GENOMIC LOCALIZATION OF PLASMID pAP27 fin N SYSTEM

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Traj-independent transfer of plasmid F is regulated by fin OP, fin Q, fin U, fin V, fin W, and fin C genetic systems contained in the genomes of individual F-like and non-F-like plasmids [8]. Meanwhile studies of plasmids of different origin have led to the suggestion that systems of genetic regulation of plasmid transfer differing from those described previously exist. One such plasmid has been found to be the F-like conjugative R-plasmid pAP27 Ap Tc Sm Su of the rd type, containing the fin N genetic regulatory system in its genome [2].

To establish the localization of the fin N system on the genome, we cloned it. The results of molecular cloning of this system are described in this paper.

EXPERIMENTAL METHOD

DNA of the test plasmid pAP27 and the vector plasmid was isolated by the method of Meagher et al. [7] and by the "alkaline" method [5]. Molecular cloning of the BamHI enzymes of plasmid pAP27 was carried out by the method of Maniatis et al. [6]. As the genetic vector we used nonconjugative plasmid pUC19 Ap $lacZ^+$. The indicator strain for this plasmid was *E. coli* strain JM83 ara, Δlac, pro, strA, thi, Θ80 lacZ, ΔM15. Activity of the fin N system was determined with respect to inhibition of transfer functions of F-like transfer factor pAP11-2:Tn5 (the test plasmid). Conjugative crossings of bacteria, their sensitivity to pili-specific phage f2, and also the rising phage titer test (RPTT) were carried out by standard methods [1, 3, 4]. Indices of inhibition (II) of the frequency of plasmid transfer and the rising phage titer index (RPTI) in cultures of diplasmid cells were calculated as the ratio of the corresponding parameters for monoplasmid and diplasmid cell cultures.

EXPERIMENTAL RESULTS

In the experiments immediately before cloning the character of restriction of plasmid pAP27 DNA was studied. For this purpose DNA of plasmid pAP27 was isolated and treated with restriction endonucleases BamHI and Hind III, after which it was studied by agarose (0.8%) gel electrophoresis.

The results of these experiments are given in the restriction maps in Fig. 1.

It will be clear from Fig. 1 that plasmid pAP27 DNA has seven recognition sites for restriction endonuclease BamHI and six sites for restriction endonuclease Hind III. By hydrolysis of the test DNA by these enzymes separately and together it was possible also to calculate the molecular mass of plasma pAP27, which was found to be 50133 base pairs (bp).

In view of the results of restriction of plasmid pAP27 DNA, we cloned the BamHI-restriction fragments of this plasmid, for which purpose each of the seven BamHI-fragments was ligated with DNA of vector plasmid pUC19, previously degraded by the same enzyme, and then we transformed cells of strain JM83 with the ligated mixtures.

