SYSTEMS CONTROLLING Tra GENES OF DEREPRESSED F-LIKE PLASMIDS

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

UDC 579.842.1/.2 : 579.252.5

KEY WORDS: plasmid; control system; Fin-group; transfer genes.

Investigation of plasmids identified in natural populations of different species of intestinal bacteria has led to the establishment of six different genetic systems (Fin-systems), whose products inhibit the functions of transfer (Tra) genes of plasmid Flac [7, 8]. However, the role of these systems in regulation of genetic transfer (Tra) functions and surface exclusion of other F-like plasmids, identified in cells of different bacteria, still remains unexplained.

In the investigation described below features of control systems of four previously characterized [1-3, 6] derepressed F-like plasmids (pAP22-2, pAP38, pAP43, pAP53) were studied with the aid of standard repressed plasmids, representatives of six different genetic control groups Fin-groups).

EXPERIMENTAL METHOD

Standard strains of E. coli K-12, carrying chromosomal genes of resistance to streptomycin (C600 str), nalidixic acid (AP132), or rifampicin (C600 rif), containing (or not containing) one of the derepressed plasmids for testing, marked by different transposons: pAP22-2 : Tn1, pAP38 :: Tn9, pAP43 :: Tn5, pAP53 :: Tn9, were used. The original strains of bacteria containing standard plasmids of six different Fin-groups were obtained from Willetts' laboratory (England).

The sensitivity of the bacteria to donor-specific phage MS2, conjugation transmission of the plasmids, and their ability to inhibit mutual transfer functions and surface exclusion were determined by standard methods [1, 4, 5].

EXPERIMENTAL RESULTS

To determine the possible effect of the test derepressed plasmids on Tra-functions of the reference plasmid transconjugants containing two plasmids (test and standard) were obtained. The study of these transconjugants showed that plasmids pAP38 :: Tn9 and pAP43 :: Tn5 (like the original repressed variants of these plasmids) inhibit transfer of plasmid Flac into cells of plasmid-free strains. Inhibition indices (II) of transfer were $5 \cdot 10^2$–$5.6 \cdot 10^2$ and $1.5 \cdot 10^2$–$3.0 \cdot 10^2$ respectively. Meanwhile plasmids pAP22-2 :: Tn1 and pAP53 :: Tn9 did not possess this property (II was 0.8-0.9 and 1.3-3.0 respectively). The results are evidence that plasmids pAP38 :: Tn9 and pAP43 :: Tn5 contain genes determining synthesis of a repressor, active against plasmid Flac, but not against the intrinsic system of tra-genes of these plasmids.

To determine the inhibitory capacity of the standard plamids of six different Fin-groups (R100 – FinOP; TP108, R62 – FinQ, JR66a – FinU; R485 – FinV; R455 – FinW, CloDF13 cop 3 – FinC) double transconjugants containing one of the above-mentioned standard plasmids and one of the derepressed plasmids were obtained in conjugation experiments and investigated. Isogenic plasmid-free strains of E. coli K-12 and also strains containing one derepressed or one standard plasmid were used as controls. The efficiency of transmission of derepressed plasmids into cells of plasmid-free strains of E. coli K-12 was characterized by a frequency of about $0.6 \cdot 10^{-1}$–$6.5 \cdot 10^{-1}$ (per single donor cell), whereas standard repressed plasmids were transferred with a frequency of $<10^{-5}$. To transmit nonconjugative plasmid Clo DFB cop 3, it was mobilized by plasmid Flac and the conjugated plasmid was subsequently removed from cells of the double transconjugants obtained.

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow.
(Presented by Academician of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 8, pp. 226-227, August, 1985. Original article submitted June 26, 1984.

