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EFFECT OF TRANSPOSONS Tn1 AND Tn9 ON GENETIC CONTROL SYSTEM OF TRANSFER AND INCOMPATIBILITY FUNCTIONS OF pAP19-1 Col-PLASMID

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UDC 579.252.5

KEY WORDS: Col-plasmid; induction; mutants; transfer function; incompatibility.

The writers previously showed that derepressed (drd) variants of the F-like Ent[1]-plasmid pAP10-2 can be obtained by incorporating transposon Tn9 into its structure [2]. The object of this investigation was to induce drd-mutants of F-like Col-plasmid pAP19-1 with the aid of transposons Tn1 and Tn9 and also to study regulation of transfer (Tra) function and compatibility with F-like plasmids of different incompatibility groups in these mutants.

EXPERIMENTAL METHOD

Standard strains of *Escherichia coli* K-12 with chromosomal genes of resistance to streptomycin (C600, AP106) or to nalidixic acid (AP115, AP132) were used.

Genetic markers of the test plasmids (resistance to antibiotics, transmissibility, sensitivity to donor-specific phages), and conjugation transmission of plasmids and their ability to mutually inhibit transfer functions were undertaken by standard methods [3, 5]. Transposons Tn1 and Tn9 were incorporated into the structure of the test plasmid by schemes worked out previously [1, 4]. Compatibility (incompatibility) of the plasmids was determined by the standard method [7]. The plasmids and their genetic markers were named in accordance with the recommendation of Novick et al. [9].

EXPERIMENTAL RESULTS

Plasmid pAP19-1, discovered previously [6] in cells of conditionally pathogenic strain *E. coli* AP53 (serogroup 0141) was transmitted into cells of strains *E. coli* K-12. After incorporation of transposon Tn1 or Tn9 into the structure of plasmid pAP19-1, ten drd-mutants of this plasmid containing one of the above-mentioned transposons were selected. All mutants obtained were able to make the cells containing them sensitive to donor-specific phage MS2 and highly efficient in conjugation transmission of plasmid markers. They were also sensitive to the inhibitory action of plasmid R1, with repressed Tra functions. Typical clones of bacteria with plasmid mutants, carrying transposons Tn1 and Tn9, and designated pAP19-2 and pAP19-3 (respectively), were selected for further experiments.

The results of the study of systems controlling Tra functions of these plasmids, given in Table 1, show that mutant plasmids pAP19-2 and pAP19-3 (like the original plasmid pAP19-1 and its Tn9-marked variant of repressed type, pAP19-4), are unable to inhibit transfer functions of derepressed reference plasmid Flac. Meanwhile Tra functions of drd plasmids pAP19-2 and pAP19-3 are inhibited by reference plasmids R1 and R100, which as we know are carriers of

Department of Biology and General Genetics, P. Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 332-334, March, 1984. Original article submitted April 30, 1983.