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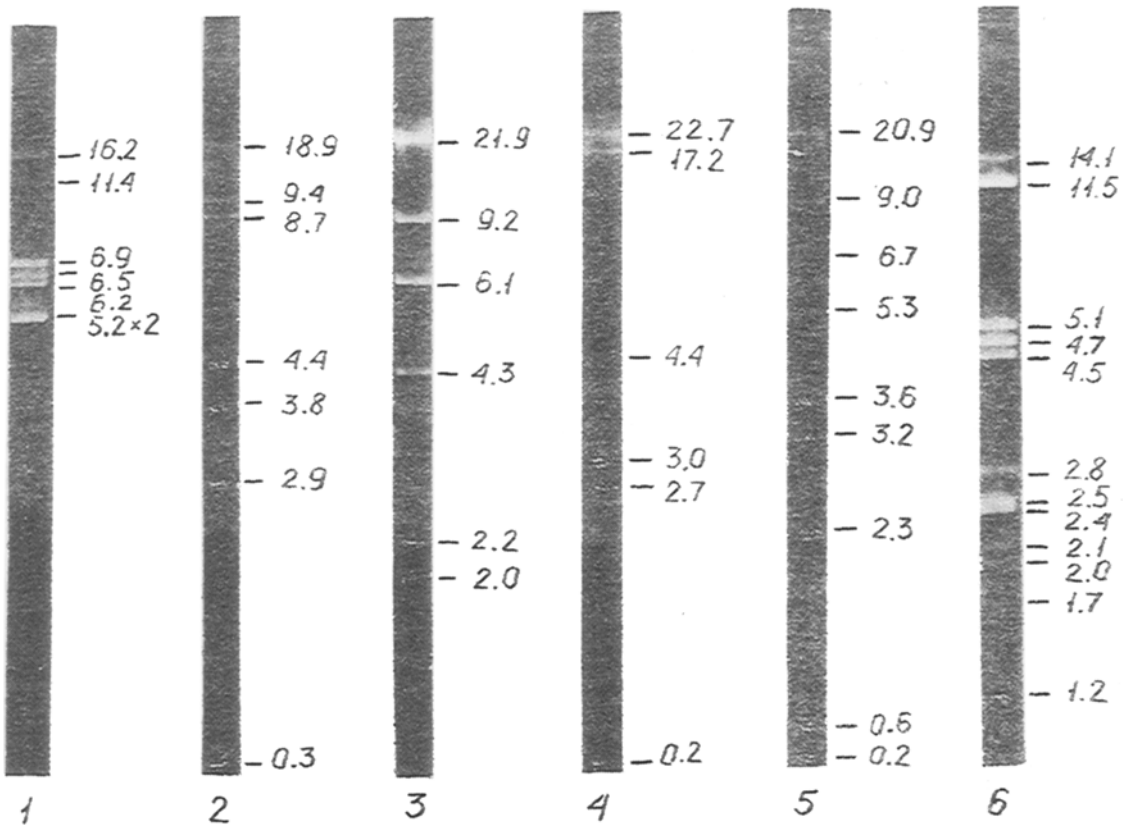

Fig. 1. Electrophoresis of restriction products of plasmid pAP27 DNA (dimensions of fragments given in kilo-base-pairs). 1) DNA of phage λ, BamHI; 2) DNA of plasmid pAP27, BamHI; 3) DNA of phage λ, Hind III; 4) DNA of plasmid pAP27, Hind III; 5) DNA of plasmid pAP27, BamHI + Hind III; 6) DNA of phase λ, Pst I.

Transformants containing recombinant plasmids were selected for markers Str^rAp^r and Lac^-, for incorporation of a fragment of foreign DNA into the BamHI restriction site of plasmid pUC19, located in the polylinker between the promoter and the structural part of the LacZ gene, ought to be accompanied by disturbance of the expression of this gene and, consequently, by termination of synthesis of β-galactosidase, which is involved in lactose utilization.

Having selected a number of transformants. we regarded them as cells containing recombinant plasmids. However, to test this assumption, plasmid DNA was isolated from individual transformants and tested by agarose (1%) gel electrophoresis in the native form and after treatment with restriction endonucleases.

Figure 2 gives the results of electrophoresis of native DNA and of DNA restricted to enzymes SalGI, PvuII, and HpaI, of two plasmids obtained on the basis of DNA of vector plasmid pUC19, and of BamHI restriction fragment f3 of plasmid pAP27 (8.7 kbp), and designated pAP112 and pAP113.

As Fig. 2 shows, the number of three-dimensional isomers of native DNA of plasmids pAP112 and pAP113, on the one hand, and pUC19, on the other hand, differed: DNA of the recombinant plasmids formed four topo-isomers, DNA of the vector only two. The restriction maps of DNA of plasmids pAP112 and pAP113, treated with enzymes SalGI, Pvu II, and HpaI, indicate the presence of additional fragments in their genomes. The results thus confirmed the formation of recombinant plasmids.

