GENETIC REGULATION OF FUNCTIONS OF *E. coli* PLASMID pAP42 TRANSFER GENES

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The study of the ability of various conjugative plasmids to inhibit the transfer (tra) functions of plasmid F-lac has led to the establishment of six fertility inhibition systems (Fin-groups) of this plasmid [7]. As regards other F-like plasmids of the transfer factor type, these systems have not been investigated.

This paper describes the study of the system regulating expression of tra-genes of derepressed F-like plasmid (transfer factor) pAP42, identified in cells of conventionally pathogenic *E. coli*, and marked by transposons Tn1 and Tn9 [5].

EXPERIMENTAL METHOD

Plasmids pAP42::Tn1 and yAP42::Tn9, contained in cells of *E. coli* K-12 (AP132, Nal, C600 Rif), were used. The strains of bacterial containing reference plasmids of the different Fin-groups were obtained from N. Willetts' Laboratory (England). A mutant variant of plasmid R445 (ApCmTc), ensuring stability of the bacteria carrying it only to tetracycline — R445 (Tc) was reared by us by the standard method [2] after treatment of AP132 (R455) cells with N-methyl-N'-nitro-N-nitrosoguanidine.

The efficiency of formation of donor-specific pili in bacteria containing the plasmids was determined by their sensitivity to donor-specific phage MS2 by the agar layers method [6].

Conjugation transmission of the plasmids, and also ability to inhibit transfer functions and to engage in surface exclusion, were determined by standard methods [3, 4]. Indices of inhibition of the frequency of transfer and surface exclusion were calculated by equations given in [1]. The index of inhibition of pilum formation was determined as the ratio between the mean number of phage-induced zones of lysis for cells containing one test plasmid only and the corresponding parameter for cells of double plasmid transconjugants.

EXPERIMENTAL RESULTS

For the main experiments clones of *E. coli* AP132 containing plasmids pAP42::Tn1 and pAP42:Tn9 were selected beforehand; these clones had the greatest ability to inhibit transfer of plasmid F-lac in the presence of plasmid F-lac (index of inhibition of frequency of transfer of plasmid F-lac in the presence of plasmid pAP42::Tn1 was 98.5 ± 8.1, and in the presence of pAP42::Tn9 it was 83.9 ± 5.7). The results showed that plasmid F-lac itself does not affect functions of transfer, pilus-formation, and surface exclusion of the test plasmids (in the presence of plasmid F-lac the index of inhibition of the frequency of transfer of plasmic pAP42::Tn1 was 2.2 ± 1.1, and of plasmids pAP42::Tn9 it was 3.1 ± 1.2).

For subsequent determination of the effect of inhibitors of reference plasmids of the six known Fin-groups Fin OP, Fin V, Fin U, Fin W, Fin Q, Fin C) on function of the tra-genes of plasmid pAP42, marked by transposons Tn1 and Tn9, double plasmid transconjugants containing the reference and test plasmids were obtained. The results of the study of these transconjugants (Table 1) showed that reference plasmids belonging to groups Fin U and Fin V inhibited those functions of the tra-genes of the test plasmids which are inhibited by them in plasmid F-lac.

For instance, reference plasmid JR66a (of the Fin U group) inhibited transfer frequency, surface exclusion, and efficiency of pilus formation in the test plasmids, whereas plasmid

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TABLE 1. Effect of Standard Plasmids of Different Fin-Groups on Functions of tra-Genes of Plasmids pAP42::Tn1 and pAP42::Tn9

Reference plasmid (marker)	Inhibition system	Plasmid studied	Index of inhibition of tra-genes of test plasmids		
			Frequency of transfer	Surface exclusion	Pilus formation
R100 (Tc)	FinOP	pAP42::Tn1	0,097±0,001	464,0±87,9	10,2±1,8
		pAP42::Tn9	171,0±11,8	68,8±14,2	+
JR66a (Km)	FinU	pAP42::Tn1	260,0±71,7	20,7±5,8	+
		pAP42::Tn9	1028,0±171,0	27,5±8,2	+
R455 (Tc)	FinW	pAP42::Tn1	1,4±0,5	0,8±0,2	3,2±0,5
		pAP42::Tn9	1,4±0,8	1,0±0,3	0,9±0,3
R485 (Su)	FinV	pAP42::Tn1	293,0±48,4	0,6±0,2	+
		pAP42::Tn9	160,0±47,3	3,2±0,3	+
TP108 (Km)	FinQ	pAP42::Tn1	0,27±0,016	5,5±1,2	1,7±0,2
		pAP42::Tn9	0,091±0,016	17,3±2,2	3,2±0,6
CloDE13 (Clo)	FinC	pAP42::Tn1	0,7±0,2	1,0±0,0	1,9±0,05
		pAP42::Tn9	1,6±0,3	2,3±0,6	1,4±0,1

Legend. Standard statistical deviation for indices of inhibition were calculated for three clones: +) sensitivity to phage MS2 completely absent.

R485 (of the Fin V group) inhibited only transfer frequency and efficiency of pilus formation.

In the case of plasmid R100 (group Fin OP) a different effect was observed on the two plasmids studied. When plasmid pAP42::Tn1 was introduced into cells of the AP132 strain, containing plasmid R100, only 1.3% of cells preserved the resident plasmid, whereas when plasmid pAP42::Tn9 was introduced, the number was 92%, evidence that incorporation of each of the transposons has a different effect on the compatibility functions of plasmid pAP42. Investigation of transconjugants containing plasmid pAP42::Tn1 and R100 revealed a very small decrease in sensitivity to phage MS2, and inhibition of surface exclusion but not of transfer frequency of plasmids pAP42::Tn1, which was increased by 12-56 times. In the case of plasmid pAP42::Tn9, the inhibitor of plasmid R100 inhibited all three functions characteristic of tra-genes: transfer frequency, surface exclusion, and pilus formation (Table 1).

Reference plasmids TP108 (of the Fin Q group) had a weak inhibitory action only on the surface exclusion function of the test plasmids, which distinguishes them from F-lac plasmids.

Reference plasmids of the Fin W and Fin C groups had no action on the tra-functions of the test plasmids.

The results can be summed up in the conclusion that the tra-region of plasmids pAP42::Tn1 and plasmid pAP42::Tn9, unlike that of plasmid F-lac, is sensitive to fertility inhibitors of only three types, namely Fin OP, Fin V, and Fin U. The characteristics of plasmids pAP42::Tn1 and pAP42::Tn9 with respect to inhibition of functions of the tra-genes and incompatibility with plasmid R100 (of the Fin OP group) thus revealed are probably linked with the different effects of transposons Tn1 and Tn9 incorporated into the structure of plasmids pAP42.

LITERATURE CITED

1. N. I. Buyanova, V. P. Shchipkov, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 8, 92 (1982).
2. J. H. Miller, in: Experiments in Molecular Genetics, Cold Spring Harbor Lab., New York (1972).
3. A. P. Pekhov, V. P. Shchipkov, E. V. Gubar', et al., Zh. Mikrobiol., No. 12, 31 (1980).
4. V. P. Shchipkov, Byull. Éksp. Biol. Med., No. 1, 70 (1982).
5. V. P. Shchipkov, V. N. Reshenikova, N. A. Drobysheva, et al., in: Abstracts of Proceedings of the 4th Working Conference on the "Plasmid" Program [in Russian], Tartu (1979), pp. 160-163.
6. A. Gratia, Ann. Inst. Pasteur, 57, 652 (1936).
7. N. S. Willetts and R. Skurray, Annu. Rev. Genet., 14, 41 (1980).