GENETICS

Activity of Fin-Systems of Artificial Cointegrative pAP42/pRSF2124 and pAP42/pUB781 Plasmids

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The fin-systems finV and finU in cointegrative plasmids are shown to produce a much stronger transfer-inhibitory effect as compared to the sum of effects produced by FinU (in transfer factor pAP42) and FinV (in nonconjugative plasmids pRSF2124 and pUB781) separately.

Key Words: *transfer inhibitor; cointegrative plasmids; transfer regulation systems*

The genetic transfer of F and other F-like plasmids is under the control of genetic regulation systems (fin-systems) present in the genome of both conjugative and nonconjugative plasmids [3,7]. At present several systems of such regulation are known, notably finOP, finU, finQ, finV, finW, finK, finL, finM, and finN [1,6].

Modeling the process of cointegrative plasmid formation, we found that the cointegrates based on the pAP42 transfer factor and pRSF2124 or pUB781 nonconjugative plasmid contain two fin-systems, one of them a "contribution" of pAP42 and the other of pRSF2124 or pUB781 (finU and finV, respectively).

Since in the process of complex construction both finU and finV proved to be active, we undertook an investigation of the features of their expression in the constructed pAP42/pRSF2124 and pAP42/pUB781 cointegrative plasmids [2]. The results are presented in this report.

MATERIALS AND METHODS

The following plasmids were used: pAP42, pRSF2124 ApColE1, pUB781 HgColE1, and the cointegrative plasmids pAP42/pRSF2124 ApColE1 and pAP42/pUB781 HgColE1. For a study of the effect of a complex inhibitor (FinU + FinV), two variants of plasmids were used: a) F-like drd-plasmids (pAP22-2::Tn5 and pAP18-1::Tn5) sensitive to the FinV-type transfer inhibitor, and b) pAP11-2::Tn9 and pAP19-1::Tn9 sensitive both to FinV and FinU. In the conjugative crossings *E. coli* K12 cells served as host cells (strains AP115 Nal, AP132 Nal, C600 Str, and C600 Rif).

Crossing of bacteria and determination of the sensitivity to Qβ phage were performed routinely [4,5]. The index of plasmid transfer inhibition (PTI) was estimated as the ratio of the corresponding values for monoplasmid and diplasmid cell cultures of the same strain.

RESULTS

The study of characteristic effects of the complex inhibitor (FinU+FinV) encoded by the pAP42/pRSF2124 and pAP42/pUB781 cointegrative plasmids was begun with an investigation of transfer function inhibition using the F-like, FinV-type-sensitive drd-plasmids (pAP22-2::Tn5 and pAP18-1::Tn5) and the plasmids sensitive to both FinU and FinV-type inhibitors (pAP11-2::Tn9 and pAP19-1::Tn9). For this purpose we selected

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TABLE 1. Effect of Complex (FinU+FinV) Inhibitor Synthesized under the Control of Cointegrative pAP42/pRSF2124 and pAP42/pUB781 Plasmids on the Transfer of drd−Plasmids

Plasmid tested (type of transfer inhibitor)	Inhibition of F-type drd-plasmid transfer (type of sensitivity to transfer inhibitor factor)							
	pAP22-2::Tn5 (V)		pAP18-1::Tn5 (V)		pAP11-2::Tn9 (OP,U,V)		pAP19-1::Tn9 (OP,U,V)	
	1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
pAP42 (U)	1.5−4.2	−	0.8−1.0	−	50.0−70.0	+	22.0−25.0	+/−
pRSF2124 (V)	$(0.6-1.0)\times10^2$	+/−	$(1.7-6.0)\times10^3$	+	$(0.8-1.0)\times10^2$	+	$(1.2-1.5)\times10^2$	+/−
pUB781 (V)	$(2.8-3.0)\times10^2$	+	$(1.4-3.2)\times10^2$	+	$(1.2-2.0)\times10^2$	+/−	$(1.6-2.1)\times10^2$	+/−
pAP42/pUB781 (U+V)	$(7.4-8.0)\times10^2$	+	$(1.2-3.8)\times10^2$	+	$(2.0-2.3)\times10^4$	+	$(2.3-4.0)\times10^4$	+
pAP42/pRSF2124 (U+V)	$(0.4-1.5)\times10^2$	+/−	$(2.5-3.0)\times10^2$	+/−	$(2.2-4.0)\times10^3$	+	$(0.8-1.3)\times10^3$	+

Note. 1) index of transfer inhibition for a drd−plasmid; 2) functioning of pili formation by drd−plasmids; + and − denote inhibition and lack of inhibition, respectively, of pili formation; +/− denotes moderately inhibiting effect.

monoplasmid and diplasmid transconjugates C600 Str containing one of the examined and/or standard drd-plasmids. The obtained bacterial strains were assessed for sensitivity to Qβ phage and efficiency of conjugational transfer of standard drd-plasmid during crossing with recipient cells of the C600 Rif strain. In control experiments the inhibitory effect on the transfer of the same drd-plasmids was evaluated by separate use of the FinU-system (pAP42 transfer factor) and FinV-system (nonconjugative pRSF2124 and pUB781 plasmids).

The results reflected in Table 1 show that the PTI values of standard plasmids (pAP22-2::Tn5 and pAP18-1::Tn5) by cointegrative plasmids (pAP42/pRSF2124 and pAP42/pUB781) differ only slightly from PTI for nonconjugative plasmids (pRSF2124 and pUB781). Thus, regarding the transfer of drd-plasmids sensitive to FinV-type inhibitor only, the levels of inhibition produced by cointegrative plasmids (FinV+FinU) and the initial nonconjugative plasmids are comparable.

The use of standard drd-plasmids (pAP11-2::Tn9 and pAP19-1::Tn9) sensitive to both FinV- and FinU-type transfer inhibitors yielded different results. Both the Fin-system of pAP42 transfer factor (FinU) and the Fin-systems of nonconjugative plasmids pRSF2124 and pUB781 (FinV) administered separately produced an inhibitory efect. However, the inhibitory effect of complex inhibitor (FinV+FinU) produced by cointegrative plasmids proved to exceed strongly the sum of effects produced by both inhibitors separately. Thus, the complex inhibitor in cointegrative pAP42/pUB781 plasmid exceeded the summated effect of separately acting FinU (pAP42) and FinV (pUB781) 85-120-fold relative to the drd-plasmid pAP11-2::Tn9 and 125-170-fold relative to pAP19-1::Tn9. In the case of the complex inhibitor produced by pAP42/pRSF2124 and Fin-systems of pAP42 and pRSF2124 the excess was equal to 4.5-7.5 times and 17.0-24.0 times vis-a-vis the standard pAP11-2::Tn9 and pAP19-1::Tn9 plasmids, respectively.

Summarizing the data obtained, we can conclude that the two genetic systems of regulation (FinU and FinV) residing together in the genomes of cointegrative pAP42/pUB781 and pAP42/pRSF2124 plasmids produce a summated effect. However, this effect is much stronger than the simple sum of separately introduced inhibitors, i.e., FinU and FinV under the control of pAP42 transfer factor and pUB781 and pRSF2124 nonconjugative plasmids. In light of these results, the cause of such a drastic increase of the expressivity of plasmid cointegrate-inserted Fin-genes remains unclear, and further investigation is required.

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