In subsequent experiments all the selected recombinant plasmids were tested for donor activity. As the experiments showed, transformants containing recombinant plasmids were characterized by phage-resistance, they did not give RPTT, and did not conjugate with AP115 recipient cells, evidence of the absence of ability for conjugative transfer in the recombinant plasmids.

To discover in which of the seven cloned BamHI fragments of plasmid pAP27 DNA the fin N system is located, test plasmid pAP11-2::Tn5 was introduced into selected transformants containing recombinant plasmids. The diplasmid cells were then tested in RPTT and for donor activity in crosses with recipient cells of the AP115 strain.

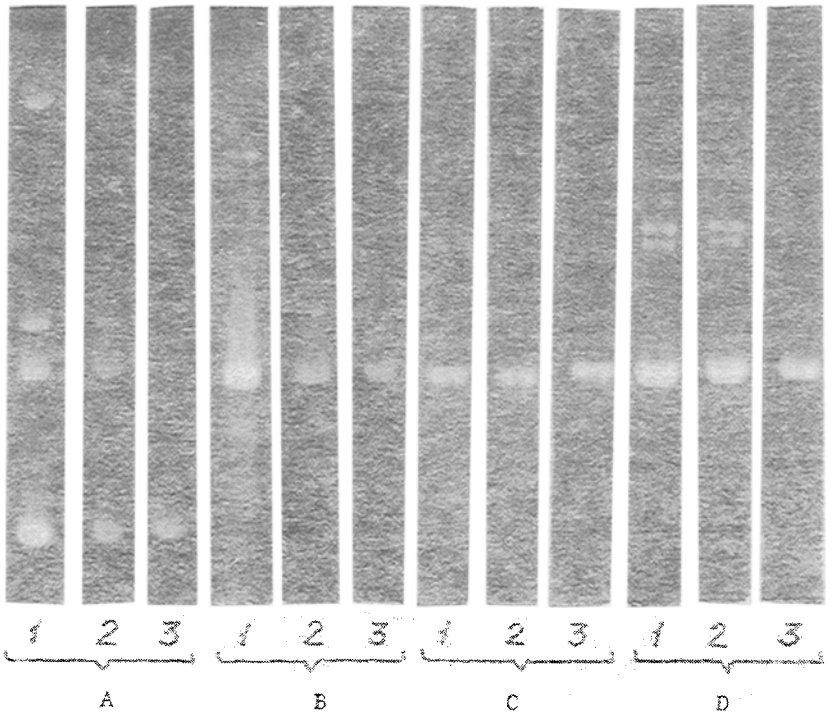

Fig. 2. Electrophoresis of native DNA and restriction products of DNA of plasmids pAP112, pAP113, and pUC19: 1) DNA of plasmid pAP112; 2) DNA of plasmid pAP113; 3) DNA of plasmid pUC19; A) native form of DNA; B) DNA, HpaI; C) DNA, SalGI; D) DNA, Pvu II.

Values of II of RPTI in cultures of diplasmid cells and II of frequencies of test plasmid transfer, obtained in independent experiments, are shown in Table 1.

As Table 1 shows, only those recombinant plasmids which were constructed on the basis of DNA of vector pUC19 and the BamHI-fragment f3 of plasmid pAP27, i.e., pAP112, pAP113, and pAP114 (the recombinant nature of the latter was determined by a genetic test). The Fin-effect was maximal for plasmid pAP112 and it exceeded the Fin-effect due to plasmid pAP27 (by 1.8-4.0 times according to transfer, by 1.8-66.6 times for RPTT). The Fin-effect of plasmid pAP113 was virtually indistinguishable from the corresponding effect of plasmid pAP27. The Fin-effect of plasmid pAP114 was exhibited only in relation to pilus formation, and not to transfer of the test plasmid.

Since we know from our experiments that vector plasmid pUC19 itself has an inhibitory effect on transfer function of the test plasmid (Table 1), and that BamHI-hydrolysis of the DNA of this plasmid leads to inactivation of its genetic regulatory system, involvement of the latter in the Fin-effect of plasmids pAP112, pAP113, and pAP114 could be excluded, i.e., this effect could be considered to be due to the cloned fin N system. Under these circumstances, however, the possibility of formation of a "hybrid" inhibitor, programmed by recombinant plasmids, and also the effect of the fin-system of plasmid pUC19 on the fin N system, would also have to be ruled out.

