EFFECT OF TRANSPOSONS ON THE REGULATORY SYSTEM OF DEREPRESSED PLASMID Tra GENES

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The writers previously [3, 7] characterized systems regulating transfer functions (Tra-functions) of a number of derepressed (drd) F-like plasmids and demonstrated their difference from the system regulating transfer of F plasmid [8, 10].

Meanwhile data are available to show how changes may arise in these systems on incorporation of individual transposons into the structure of F-like plasmids [2, 3].

The aim of the present investigation was accordingly to study the effect of several transposons (Tn1, Tn5, Tn9, Tn1721) on the sensitivity of individual F-like drd plasmids of the genetic transfer factor type to standard inhibitors of Tra-functions of six different genetic systems (Fin-systems), known in the case of F plasmid [8, 10].

EXPERIMENTAL METHOD

Standard strains of Escherichia coli K-12 with chromosomal genes of resistance of streptomycin (C600 str), rifampicin (C600 rif), and malidixic acid (AP132 nal), containing or not containing standard and test plasmids, were used.

Genetic marking of plasmids by incorporation of individual transposons into their structure and also conjugation transmission of plasmids and the study of their ability to inhibit transfer functions of one another were carried out by standard methods [4, 6, 9]. The inhibitory effect of the standard plasmid relative to Tra-functions of the drd-plasmid under investigation was determined from the decrease in frequency of conjugation transmission of the drd-plasmid from cells of two-plasmid transconjugants, containing the standard and test plasmid, compared with the frequency of its transmission from monoplasmid cells, containing only the test plasmid, and also by the decrease in effectiveness of formation of plasmid-specific pili of S-type by two-plasmid cells, as judged by their sensitivity to donor-specific phage MS2 [6].

EXPERIMENTAL RESULTS

F-like drd-plasmids pAP11-2 (Col), pAP18-1 (Col V, Tc), pAP22-2 ("pure" transfer factor), pAP53 (Col B), and also transposon-containing drd-variants of plasmids obtained previously – pAP11-2::Tn1, pAP 11.2::Tn9 [1], pAP19-1::Tn1, pAP19-1::Tn9 [2], pAP41::Tn1721, pAP41::Tn9::Tn1721 [5], pAP22-2::Tn1, pAP53::Tn9 [7], were studied. During the course of the invesitgation the following transposon-marked drd-variants of plasmids were obtained: pAP11-2::Tn5, pAP18-1::Tn5, pAP18-1::Tn9, pAP22-2::Tn5, pAP22-2::Tn1::Tn5.

To determine the ability of standard (self-repressed) plasmids of six known Fin-groups to inhibit the conjugation transfer of the test drd-plasmids and synthesis of plasmid-specific ("sex") pili coded by them, two-plasmid transconjugants containing one of the test plasmids and one of the standard plasmids were obtained and investigated. The results of analysis of these transconjugants are given in Table 1.

It will be clear from Table 1 that, unlike plasmid F_{lac}, sensitive to inhibitors of Tra-functions of all six types [8, 10], the test F-like drd-plasmids form sensitivity only

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TABLE 1. Ability of Standard Plasmids of Six Fin-Groups to Inhibit Functions of Tra-Genes of F-like drd-Plasmids

Standard plasmid (type of inhibitor)	Inhibited functions	drd-Plasmids																		
		Flac	pAP11-2	pAP11-2::Tn9	pAP11-2::Tn1	pAP11-2::Tn5	pAP18-1	pAP18-1::Tn9	pAP18-1::Tn5	pAP19-1::Tn1	pAP19-1::Tn9	pAP22-2	pAP22-2::Tn1	pAP22-2::Tn5	pAP22-2::Tn1::Tn5	pAP41::Tn1721	pAP41::Tn9::Tn1721	pAP53	pAP53::Tn5	pAP53::Tn9
R100 (Fin OP)	1	+	N.d.	+	+	+	—	—	—	+	+	N.d.	—	—	—	+	+	N.d.	—	—
	2	+	+	+	+	+	—	—	—	+	+	+	—	—	—	+	+	—	—	—
TP108	1	+	N.d.	—	+	—	—	—	—	+	—	N.d.	—	—	—	—	—	N.d.	—	—
R820a (FinQ)R62	2	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
JR66a (Fin U)	1	+	N.d.	+	+	+	—	—	—	+	+	N.d.	+	—	—	+	+	N.d.	—	—
	2	+	+	+	+	+	—	—	—	+	+	—	+	—	—	+	+	—	—	—
R485 (Fin V)	1	+	N.d.	+	+	+	+	+	+	+	+	N.d.	+	+	+	+	+	N.d.	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R455 (Fin W)	1	+	N.d.	—	—	—	+	+	—	+	—	N.d.	—	—	—	+	—	N.d.	—	—
	2	—	+	+	+	—	—	+	—	+	—	—	—	—	—	+	—	—	—	—
CloDF13 cop	1	+	N.d.	—	—		—	—	—	—	—	N.d.	—	—	—	—	—	N.d.	—	—
3 (Fin C)	2	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—

Legend. 1) Conjugation transmission. 2) Formation of F-pili. +) Ability, −) inability of standard plasmid to inhibit Tra-Functions of test drd-plasmid; N.d.) Not determined.

to certain inhibitors. Meanwhile the type of sensitivity of individual plasmids can change as a result of incorporation of different transposons into their structure. For example, F-like plasmid pAP22-2, which is a "pure" transfer factor, is sensitive to inhibitors of two types (Fin OP and Fin V), whereas its marked variant pAP22-2::Tn1 is sensitive to inhibitors of Fin U and Fin V types, but marked variants of this same plasmid pAP22-2::Tn5 and pAP22-2::Tn1::Tn5 are sensitive only to inhibitors of the Fin V type. In the case of Col-plasmid pAP11-2 and its marked variant pAP11-2::Tn9 sensitivity to inhibitors of Fin OP, U, V, and W types is observed, whereas the marked variant pAP11-2::Tn1 has sensitivity of the Fin OP, Q, U, V and W types, but pAP11-2::Tn5 has sensitivity of the Fin OP, U and V type.

It will also be clear from Table 1 that, unlike plasmid pAP18-1 and its marked variant pAP18-1::Tn9, which are sensitive to inhibitors of the Fin V and Fin W types, another marked variant of this plasmid pAP18-1::Tn5 is sensitive only to Fin V inhibitor. Differences were found in the sensitivity of transposon-induced drd-mutants of Col B-plasmid pAP19-1, namely pAP19-1::Tn1 (Fin OP, Q, U, V, W) and pAP19-1::Tn9 (Fin OP, U, V), and also of the "pure" transfer factor TnP41, namely pAP41::Tn1721 (Fin OP, U, V, W) and pAP41::Tn1721 (Fin OP, U, V). Meanwhile incorporation of transposons Tn5 and Tn9 into the structure of drd-plasmid pAP53 does not change the type of its sensitivity (Fin V).

These data are evidence of a possible change in the type of sensitivity to inhibitors of Tra-functions in individual F-like plasmids in response to incorporation of different transposons into their structure. It can be tentatively suggested that this phenomenon is based on inactivation of separate sites in the structure of the plasmid DNA, on which certain inhibitors act, or the appearance of new sites, determining sensitivity to inhibitors of other types. These data also suggest that one cause of the diversity of regulatory systems of Tra-functions of F-like plasmids, identified in natural populations of bacteria, is the incorporation of different transposons into their structure.

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