LITERATURE CITED

1. I. A. Alov, Vest. Akad. Med. Nauk SSSR, No. 11, 58 (1965).
2. I. A. Alov, in: Cytophysiology and Pathology of Mitosis [in Russian], Moscow (1972).
3. I. A. Alov, in: Elements of Cell Pathology. Progress in Science and Technology, Series Cytology [in Russian], Vol. 3, Moscow (1976), pp. 40-66.
4. I. A. Alov, M. E. Aspiz, and O. M. Zapara, Byull. Éksp. Biol. Med., No. 7, 874 (1976).
5. M. E. Aspiz, "Formation of the mitotic apparatus and pathology of mitosis in normal and tumor cells," Author's Abstract of Doctoral Dissertation, Moscow (1974).
6. N. A. Graevskaya, N. M. Strizhachenko, V. Ya. Karmysheva, et al., Vopr. Onkol., No. 5, 79 (1972).
7. I. A. Kazantseva, in: Proceedings of the 6th All-Union Congress of Pathological Anatomists [in Russian], Moscow (1977), p. 27.
8. V. A. Rozenblat, "Investigation of the mechanisms of resistance of tumor cells to colchicine," Author's Abstract of Candidate's Dissertation, Leningrad (1974).
9. A. A. Stavrovskaya, "Mechanisms of origin of genome mutations in tumor cells," Author's Abstract of Doctoral Dissertation, Moscow (1975).
10. A. A. Stavrovskaya, E. S. Kakpakova, and T. S. Stromskaya, Tsitologiya, No. 6, 739 (1973).
11. N. H. Lewis, Ann. N. Y. Acad. Sci., 51, 1284 (1951).
12. W. McLean and P. Keen, Exp. Cell Res., 80, 345 (1973).
13. L. Roth and Y. Shigenska, J. Ultrastruct. Res., 31 356 (1970).

ATYPICAL INCOMPATIBILITY OF F-LIKE GENETIC TRANSFER FACTORS pAP22-4, pAP39, AND pAP41 WITH F-GROUP INCOMPATIBILITY PLASMIDS

A. P. Pekhov, V. P. Shipkov,
V. N. Reshetnikova, E. V. Gubar',
and N. A. Drobysheva

UDC 576.851.48.095.57:575.133

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

When studying the plasmid complex found in cells of serologically typed (serogroup 06) strain Escherichia coli AP1 [1-3], we identified in it a genetic transfer factor pAP22-4 with a molecular weight of $39.3 \cdot 10^6$ daltons. Meanwhile in bacteria of strains AP25 (serogroup 076) and AP26 (serogroup 0121) transfer factors pAP39 and pAP41 with molecular weights of $42.6 \cdot 10^6$ and $90 \cdot 10^6$ daltons, respectively, were identified. All these factors are F-like plasmids.

The relationship of the identified transfer factors to F-group incompatibility plasmids was studied in the present investigation.

EXPERIMENTAL METHOD

Genetic marking of the transfer factors was carried out in crosses in which the donors were cells of E. coli 1553, carrying mutant temperature-sensitive plasmid RP4 [4] with transposon Tn1, containing the gene of resistance to ampicillin (Ap), served as donors and E. coli AP105 cells carrying one of the transfer factors under investigation, served as recipients. The transfer factors marked by the Tn1 transposon (resistance to ampicillin) were designated pAP22-4:: Tn1, pAP39:: Tn1, and pAP41:: Tn1. In experiments to determine compatibility (incompatibility) of the transfer factors with F-like plasmids, plasmids R386, R1-19, ColB-R3, R124, F_0lac, and Hly-P212, reference plasmids of the incompatibility group FI, FII, FIII, FIV, FV, and FVI respectively, were used. These experiments were carried out by a method according to which a given transfer factor

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 8, pp. 205-208, August, 1980. Original article submitted December 19, 1979.