To solve the problems arising, in comparative experiments we tested the systems regulating transfer of vector plasmid pUC19 and recombinant plasmid pAP112 relative to the transfer function of the F factor in accordance with the standard scheme. These experiments showed that these plasmids, like plasmid pAP27, inhibit neither pilus formation nor conjugative transfer of the F factor. Experiments to test the second hypothesis showed that in a culture of diplasmid JM83 cells, carrying plasmids pUC19 and pAP27, as a result of the Fin-effect of plasmid pUC19 the RRTT determined by conjugative plasmid pAP27 was absent, nor were transconjugants formed when these cells were crossed with AP115 recipient cells. Comparison of levels of inhibition of transfer of RD-plasmid pAP27 fin N and the Drd-plasmid pAP11-2::Tn5 by the fin-system of plasmid pUC19 showed that with respect to inhibition of pilus formation the effect was the same (RPTT was inhibited virtually completely), but with respect to transfer of plasmid pAP11-2::Tn5 the Fin-effect was exhibited much less strongly than for transfer of plasmid pAP27. Assuming,

TABLE 1. Level of Inhibition of Transfer Functions of Factor pAP11-2::Tn5 by Recombinant Plasmids

Recombinant plasmid	Inhibition relative to transfer functions of pAP11-2::Tn5	
	Pilus formation (II of RPTI)	transfer (II of transfer frequency)
pAP112	+(from 27,5 to 1,0× ×10^3)	+(from 12,4 to 31,2)
pAP113	+(from 0,7 to 50,0)	+(from 1,0 to 5,1)
pAP114	+(from 1,0 to 50,0)	+/−(from 0,2 to 2,1)
Remaining recombinant plasmids	−(0,6)	−(0,1)
Control:		
pAP27	+(15,4±0,3)	+(7,8±0,2)
pUC19	+((3,7±0,8)×10^7)	+((4,3±1,3)×10^2)

on the basis of these results, the possibility of summation-of Fin-effects due to plasmids pUC19 and pAP27, we tested their combined effect on the transfer function of the test plasmid. For this purpose, plasmid pAP11-2::Tn5 was introduced into JM83 (pUC19, pAP27) cells, and the transconjugants thus obtained were then tested for phage-sensitivity, in the RPTT, and for conjugativeness in crosses with AP115 recipient cells. It was found that triplasmid cells are characterized by phage-sensitivity with an approximately tenfold decrease in RPTT, but the same level of inhibition of the pilus formation function of plasmid pAP11-2::Tn5, as was shown above, is characteristic of the fin-system of plasmid pAP27. The transfer function of the test plasmid was inhibited at a level characteristic of inhibition of the fin-system of plasmid pUC19, for II of the frequency of transfer of the test plasmid from the three plasmid cells $[(3.2 \pm 0.05) \cdot 10^2]$ is comparable with this parameter for diplasmid cells with plasmid pUC19 $[(4.3 \pm 1.3) \cdot 10^2]$, but not with plasmid pAP27 (7.8 ± 0.2). These results show that effects of the fin-system of plasmids pUC19 and pAP27 relative to the transfer function of factor pAP11-2::Tn5 are not additive, and their interaction is dissimilar in character.

If the results are discussed as a whole it can be concluded that the fin N-system of regulation of the conjugative functions of F-like plasmid pAP27 (50.1 kbp) is located on its BamHI-restriction fragment f3 (length 8.7 kbp). Since nonconjugative plasmids used as genetic vectors may possess their own system of plasmid transfer regulation, as has been found, for example, for plasmid pUC19, this offers the possibility of studying interaction between plasmid transfer regulatory systems of different origin in recombinant plasmids.

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