GENETIC PROPERTIES OF TRANSPOSONS Tn6-1, Tn6-2, AND Tn19-1

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UDC 612.6.05:577.251

KEY WORDS: plasmid, plasmid transfer, transposon, transposition.

Transposons of prokaryotes are mobile DNA segments that are capable of independent transfer from one site to another within the same genome (replicon) or of transfer from one genome (plasmid, bacterial, phage) to another [3, 4].

Continuing the search for nonconjugative plasmids in cells of natural antibiotic-resistant strains of *E. coli* and determination of their transposon content, we identified transposons Tn6-1 and Tn19-1, determining resistance to trimethoprim, and Tn6-2, determining resistance to ampicillin.

In this paper we give the results of a study of the genetic properties of these transposons (range and frequency of transposition, effect on plasmid transfer and mobilization of nonconjugative plasmids for transfer, and also the levels of resistance of bacteria to trimethoprim and ampicillin).

EXPERIMENTAL METHOD

Derivatives of strain *E. coli* K-12 resistant to nalidixic acid (*E. coli* AP115Nal^r), streptomycin (*E. coli* C600Str^r), or rifampicin (*E. coli* C600Rif^r) were used in the experiments. The donors of transposons Tn6-1, Tn6-2, and Tn19-1 were *E. coli* C600Rif^r cells. As "targets" for the transposons studied, we used conjugative R-plasmids of drug resistance

TABLE 1. Range and Frequency of Transposition of Transposons Tn6-1, Tn6-2, and Tn19-1

Transposon	Target plasmid	Frequency of transposition
Tn6-1	R27	$2{,}0\cdot10^{-3}$
	F'lac	$9{,}9\cdot10^{-3}$
	pAP41	Not determined
	pAP41::Tn6	$1{,}0\cdot10^{-1}$
	pAP41::Tn9	$1{,}0\cdot10^{-1}$
	pAP41::Tn1721	$1{,}0\cdot10^{-1}$
	pAP41::Tn9 Tn1721	$2{,}0\cdot10^{-1}$
Tn6-2	R386	$8{,}8\cdot10^{-3}$
	R15	$3{,}3\cdot10^{-4}$
	pJA6012	$2{,}4\cdot10^{-5}$
	R64	$9{,}3\cdot10^{-6}$
	R621a	$8{,}4\cdot10^{-6}$
	R446b	$1{,}4\cdot10^{-4}$
	F'lac	$1{,}9\cdot10^{-1}$
	pAP41	Not determined
	pAP41::Tn5	$1{,}4\cdot10^{-3}$
	pAP41::Tn9	$9{,}1\cdot10^{-2}$
	pAP41::Tn1721	$2{,}0\cdot10^{-3}$
	pAP41::Tn9 Tn1721	$2{,}5\cdot10^{-3}$
Tn19-1	pJA6012	$2{,}4\cdot10^{-5}$
	R64	$2{,}5\cdot10^{-3}$
	R621a	$9{,}1\cdot10^{-3}$
	F'lac	$5{,}8\cdot10^{-2}$
	pAP41	Not determined
	pAP41::Tn5	$3{,}3\cdot10^{-2}$
	pAP41::Tn9	$9{,}4\cdot10^{-3}$
	pAP41::Tn1721	$1{,}7\cdot10^{-3}$
	pAP41::Tn9 Tn1721	$5{,}6\cdot10^{-2}$

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 347-349, September, 1988. Original article submitted April 20, 1988.

