COMPLEMENTATION ANALYSIS OF DEREPRESSIVE MUTANTS OF F-LIKE PLASMIDS OF *Escherichia coli*

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Conjugative plasmid transfer is under genetic control. Six genetic systems (fin-systems) inhibiting transfer of F and F-like plasmids are now known [4-7], but the genetic organization of the fin-regions of these plasmids has not yet been studied.

This paper describes an attempt to undertake complementation analysis of the fin-region of a number of F-like plasmids, derepressed (drd) for transfer functions, and belonging to the category of R-, Col-plasmids, which are genetic transfer factors and are natural drd-mutants or drd-mutants induced by incorporation of transposons or by treatment with nitrosoguanidine [1-5].

EXPERIMENTAL METHODS

The following F-like drd-plasmids with a known type of sensitivity to inhibitors of the various fin-systems were used: pAP11-2::Tn1 (Fin OP, Q, U, V), pAP18-1 (Fin V, W), pAP18-1::Tn5 (Fin V), pAP22-2::Tn1 (Fin U, V), pAP22-2::Tn5 (Fin V), pAP41::Tn9::Tn1721 (Fin OP, U. V), pAP42::Tn1 (Fin U, V), pAP 42::Tn5 (Fin U, V, W), pAP42::Tn9 (Fin OP, U, V), pAP53::Tn5 (Fin V), pAP53::Tn9 (Fin V). Cells of strains *E. coli* K-12 (AP132 Nal, C600 Str, C600 Rif) served as hosts of the plasmids.

The ability (inability) of the different drd-plasmids to mutually inhibit the functions of formation of plasmid-specific pili of the F-type was judged on the basis of resistance (sensitivity) of the corresponding two- or three-plasmid transconjugants to the pilus-specific phage MS2. In control tests with phage MS2 cells containing plasmids of one pattern chosen for study (one-plasmid transconjugants) were used. Conjugative transfer of the plasmids and their ability to inhibit transfer functions of each other were determined by standard methods [4, 5]. In each case the index of inhibition of the frequency of transmission of each of the test plasmids was calculated as the ratio of the frequency of conjugative transfer of that plasmid from cells on one-plasmid transconjugants to the corresponding parameters for cells of two- or three-plasmid transconjugants.

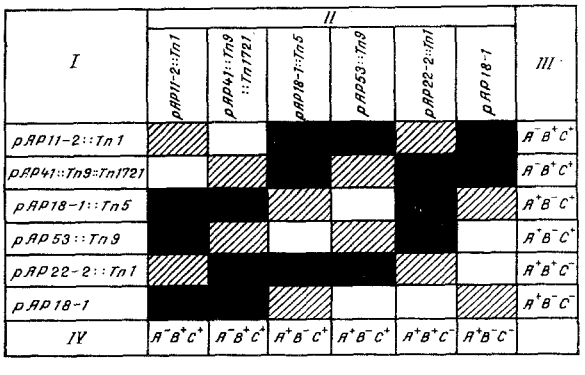

Fig. 1. Complementation analysis of F-like drd-plasmids, mutants for genes of the fin V region. Black rectangles denote complementary inhibition of conjugative plasmid transfer; white rectangles — absence of inhibition; obliquely shaded rectangles — production of two-plasmid transconjugants technically impossible. I) 1st plasmid; II) 2nd plasmid; III, IV) probable genetic structure of fin V region of plasmid.

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TABLE 1. Complementary Inhibition of Conjugative Transfer of F-Like drd-Plasmids in Two- and Three-Plasmid Complexes of *E. coli* AP132 Transconjugants