TABLE 1. Colonial Test of Compatibility (Incompatibility) of Transfer Factors pAP22-4 and pAP41 with F-Group Plasmids in E. coli AP115

Plasmid		Selective marker	Frequency of transfer (per donor cell)	Index of surface exclusion	Number of colonies (in %) whose cells contain		
introduced	resident				introduced plasmid	resident plasmid	both plasmids
pAP22-4::Tn1	R1-19 (FII)	Ap	$3,3 \cdot 10^{-1}$	2,5	100	75	75
pAP22-4::Tn1		Ap	$8,3 \cdot 10^{-1}$		100		
R1-19 (FII)	pAP22-4::Tn1	Km	$3,9 \cdot 10^{-1}$	0,8	100	100	100
R1-19 (FII)		Km	$3 \cdot 10^{-1}$		100		
pAP22-4::Tn1	ColB-R3 (FIII)	Ap	$7,7 \cdot 10^{-1}$	1,1	100	64	64
ColB-R3 (FIII)	pAP22-4::Tn1	Lm	$3 \cdot 10^{-2}$	0,9	100	50	50
ColB-R3 (FIII)		Lm	$2,8 \cdot 10^{-2}$		100		
pAP22-4::Tn1	R124 (FIV)	Ap	$2,2 \cdot 10^{-1}$	3,8	100	100	100
R124 (FIV)	pAP22-4::Tn1	Tc	$6 \cdot 10^{-3}$	1,2	100	77	77
R124 (FIV)		Tc	$7 \cdot 10^{-3}$		100		
pAP22-4::Tn1	F_0lac (FV)	Ap	$1,7 \cdot 10^{-3}$	480	100	80	80
F_0lac (FV)	pAP22-4::Tn1	Lac	$3 \cdot 10^{-7}$	7,7	100	84	84
F_0lac (FV)		Lac	$2,3 \cdot 10^{-6}$		100		
pAP41::Tn1	R386 (FI)	Ap	$3 \cdot 10^{-3}$	1,7	100	59	59
pAP41::Tn1		Ap	$5 \cdot 10^{-3}$		100		
R386 (FI)	pAP41::Tn1	Tc	$5 \cdot 10^{-2}$	1,6	100	85	85
R386 (FI)		Tc	$8 \cdot 10^{-2}$		100		
pAP41::Tn1	R124 (FIV)	Ap	$2 \cdot 10^{-4}$	25	100	75	75
R124 (FIV)	pAP41::Tn1	Tc	$1 \cdot 10^{-4}$	60	100	100	100
R124 (FIV)		Tc	$6 \cdot 10^{-3}$		100		

was introduced by conjugation crosses into cells of E. coli AP115, containing as resident plasmid the reference plasmid of one of the incompatibility F-groups and, conversely, a given reference plasmid was introduced into cells containing one of the transfer factors as resident plasmid. The conjugation mixtures of bacteria were seeded on selective media, by means of which transconjugants for the introduced plasmid could be selected. To determine the plasmid content of the conjugants from each cross, 100 transconjugant colonies were selected and seeded on two media with additives, one of which enabled the introduced plasmid, the other the resident plasmid, to be detected in the bacteria (the colonial test). Depending on the results, the next step was to carry out clonal tests and to study transfer of plasmids from the transconjugants to the recipient bacteria. Data on surface exclusion of plasmids were obtained in control crosses, in which the transfer factors and reference plasmids were introduced separately into plasmid-free AP115 cells.

EXPERIMENTAL RESULTS

To introduce marked transfer factors into E. coli AP115 cells containing reference plasmids of F-groups as resident plasmids, and vice versa, 36 direct and reciprocal crosses were carried out, from which 3600 transconjugant colonies were selected. As the colonial tests showed, colonies isolated from different crosses were distributed among three types depending on the plasmids contained in their cells.

The first type consisted of transconjugant colonies, the cells of which contained the introduced plasmid, but were partly without the resident plasmid. These were found to be colonies isolated from crosses in which the relationship of transfer factor pAP22-4:: Tn1 to reference plasmids of groups FII, FIII, FIV, and FV and of transfer factor pAP41 to reference plasmids of groups FI and FIV were analyzed (Table 1).

It will be clear from Table 1 that among each 100 colonies isolated from the above crosses there were some whose cells had lost the resident plasmid. The only exceptions were colonies from crosses in which the relationship of transfer factors pAP22-4:: Tn1 and pAP41:: Tn1 to reference plasmids R1-19 (FII) and R124 (FIV) was analyzed.