0007-4888/88/0009-1325$12.50

TABLE 2. Effect of Transposons on Plasmid Transfer

Transposon	Target plasmid	Frequency of self-transfer of		Frequency of mobilization of	
		target plasmid with transposon	target plasmid	target plasmid with transposon	target plasmid
Tn6-1	R27	$3,3 \cdot 10^{-1}$	$1,5 \cdot 10^{-7}$	—	—
	F'lac	$8,8 \cdot 10^{-1}$	$7,0 \cdot 10^{-1}$	—	—
	pAP41	$4,5 \cdot 10^{-1}$	Not determined	0	$1,3 \cdot 10^{-1}$
	pAP41::Tn5	$9,3 \cdot 10^{-2}$	$4,1 \cdot 10^{-3}$	$1,5 \cdot 10^{-1}$	$5,0 \cdot 10^{-3}$
	pAP41::Tn9	$1,1 \cdot 10^{-1}$	$3,6 \cdot 10^{-4}$	0	$2,3 \cdot 10^{-1}$
	pAP41::Tn1721	$1,6 \cdot 10^{-1}$	$2,9 \cdot 10^{-1}$	$2,0 \cdot 10^{-7}$	$5,5 \cdot 10^{-2}$
	pAP41:Tn9 Tn1721	$1,1 \cdot 10^{-1}$	$4,4 \cdot 10^{-2}$	0	$0,2 \cdot 10^{-1}$
Tn6-2	R386	$2,1 \cdot 10^{-2}$	$2,0 \cdot 10^{-1}$	—	—
	R15	$2,3 \cdot 10^{-5}$	$4,3 \cdot 10^{-2}$	—	—
	pJA6012	$1,1 \cdot 10^{-1}$	$1,0 \cdot 10^{-1}$	—	—
	R64	$1,0 \cdot 10^{-1}$	$9,5 \cdot 10^{-2}$	—	—
	R621a	$8,4 \cdot 10^{-1}$	$7,3 \cdot 10^{-3}$	—	—
	R446b	$1,7 \cdot 10^{-1}$	$4,7 \cdot 10^{-2}$	—	—
	F'lac	$8,9 \cdot 10^{-1}$	$7,0 \cdot 10^{-1}$	—	—
	pAP41	$2,3 \cdot 10^{-1}$	Not determined	$7,4 \cdot 10^{-6}$	$1,3 \cdot 10^{-1}$
	pAP41::Tn5	$1,2 \cdot 10^{-6}$	$4,1 \cdot 10^{-3}$	$1,7 \cdot 10^{-4}$	$5,0 \cdot 10^{-3}$
	pAP41::Tn9	$4,0 \cdot 10^{-4}$	$3,6 \cdot 10^{-4}$	$1,0 \cdot 10^{-1}$	$2,3 \cdot 10^{-1}$
	pAP41::Tn1721	$1,7 \cdot 10^{-1}$	$2,9 \cdot 10^{-1}$	$1,7 \cdot 10^{-8}$	$5,5 \cdot 10^{-2}$
	pAP41::Tn9 Tn1721	$3,5 \cdot 10^{-2}$	$4,4 \cdot 10^{-2}$	$4,5 \cdot 10^{-4}$	$0,2 \cdot 10^{-1}$
Tn19-1	pJA6012	$2,4 \cdot 10^{-1}$	$1,0 \cdot 10^{-1}$	—	—
	R64	$1,5 \cdot 10^{-4}$	$9,5 \cdot 10^{-2}$	—	—
	R621a	$0,8 \cdot 10^{-1}$	$7,3 \cdot 10^{-3}$	—	—
	F'lac	$4,6 \cdot 10^{-1}$	$7,0 \cdot 10^{-1}$	—	—
	pAP41	$9,4 \cdot 10^{-1}$	Not determined	0	$1,3 \cdot 10^{-1}$
	pAP41::Tn5	$1,1 \cdot 10^{-1}$	$4,1 \cdot 10^{-3}$	$3,9 \cdot 10^{-1}$	$5,0 \cdot 10^{-3}$
	pAP41::Tn9	$4,7 \cdot 10^{-1}$	$3,6 \cdot 10^{-4}$	0	$2,3 \cdot 10^{-1}$
	pAP41::Tn1721	$1,6 \cdot 10^{-1}$	$2,9 \cdot 10^{-1}$	$8,0 \cdot 10^{-8}$	$5,5 \cdot 10^{-2}$
	pAP41::Tn9 Tn1721	$1,9 \cdot 10^{-1}$	$4,4 \cdot 10^{-2}$	$1,4 \cdot 10^{-6}$	$0,2 \cdot 10^{-1}$

R386 (incFI), R1 (incFII), pJA6012 (incA), R16 (incB;, R27 (incH), R64 (incIα), R621a (incIγ), R446b (incM), R15 (incN), S-a (incW) and also transfer factors F'lac, pAP41 and its transposon-containing variants pAP41::Tn5, pAP41::Tn9, pAP41::Tn1721, pAP41::Tn9Tn1721.

The range of transposition of the transposons was determined by introducing them into conjugative plasmids in "three-parent" crosses [2]. The frequency of transposition was determined as the ratio of the number of transconjugants formed in the second cross and inheriting resistance to the number of cells formed in the first cross, and carrying the transposon and nonconjugative plasmid. The conjugativeness of the plasmids and the effect of the transposons on the mobilization ability of the plasmids were determined by standard methods [2].

EXPERIMENTAL RESULTS

The study of the genetic properties of transposons Tn6-1, Tn6-2, and Tn19-1 began with determination of their transposition range.

The experiments showed that the transposons studied are transposed to nearly all plasmids (Table 1). It will be clear from Table 1 that the widest range of transposition was characteristic of transposon Tn6-2, which was transposed into genomes 12 and 16 of the conjugative plasmids used. Transposon Tn19-1 was incorporated into the genomes of nine plasmids, and transposon Tn6-1 into the genome of seven plasmids.

Determination of the frequencies of transposition showed that they were quite high for transposons Tn6-1 and Tn19-1 ($2.0 \cdot 10^{-1}$-$2.0 \cdot 10^{-3}$ and $3.3 \cdot 10^{-2}$-$1.4 \cdot 10^{-4}$ respectively), whereas transposon Tn6-2 was transposed with a lower frequency ($1.9 \cdot 10^{-1}$-$8.4 \cdot 10^{-6}$).