 0007-4888/84/9703-0320$08.50

TABLE 1. Properties of Double Plasmid Transconjugants

Introduced plasmid	Resident plasmid	Frequency of transmission of introduced plasmid	Sensitivity of phage	Analysis of transconjugants: frequency of transmission of plasmids into C600 cells		Index of reduction of frequency of transmission of plasmids	
				introduced	resident	introduced	resident
Flac	pAP19-1	$1 \cdot 10^{-1}$	+	$7 \cdot 10^{-1}$—$7{,}2 \cdot 10^{-1}$	—	0,03—0,3	—
Flac	pAP19-2	$1 \cdot 10^{-1}$	+	$4 \cdot 10^{-2}$—$0{,}7 \cdot 10^{-1}$	$1 \cdot 10^{-1}$—$2{,}4 \cdot 10^{-1}$	2,8—5	—
pAP19-3	Flac	$4 \cdot 10^{-3}$	+	$1{,}2 \cdot 10^{-1}$—$1{,}3 \cdot 10^{-1}$	$1{,}9 \cdot 10^{-1}$—$2 \cdot 10^{-1}$	—	5—6
pAP19-4	Flac	$1{,}5 \cdot 10^{-4}$	+	$1{,}4 \cdot 10^{-1}$—$1{,}7 \cdot 10^{-1}$	$3{,}8 \cdot 10^{-1}$—$4 \cdot 10^{-1}$	—	2—2,5
R1	pAP19-3	$8{,}5 \cdot 10^{-5}$	—	$2 \cdot 10^{-3}$—$1{,}6 \cdot 10^{-2}$	$3 \cdot 10^{-3}$—$2{,}7 \cdot 10^{-2}$	—	25—150
R100	pAP19-3	$1 \cdot 10^{-3}$	—	$5{,}4 \cdot 10^{-4}$—$1 \cdot 10^{-3}$	$0{,}6 \cdot 10^{-3}$—$1 \cdot 10^{-3}$	—	120—200
R100	pAP19-2	$1{,}3 \cdot 10^{-1}$	—	$8 \cdot 10^{-4}$—$1 \cdot 10^{-3}$	$1{,}3 \cdot 10^{-2}$—$1{,}4 \cdot 10^{-2}$	—	11
pAP17-1	pAP19-3	$1 \cdot 10^{-2}$	—	$1 \cdot 10^{-3}$—$4 \cdot 10^{-3}$	$0{,}5 \cdot 10^{-6}$—$4{,}6 \cdot 10^{-6}$	—	$1 \cdot 10^{5}$—$9 \cdot 10^{5}$
—	Flac	—	+	|—	$2 \cdot 10^{-1}$—$10 \cdot 10^{-1}$		
—	pAP19-2	—	+	—	$1{,}6 \cdot 10^{-1}$—$1{,}7 \cdot 10^{-1}$		
—	pAP19-3	—	+	—	$1{,}2 \cdot 10^{-1}$—$4{,}5 \cdot 10^{-1}$		
—	pAP19-4	—	—	—	$4 \cdot 10^{-2}$—$4{,}5 \cdot 10^{-2}$		

Legend. In each case at least 3 clones of transconjugants were studied. Index of reduction of frequency of plasmid transmission was determined as the quotient from dividing the frequency of transmission of this plasmid from cells of the control strain (containing only one plasmid) by the frequency of its transmission from double plasmid transconjugants [5].

an inhibition system of the fin OP type [8]. The strongest inhibitory effect was found in the case of Hly-plasmid pAP17-1 (Table 1).

These results suggest that plasmid pAP19-1 and its transposon-marked derivatives do not contain inhibition systems that are functionally active against Tra functions of plasmid Flac, but are sensitive to the action of an inhibition system of fin OP type of other plasmids. At the same time, plasmid pAP19-1, which is self-repressed in relation to Tra functions, probably possesses its own genetic control system, which can be derepressed as a result of transposon incorporation into its structure.

The effect of transposons Tn1 and Tn9 on incompatibility of plasmid pAP19-1 was studied by tests of compatibility (incompatibility) of this plasmid and its marked variants with standard reference plasmids of nine known incomptability groups of F-like plasmids. It was shown for plasmids pAP19-1 and pAPL9-2 that they are compatible with reference plasmids of all nine groups. Indices of compatibility, i.e., the percentage of clones of double plasmid transconjugants preserving markers of both plasmids after passage were found to be 71-100% for plasmid pAPL9-1 and 80-100% for plasmid pAP19-2. On the basis of these data it can be postulated that plasmid pAP19-1 is a representative of a new incompatibility group (FX).

As regards plasmid pAP19-3, it was found to be compatible with plasmid pAP19-2 and with plasmids of incompatibility groups FI, FII, FIV, FV, FVI, FVIII, and FIX (indices of compatibility 89-100%). Meanwhile, during transmission of plasmid pAP38-1 (group FVII) into cells containing plasmid pAP19-3, incompatibility of these plasmids was observed (indices of compatibility for individual clones of double plasmid transconjugants varied from 0 to 6%).

It will be clear from the results that induction of drd mutants of Col-plasmid pAP19-1 is possible as a result of incorporation of both Tn1 and Tn9 into its structure. On incorporation of transposon Tn9 into the structure of this plasmid, simultaneous modification of the functions of the two different genetic systems controlling Tra functions and incompatibility is possible. This state of affairs is of essential importance in connection with the problem of classification of plasmids on the basis of their incompatibility.

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