0007-4888/85/0008-1113$09.50

TABLE 1. Ability of Plasmids of Six Standard Fin-Groups to Inhibit Functions of tra-Genes of Derepressed Plasmids

Standard plasmid	Test plasmid	II of frequency of transmission of test plasmid	Ability of standard plasmids to inhibit functions of test plasmid: formation of donor-specific pili	Ability of standard plasmids to inhibit functions of test plasmid: surface exclusion
R 100 (FinOP)	pAP22-2::Tn 1	1,1—2,3	—	—
	pAP38::Tn 9	0,72—0,8	—	—
	pAP43::Tn 5	0,5—1,0	—	—
	pAP53::Tn 9	0,5—1,1	—	—
TP108 (FinQ)	pAP22-2::Tn 1	1,5—1,6	—	—
	pAP38::Tn 9	0,8—1,0	+	—
R 62	pAP43::Tn 5	0,5—1,5	—	—
	pAP53::Tn 9	2,0—2,1	—	—
JR66a (FinU)	pAP22-2::Tn 1	320—3200	+	+
	pAP38::Tn 9	1,4—1,9	+	+
	pAP43::Tn 5	1,2—2,0	+	+
	pAP53::Tn 9	0,8—1,0	+	+
R 485 (FinV)	pAP22-2::Tn 1	$270—1,0 \cdot 10^4$	+	+
	pAP38::Tn 9	19—180	+	+
	pAP43::Tn 5	0.8—2,0	+	+
	pAP53::Tn 9	$2,0 \cdot 10^3—3,0 \cdot 10^3$	+	+
R 455 (FinW)	pAP22-2::Tn 1	1,6—2,0	+	+
	pAP38::Tn 9	0,3—1,1	+	+
	pAP43::Tn 5	0,3—0,4	+	+
	pAP53::Tn 9	1,1—1,19	—	+
ClodF13cop3 13cop3 (FinC)	pAP22-2::Tn 1	0,2—0,6	—	—
	pAP38::Tn 9	0,4—1,0	—	—
	pAP43::Tn 5	0,4—0,6	—	—
	pAP53::Tn 9	0,7—1,1	—	—

The results are evident that standard plasmids of the FinOP and FinC groups have no effect on the efficiency of conjugation transfer or on synthesis of donor-specific pili (the efficiency of which was judged by the sensitivity of the cells to donor-specific phase MS2 and surface exclusion of all four derepressed plasmids (Table 1)). A plasmid of the FinQ group inhibited only the formation of donor-specific pili, controlled by plasmid pAP38:: Tn9. A plasmid of the FinU group inhibited the formation of donor-specific pili of plasmids pAP22-2:: Tn1, pAP38:: Tn9, pAP43:: Tn5 and transfer of plasmid pAP22-2:: Tn1, but did not affect the surface exclusion functions. A plasmid of the FinV group inhibited the formation of pili of all four plasmids and transfer of plasmids pAP22-2:: Tn1, pAP38:: Tn9, pAP53:: Tn9. A plasmid of the FinW group inhibited pilus formation controlled by plasmid pAP43:: Tn5 and the surface exclusion functions of plasmid pAP53:: Tn9. The results are evidence of significant differences between the control systems of tra-genes of the four test F-like plasmids and that of standard plasmid Flac, transfer of which is known to be inhibited by standard plasmids of all six known Fin-groups [8]. The transfer system of plasmid pAP22-2:: Tn1 is sensitive only to inhibitors of FinU and FinV types, that of plasmids pAP38:: Tn9 and pAP53:: Tn9 to FinV. The formation of donor-specific pili in the case of plasmid pAP38:: Tn9, moreover, is inhibited by plasmids of FinQ, FinU, and FinV groups, and in the case of plasmid pAP43:: Tn5, by plasmids of the FinU, FinV, and FinW. On the practical plane, the results are useful for the development of a classification of plasmids identified in natural populations of bacteria, on the basis of specific differences in the systems controlling tra-genes of these plasmids.

LITERATURE CITED

1. N. I. Buyanova, V. P. Shchipkov, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 8, 92 (1982).
2. D. Miller, Experiments in Molecular Genetics, Cold Spring Harbor (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. Reshetnikova, 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. Gratis, Ann. Inst. Pasteur, 57, 652 (1936).
7. N. S. Willetts and R. Skurray, Annu. Rev. Genet., 14, 41 (1980).