To study the effect of transposons Tn6-1, Tn6-2, and Tn19-1 on genetic transfer of plasmids, we investigated their effect separately on self-transfer of plasmids R386, R15, pJA6012, R64, R621a, R27, R466b, F'lac, pAP41::Tn5, pAP41::Tn9, pAP41::Tn1721, pAP41::Tn9Tn1921 and on the mobilization capacity of plasmid pAP41 and its transposon-containing variants.

The effect of the transposons on self-transfer of plasmids was determined by comparing the frequencies of the transconjugants formed in crosses in which the donor cells contained one of the plasmids mentioned above, with one or other of the transposons for study incorporated into it, with frequencies of transconjugants in crosses in which the donor cells

contained this same plasmid, but without the transposon. It was found that incorporation of all transposons (Tn6-1, Tn6-2, and Tn19-1) into the plasmid genomes was accompanied, as a rule, by a decrease or increase in the frequencies of transfer of the latter (Table 2). Reduction of the frequencies of plasmid transfer probably took place as a result of integration of transposons into the tra region of the plasmids.

To study the possible effect of transposons Tn6-1, Tn6-2, and Tn19-1 on the mobilization capacity of transfer factor pAP41 and its variants, three-parent crosses were undertaken, in which nonconjugative plasmid pAP57 Hly [1] was used as the mobilized plasmids, namely:

C600RifpAP41::Tn5×AP115pAP57×C600Str,
C600RifpAP41::Tn5Tn6-1×AP115pAP57×C600Str,
C600RifpAP41::Tn5Tn6-2×AP115pAP57×C600Str,
C600RifpAP41::Tn5Tn19-1×AP115pAP57×C600Str,
C600RifpAP41::Tn6-1×AP115pAP57×C600Str,
C600RifpAP41::Tn6-2×AP115pAP57×C600Str,
C600RifpAP41::Tn19-1×AP115pAP57×C600Str,
C600RifpAP41×AP115pAP57×C600Str,
C600RifpAP41::Tn9Tn6-1×AP115pAP57×C600Str,
C600RifpAP41::Tn9Tn6-2×AP115pAP57×C600Str,
C600RifpAP41::Tn9Tn19-1×AP115pAP57×C600Str,
C600RifpAP41::Tn9×AP115pAP57×C600Str,
C600RifpAP41::Tn1721×AP115pAP57×C600Str,
C600RifpAP41::Tn1721Tn6-1×AP115pAP57×C600Str,
C600RifpAP41::Tn1721Tn6-2×AP115pAP57×C600Str,
C600RifpAP41::Tn1721Tn19-1×AP115pAP57×C600Str,
C600RifpAP41::Tn9Tn1721×AP115pAP57×C600Str,
C600RifpAP41::Tn9Tn1721Tn6-1×AP115pAP57×C600Str,
C600RifpAP41::Tn9Tn1721Tn6-2×AP115pAP57×C600Str,
C600RifpAP41::Tn9Tn1721Tn19-1×AP115pAP57×C600Str.

The results obtained in these experiments are shown in Table 2, from which it will also be clear that incorporation of transposons into the genome of different variants of plasmid pAP41 changed their ability to mobilize the nonconjugative plasmid for transfer in virtually all cases. Table 2 also shows that changes in conjugativeness and mobilization capacity of the transposon-containing variants of plasmids pAP41 are independent of each other, i.e., the tra region and the region responsible for mobilization for transfer of nonconjugative plasmids, are independent.

In the final experiments we studied levels of resistance of bacteria to trimethoprim and ampicillin, depending on the presence of transposons in the plasmid or bacterial genome.

The experiments showed that the presence of transposon Tn6-1 in plasmid genomes (R27 and F'lac) led to resistance of the bacteria to trimethoprim in concentrations of 50 and 550 μg/ml. Transposon Tn6-2, if present in plasmid genomes (R15, R386, pJA6012, R64, R621a, R446b and F'lac), endowed the cells with resistance to ampicillin in concentrations of 25 to 400 μg/ml. As regards transposon Tn19-1, when incorporated into the genomes of plasmids (R64, pJA6012, R621a, and F'lac), endowed the cells with resistance to the action of trimethoprim in concentrations of 50-600 μg/ml.

The presence of transposons Tn6-1, Tn6-2, and Tn19-1 in the chromosomal genomes led to the appearance of resistance of the bacterial cells to trimethoprim in a concentration to 50 μg/ml, ampicillin in a concentration of 400 μg/ml, and trimethoprim in a concentration of 400 μg/ml respectively.

It can be concluded from the results of these experiments that the identified transposons differ in their frequency and range of transposition. They also differ in their effect on transfer of conjugated plasmids and mobilization of nonconjugative plasmids for transfer.

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