Nos. of plasmids in complex	Plasmid composition of complex	Type of sensitivity of plasmid to inhibitors of fin-systems	Inhibition of transfer of plasmids of complex			Possible type of complementary Fin-product
			1	2	3	
1	pAP11-2 : : Tn1	OP, Q, U, V	+			
2	pAP18-1			+		V
1	pAP11-2 : : Tn1	OP, Q, U, V	−			
2	pAP41 : : Tn9 : : Tn1721	OP, U, V		−		−
1	pAP11-2 : : Tn1	OP, Q, U, V	+			
2	pAP53 : Tn9	V		+		V
1	pAP18-1	V, W	+			
2	pAP22-2 : : Tn5	V		+		
3	pAP42 : : Tn9	OP, U, V			+	V
1	pAP18-1	V, W	−			
2	pAP22-2 : : Tn5	V		−		−
1	pAP18-1	V, W	+			
2	pAP42 : : Tn9	OP, U, V		+		V
1	pAP22-2 : : Tn5	V	−			
2	pAP42 : : Tn9	OP, U, V		−		−
1	pAP18-1	V, W	+			
2	pAP42 : : Tn9	OP, U, V		+		V+OP(U)
3	pAP53 : : Tn5	V			+	
1	pAP18-1	V, W	−			
2	pAP53 : : Tn5	V		−		−
1	pAP42 : : Tn9	OP, U, V	+			OP (U)
2	pAP53 : : Tn5	V		−		
1	pAP18-1	V, W	−			−
2	pAP22-2 : : Tn1	U, V		−		
1	pAP18-1	V, W	+			
2	pAP41 : : Tn9 : : Tn1721	OP, U, V		+		V
1	pAP18-1	V, W	−			
2	pAP53 : : Tn9	V		−		−
1	pAP18-1 : : Tn5	V	+			
2	pAP22-2 : : Tn1	U, V		+		V
3	pAP41 : : Tn9 : : Tn1721	OP, U, V			+	
1	pAP18-1 : : Tn5	V	+			
2	pAP22-2 : : Tn1	U, V		+		V
1	pAP18-1 : : Tn5	V	+			
2	pAP41 : : Tn9 : : Tn1721	OP, U, V		+		V
1	pAP22-2 : : Tn1	U, V	+			V (U)
2	pAP41 : : Tn9 : : Tn1721	OP, U, V		+		
1	pAP18-1 : : Tn5	V	+			
2	pAP22-2 : : Tn1	U, V		+		V
3	pAP53 : : Tn9	V			+	
1	pAP18-1 : : Tn5	V	−			−
2	pAP53 : : Tn9	V		−		
1	pAP22-2 : : Tn1	U, V	+			
2	pAP53 : : Tn9	V		+		V
1	pAP22-2 : : Tn5	V	−			
2	pAP42 : : Tn1	U, V		+		U
3	pAP53 : : Tn9	V			−	
1	pAP22-2 : : Tn5	V	−			U
2	pAP42 : : Tn1	U, V		+		
1	pAP22-2 : : Tn5	V	−			−
2	pAP53 : : Tn9	V		−		
1	pAP42 : : Tn1	U, V	+			−
2	pAP53 : : Tn9	V		−		
1	pAP42 : : Tn1	U, V	+			
2	pAP53 : : Tn5	V		−		U
1	pAP42 : : Tn5	U, V, W	+			
2	pAP53 : : Tn9	V		+		V

EXPERIMENTAL RESULTS

To determine the possibility of formation of a complementary Fin-product, two- and three-plasmid transconjugants with different combinations of the drd-plasmids to be analyzed were obtained and investigated.

The results (Table 1) indicate that the formation of a complementary inhibitor (complex Fin-product), capable of inhibiting the transfer functions of each of these plasmids or of only one of them, probably takes place in bacterial cells with particular combinations of two different drd-plasmids. In cases in which transfer of both plasmids was inhibited, as a rule inhibition of synthesis of plasmid-specific pili also was observed.

Considering the known type of sensitivity of drd-plasmids in the composition of the two- and three-plasmid complexes obtained to inhibitors of the different fin-systems [3-5], a conclusion can be drawn regarding the possible type of the complementary Fin-product synthesized

under their control. As Table 1 shows, in most cases with a combination of two different drd-plasmids in one bacterial cell, the formation of a complementary inhibitor of the Fin V type was observed, less frequently an inhibitor of the Fin U type. For the two-plasmid complex pAP42::Tn9+pAP53::Tn5 no unequivocal conclusions can be drawn regarding the possible type of complementary Fin-product, just as in the case of the pAP22-2::Tn1+pAP41::Tn9::Tn1721 complex.

In connection with the discovery of a quite large number of drd-plasmids capable of inducing the formation of a complementary product of the Fin V type, an additional complementation analysis was made of these plasmids, in order that a conclusion could be drawn on the possible genetic structure of the Fin V region. As Fig. 1 shows, it was concluded from analysis of the results that at least three different genes, represented by the letters A, B, and C, exist in the composition of the Fin V region. Possible genotypes of the drd-plasmids studied, which are defective for one or two genes of the Fin V region, also are shown in Fig. 1.

To generalize the data given above it can be concluded that the genetic region of the plasmids coding the synthesis of components (subunits) of a transfer inhibitor of a particular type has a complex (polygenic) organization. It can also be postulated that the functioning of individual fin genes, i.e., participation of the proteins coded by them (Fin-products) in the formation of inhibitors (complex Fin-products) of different types, is pleiotropic in character. However, this hypothesis requires further experimental confirmation.

The results may be useful for analysis of the mechanisms of functioning of the various conjugation systems of bacteria, determined by plasmid complexes found in natural communities of bacteria.

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