GENETIC CHARACTERISTICS OF PLASMID pAP53 DEREPRESSED FOR TRANSFER FUNCTIONS DETECTED IN CELLS OF A CONVENTIONALLY PATHOGENIC STRAIN OF *Escherichia coli*

N. I. Buyanova, V. P. Shchipkov, and A. P. Pekhov

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Conjugative plasmids identified in cells of conventionally pathogenic strains of *Escherichia coli* isolated from patients and sick livestock can differ significantly in the effectiveness of transmission by bacteria of plasmid-free strains of *E. coli* K-12 [3, 4]. One cause of this phenomenon is probably connected with characteristics of the genetic systems of individual plasmids controlling their transfer function in the process of conjugation of the corresponding bacterial cells [7, 10, 11].

This paper describes a study of the genetic characteristics of F-like plasmid pAP53, derepressed for transfer functions, determining colicin synthesis and discovered by the writers previously in cells of a conventionally pathogenic strain of *E. coli* belonging to serogroup O128 [2].

EXPERIMENTAL METHOD

Conjugation transfer of plasmids was carried out during standard 2-h crosses [4], using strains of *E. coli* K-12 resistant to nalidixic acid (AP107, AP132) or to streptomycin (AP106, C600) as donors or recipients. The frequency of plasmid transfer was determined from the number of corresponding plasmid transconjugants, expressed per cell of the donor strain from the conjugation mixture.

Sensitivity of the bacteria to donor-specific phage MS2 was determined by the agar layers method [12]. For quantitative estimation of the efficiency of synthesis and function of the plasmid-specific "sex fimbriae" of the bacterial cells, the phage titer increase test (PTIT) also was studied in accordance with the standard scheme [8]. Indices of the phage titer increase (IPTI) were calculated as the ratio of the mean number of phage zones of lysis in the experiment (incubation of the phage-bacterial mixture at 37°C for 18 h) and their number in the control (before the beginning of incubation of the mixture). The index of the fall in frequency of transfer of the derepressed (standard) plasmid or of the IPTI level in the presence of the second (test) plasmid was determined as the quotient obtained by dividing the frequency of transfer or the IPTI level for the strain containing only one reference plasmid by the corresponding value obtained for a strain containing reference and test plasmids simultaneously.

Genetic tagging of plasmid pAP53 was carried out by incorporating transposons into its structure — Tn1 (resistance of the bacteria to ampicillin) or Tn9 (resistance to chloramphenicol) in accordance with the standard scheme [1, 5]. Compatibility (incompatibility) of the tagged plasmid was studied by the method in [6], based on the generally accepted scheme [9], by using a reference plasmid of all known incompatibility groups of F-like plasmids.

EXPERIMENTAL RESULTS

During the preliminary study of characteristics of plasmid pAP53 it was found that it behaves in cells of different strains of *E. coli* K-12 as derepressed for genetic transfer (Tra) functions. During conjugation of these bacteria they can be transferred into cells of plasmid-

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TABLE 1. Ability of Individual Plasmids to Inhibit Conjugation Transfer of Derepressed Plasmids and Synthesis of Donor-Specific Fimbriae Controlled by Them in *E. coli* AP132 Cells