Being introduced plasmids, the transfer factors eliminated the reference plasmids from the cells when the latter were resident. However, in the converse case, if they were resident, they were not eliminated by introduced reference plasmids. It will also be clear from Table 1 that surface exclusion – the indices of which were determined as the quotient from division of the frequency of transfer of the plasmid into plasmid-free cells by the frequency of transfer of the same plasmid into cells containing a resident plasmid – was not associated with subsequent elimination of the plasmids.

The results indicated neither compatibility nor incompatibility of the transfer factors with the reference plasmids. Clonal tests were therefore carried out. For this purpose, three colonies whose cells contained both plasmids were sampled from each 100 colonies obtained in the original crosses, seeded on nutrient broth, and after five subcultures (each after 18 h) the clonal transconjugant cultures were seeded on nutrient agar to

TABLE 2. Clonal Tests of Compatibility (Incompatibility) of Transfer Factors pAP22-4:: Tn1 and pAP41:: Tn1 with Reference Plasmids of Groups FI, FII, FIII, FIV, and FV

Plasmid		Selective marker	Transconjugant clones	Number of transconjugants (in %) whose cells contain		
introduced	resident			introduced plasmid	resident plasmid	both plasmids
pAP22-4: :Tn1	R1-19 (FII)	Ap	1	100	56	56
			2	100	78	78
			3	100	64	64
R1-19 (FII)	pAP22-4 : :Tn1	Km	1	86	100	86
			2	86	98	84
			3	76	92	68
pAP22-4: :Tn1	ColB-R3 (FIII)	Ap	1	100	70	70
			2	100	68	68
			3	96	76	72
ColB-R3 (FIII)	pAP22-4: :Tn1	Lm	1	80	98	78
			2	82	100	82
			3	86	100	86
pAP22-4: :Tn1	R124 (FIV)	Ap	1	56	100	56
			2	40	100	40
			3	52	100	52
R124 (FIV)	pAP22-4: :Tn1	Tc	1	86	58	58
			2	100	78	78
			3	100	80	80
pAP22-4: :Tn1	F_0lac (FV)	Ap	1	100	100	100
			2	100	100	100
			3	100	100	100
F_0lac (FV)	pAP22-4: :Tn1	Lac	1	98	98	98
			2	100	100	100
			3	100	100	100
pAP41: :Tn1	R386 (FI)	Ap	1	100	80	80
			2	100	76	76
			3	100	62	62
R386 (FI)	pAP41: :Tn1	Tc	1	98	100	98
			2	64	100	64
			3	62	96	62
pAP41: :Tn1	R124 (FIV)	Ap	1	96	88	84
			2	98	90	88
			3	76	88	64
R124 (FIV)	pAP41: :Tn1	Tc	1	78	84	62
			2	68	94	62
			3	52	98	50

obtain isolated colonies. Fifty colonies from each clonal culture were tested for the presence of introduced and resident plasmids and of both plasmids in their cells. The results of these tests are given in Table 2.

It will be clear from Table 2 that during long-term culture the cells of transconjugant colonies of nearly all crosses lost one of their plasmids. These results suggested that transfer factor pAP22-4:: Tn1 is partially incompatible with reference plasmids of groups FII, FIII, and FIV, whereas transfer factor pAP41:: Tn1 is partially incompatible with reference plasmids from groups FI and FIV. The only exceptions were clones of transconjugants obtained in crosses in which the introduced plasmid was factor pAP22-4:: Tn1 and the resident plasmid was F_0lac (FV), and vice versa. Although in the colonial test some loss of residence was observed, in the clonal test the cells preserved both plasmids; this result probably reflects a phenomenon of "crowding out," which is sometimes found with compatible plasmids [5].

To prove the hypothesis of partial incompatibility of the transfer factors with individual reference plasmids, additional experiments were carried out to study how both plasmids, contained in biplasmid transconjugants are transmitted to recipient cells in subsequent crosses (whether jointly or separately). For this purpose, one of each three transconjugant clonal cultures described above was selected and crossed with E. coli AP106. The conjugation mixtures were seeded on two media, so that separate selection of daughter transconjugants could be carried out for the marker of each plasmid. From each selective medium 10 daughter transconjugants were then selected and inheritance of the nonselective marker (the other plasmid) by them was determined. As the results of these experiments showed, for daughter conjugants except those obtained from crosses in which the relationship of transfer factor pAP22-4:: Tn1 to plasmid F_0lac was analyzed, a high frequency of inheritance of the nonselective marker (the second plasmid) was a characteristic feature. These results thus confirmed the hypothesis drawn from the results of the colonial tests.

