	Ap	1	100	100	100
pAP39::Tn1	pAP41::Tn9	Ap	1	75	30	5
			2	25	95	20
			3	25	100	25
			4	25	95	20
			5	25	95	20

nevertheless contained both plasmids, and the frequency of loss of one plasmid by cells of the remaining clones was very low. Experiments on transfer of plasmids to recipient cells showed that it takes place separately. Consequently, plasmids pAP38 and pAP22-4 are compatible. Culture of clones containing transfer factor pAP41::Tn9 as introduced plasmid and transfer factor pAP-22-4::Tn1 as resident factor also was accompanied by stable preservation of both plasmids (just as in the opposite combination). As experiments on the transfer of these plasmids to recipient cells showed, they are transmitted separately, evidence of their compatibility. Rather different data were obtained in a study of tranconjugant clones in which the introduced plasmid was transfer factor pAP39::Tn1 and the resident plasmid was transfer factor pAP41::Tn9. Cultivation of these clones was accompanied by appreciable loss of one of the plasmids, although transfer of plasmids by cells of these clones to recipient cells also takes place separately.

On evaluation of the results it can be concluded that transfer factor pAP38 is compatible with transfer factors pAP22-4, pAP39, and pAP41, and transfer factor pAP22-4 is compatible with factors pAP39 and pAP41. Conversely, transfer factors pAP39 and pAP41 are incompatible with one another, and in this case, moreover, factor pAP41 is the "superior" plasmid. This indicates that factors pAP39 and pAP41 probably share a common phylogeny.

LITERATURE CITED

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