Introduced plasmid (test)	Resident plasmid (reference)	Frequency of transfer of introduced plasmid	Analysis of transconjugants obtained: Sensitivity to phage MS2	IPTI MS2	frequency of transfer of plasmids into C600 cells: introduced	frequency of transfer of plasmids into C600 cells: resident	index of decrease: IPTI	index of decrease: of frequency of transfer of resident plasmid
pAP53 :: Tn9	Flac	$4 \cdot 10^{-2}$	+	$0{,}4 \cdot 10^6$—$2 \cdot 10^6$	$1{,}0 \cdot 10^{-3}$—$2{,}7 \cdot 10^{-3}$	0,2—0,4	0,02—0,5	1,3—3
R1	pAP53 :: Tn9	$1{,}4 \cdot 10^{-5}$	++	$0{,}4 \cdot 10^6$—$0{,}5 \cdot 10^6$	$0{,}4 \cdot 10^{-3}$—$2{,}0 \cdot 10^{-3}$	0,1—0,15	2,8—3,5	1,4—2,5
pAP17-1	pAP53 :: Tn9	$1{,}4 \cdot 10^{-3}$	+	$1{,}6 \cdot 10^6$—$4 \cdot 10^6$	$0{,}4 \cdot 10^{-3}$—$1{,}0 \cdot 10^{-3}$	1,2—2,18	0,25—0,6	0,2—0,4
pAP41 :: Tn1	pAP53 ::Tn9	$2 \cdot 10^{-6}$	—	$0{,}4 \cdot 10^4$—$0{,}9 \cdot 10^4$	$0{,}8 \cdot 10^{-2}$—$1{,}7 \cdot 10^{-2}$	$1{,}0 \cdot 10^{-2}$—$1{,}8 \cdot 10^{-2}$	200—500	44—80
	Flac	—	+	$0{,}4 \cdot 10^6$	—	0,5—0,55		
	pAP53 :: T9	—	+	$1{,}4 \cdot 10^6$	—	0,2—0,8		

TABLE 2. Results of Determination of Surface Exclusion Functions of Plasmid pAP53 in Cells of *E. coli* AP132

Introduced plasmid	Resident plasmid	Frequency of transfer of introduced plasmid	Surface exclusion index	Index of decrease of surface exclusion
	—	1,3—2,3	—	—
pAP53	pAP53	$1{,}7 \cdot 10^{-2}$—$2{,}3 \cdot 10^{-2}$	76—100	—
	pAP41:: Tn1	0,2—0,4	5,7—6,2	—
	(pAP53)(pAP41 :: Tn1)	$0{,}8 \cdot 10^{-2}$—$1{,}2 \cdot 10^{-2}$	173—208	0,4—0,5

Legend. In each case, three clones of plasmid transconjugants were investigated.

free strains and maintain their sensitivity to donor-specific phage MS2 just as effectively as the derepressed reference plasmid.

For the subsequent investigation, tagged versions of this plasmid were obtained and designated pAP53::Tn1 and pAP53::Tn9. Compatibility tests showed that these two variants are practically completely incompatible with one another (compatibility indices, i.e., the frequency of clones of double transconjugants preserving both plasmids, were 0-5%). Later experiments also showed that test plasmid pAP53 is incompatible with plasmid colB-R3 (compatibility indices 0-5%), i.e., it is a member of incompatibility group FIII. Meanwhile, partial incompatibility was observed between plasmid pAP53 and reference plasmid R1-19, belonging to the FII group (compatibility indices 61-91%). This plasmid was compatible with plasmid groups FI, FIV, FV, FVI, FVII, FVIII, FIX (compatibility indices 94-100%).

In order to study the genetic system regulating Tra functions of the tagged plasmid pAP53, double plasmid transconjugants carrying this plasmid and also one of the test plasmids were obtained and studied. Of the test plasmids, both familiar plasmids of incompatibility groups F, N, and P (F lac, R1, R46, RP4) and also plasmids identified by the writers previously [3, 4, 8] in cells of conventionally pathogenic strains of *E. coli* (pAP2, pAP17-1, pAP22-1, pAP27, pAP28, pAP29, pAP30-1, pAP30-2, pAP31, pAP32, pAP33-1, pAP33-2, pAP38, pAP39, pAP41, pAP43) were used. As Table 1 shows, plasmid pAP53 cannot effectively inhibit transfer of reference derepressed plasmid F lac from cells of strain AP132 into cells of plasmid-free strain C600, probably because of the absence of a functionally active regulator gene (or genes) in the structure of plasmid pAP53, capable of supporting synthesis of the specific transfer inhibitor for the regulating system of the Tra functions of the F lac plasmid. Meanwhile, the Tra functions of plasmid pAP53 are not inhibited in the presence of plasmid R1 (Table 1), which possesses a genetic regulation system described as a fin OP system and capable of inhibiting the Tra functions of plasmid F lac [11]. Similar results were obtained by investigation of the overwhelming majority of other test plasmids listed above.