Transconjugant colonies whose cells contained only the introduced plasmid belonged to the second type. They were colonies isolated from crosses in which the role of introduced plasmids was played by reference plasmids R386 (FI) and R124 (FIV) and the resident plasmid was transfer factor pAP39:: Tn1. However, in analogous crosses in which the introduced plasmid was the transfer factor and plasmids R386 (FI) and R124 (FIV) were residents, the result was rather different. Whereas in the first case cells of 90% of colonies con-

tained resident plasmid, in the second case resident plasmid was found in cells of only 10% of colonies. To determine how strongly the two plasmids were maintained in the cells of these colonies, clonal tests were carried out. They showed that the cells of all colonies after culture completely lost the introduced plasmid. It can therefore be concluded that transfer factor pAP39:: Tn1 is completely incompatible with plasmids of two incompatibility groups (FI and FIV) simultaneously.

Cells of transconjugant colonies of the third type contained introduced and resident plasmids. These were colonies isolated from crosses in which the introduced plasmid was transfer factor pAP22-4:: Tn1 and the resident plasmids were R386 (FI) and Hly-P212 (FVI) and vice versa, and also from crosses in which the introduced plasmids were transfer factors pAP39:: Tn1 and pAP41:: Tn1 and the resident plasmids were R1-19 (FII), ColB-R3 (FIII), F_0lac (FV), and Hly-P212 (FVI) and vice versa. Since the coexistence of these plasmids in the cells indicated their compatibility, clonal tests were carried out to verify the stability of this coexistence. The character of transfer of the coexisting plasmids in later crosses also was studied.

As the experiments showed, cells of all clonal cultures, despite a long period of culture, constantly preserved two plasmids (introduced and resident), and also transmitted them separately in subsequent crosses. Consequently, transfer factor pAP22-4:: Tn1 is compatible with plasmids of groups FI and FVI, and transfer factors pAP39:: Tn1 and pAP41:: Tn1 are compatible with plasmids of groups FII, FIII, FV, and FVI.

The results of these experiments can be summarized in the statement that F-like transfer factors are characterized by different relationships to F-group incompatibility plasmids. Transfer factor pAP22-4 is compatible with plasmids of incompatibility groups FI, FV, and FVI but is partially incompatible with plasmids of groups FII, FIII, and FIV. Transfer factor pAP41 is compatible with plasmids of groups FII, FIII, FV, and FVI, but is partially incompatible with plasmids of groups FI and FIV. This atypical incompatibility probably reflects the commencing evolution of these transfer factors. Conversely, transfer factor pAP39 is compatible with plasmids of groups FII, FIII, FV, and FVI but incompatible at the same time with plasmids of groups FI and FIV. This probably indicates that it is a natural recombinant of two transfer factors from the other groups. This explanation is supported by the discovery of a laboratory recombinant for incompatibility between plasmids of groups FI and FII [6].

LITERATURE CITED

1. N. I. Buyanova, V. P. Shipkov, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 9, 106 (1973).
2. A. P. Kalyuzhnaya, N. I. Matvienko, V. P. Shipkov, et al., Byull. Éksp. Biol. Med., No. 12, 1492 (1976).
3. A. P. Pekhov, V. P. Shipkov, and N. I. Buyanova, Dokl. Akad. Nauk SSSR, 220, 711 (1975).
4. Yu. G. Stepanshin, F. A. Amosenko, and V. N. Danilevich, in: Abstracts of Proceedings of the 4th Working Conference on the "Plasmid" Program [in Russian], Tartu (1979), pp. 135-138.
5. J. N. Coetzee, N. Datta, and R. W. Hedges, J. Gen. Microbiol., 73, 543 (1972).
6. P. Cooper, Genet. Res., 17, 151 (1971).