However, an inhibitory effect was found during the study of plasmid pAP41, identified by the writers previously and classed in the category of "pure" genetic transfer factors and tagged by means of transposon Tn1 [6]. For instance, the investigation of double transconjugants obtained by transfer of test plasmid pAP41::Tn1 into AP132 cells with resident derepressed plasmid pAP53::Tn9, a considerable decrease was found both in the frequency of transfer of the resident plasmid into C600 cells and in the functional activity of the sex fimbriae synthesized under its control, as judged from the values of IPTI (Table 1).

Taking into account existing views on connections between the genetic mechanisms controlling these two Tra functions and those regulating surface exclusion functions of plasmids [11], additional experiments were carried out to study the ability of plasmid pAP41 to inhibit the surface exclusion function of plasmid pAP53 (Table 2). In each case, the surface exclusion index was determined as the quotient from dividing the frequency of transfer of plasmid pAP53::Tn9 into cells of plasmid-free strain AP132 by the frequency of its transfer into cells of clonal strains AP132, carrying the corresponding plasmids. The index of decrease of surface exclusion was calculated by dividing the corresponding surface exclusion indices for the recipient strain with resident plasmid pAP53 and for the strain containing two plasmids (pAP53 and pAP41::Tn1) simultaneously. It can be concluded from analysis of the data in Table 2 that plasmid pAP41 is unable to inhibit the functions of plasmid pAP53 connected with its restriction of the frequency of transfer of the superinfecting isogenic plasmid pAP53::Tn9 into the corresponding bacterial cell.

On the whole, the results are evidence that plasmid pAP53 has no functionally active system of inhibiting Tra functions of the fin OP type, which is probably the system of genetic regulation most frequently found in F-like plasmids. At the same time, this plasmid is sensitive to the action of an inhibitor system of another type, for which plasmid pAP41 is a carrier. Because of these features, plasmid pAP53 can be regarded as an additional test system which can be used for the classification of new plasmids on the basis of the characteristics of their systems regulating genetic transfer functions.

LITERATURE CITED

1. N. I. Buyanova, V. P. Shchipkov, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 2, 210 (1981).
2. N. I. Buyanova, V. P. Shchipkov, and A. P. Pekhov, in: Abstracts of Proceedings of the 6th Working Conference on the "Plasmid" Program [in Russian], Moscow (1981), pp. 34-35.
3. A. P. Pekhov, V. P. Shchipkov, T. Arai, et al., Zh. Mikrobiol., No. 9, 45 (1979).
4. A. P. Pekhov, V. P. Shchipkov, and E. V. Gubar', Zh. Mikrobiol., No. 12, 31 (1980).
5. A. P. Pekhov, V. P. Shchipkov, V. N. Reshetnikova, et al., Dokl. Akad. Nauk SSSR, No. 5, 1260 (1980).
6. A. P. Pekhov, V. P. Shchipkov, V. N. Reshetnikova, et al., Byull. Éksp. Biol. Med., No. 8, 205 (1980).
7. V. P. Shchipkov, in: Abstracts of Proceedings of the 6th Working Conference on the "Plasmid" Program [in Russian], Moscow (1981), pp. 150-152.
8. V. P. Shchipkov, N. A. Drobysheva, N. I. Shchipkova, et al., Zh. Mikrobiol., No. 9, 134, (1977).
9. N. Datta, in: R-factor. Drug Resistance Plasmid, Baltimore (1977), pp. 255-272.
10. S. Dennison, J. Bacteriol., 109, 416 (1972).
11. M. J. Gasson and N. S. Willetts, J. Bacteriol., 122, 518 (1975).
12. A. Gratia, Ann. Inst. Pasteur, 57, 652 (1936).