EXPERIMENTAL GENETICS

Genetic Regulation Systems of Cointegrative Plasmid Transfer in *E.coli* K-12 Cells

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The ability of plasmids to spread in natural bacterial populations is determined by the functioning of the particular system of conjugation transfer genetic regulation (fin system) contained in the plasmid genome [3,6]. By now such regulating systems have been detected in many conjugative and nonconjugative plasmid structures [1,7], but the patterns of the development and evolution of various regulating systems of plasmid "inhabiting" bacterial populations are still not quite clear. Since the cointegration process is one of the most probable stages in conjugative plasmid regulation [2]., we attempted a study of the functioning of fin system of plasmid cointegrates constructed on the basis of pAP42 conjugative plasmid (transfer factor) and pRSF2124 and pUB781 nonconjugative plasmids.

MATERIALS AND METHODS

We used pAP42 Tra+ genetic transfer F-like factor and nonconjugative plasmids pRSF2124 ApColE1Tra$^-$ and pUB781 HgColE1Tra$^-$. Cointegrative plasmids pAP42/pRSF2124 ApColE1Tra$^+$ and pAP42/pUB781 HgColE1Tra$^+$ were constructed in the course of the present research. For the detection of transfer inhibitor types encoded by the examined plasmids a test system was used consisting of a set of derepressed (drd) F-like plasmids with a known type of sensitivity to transfer inhibitors [4]; the characteristics of these plasmids are presented in Table 2. *E.coli* K-12 (strains AP115 Nal, AP132 Nal, C600 Str, C600 Rif) cells were used as plasmid hosts in conjugation hybridization.

Standard methods were used for conjugation hybridization and assessment of bacterial cell sensitivity to pilus-specific phage Qβ [2,5]. The index of plasmid transfer frequency inhibition (IPI) and phage inoculation relative efficacy (IRE) were defined as the ratio of the relevant parameters for monoplasmid and diplasmid cell cultures of the same strain.

RESULTS

The first task was to investigate the ability of cointegrative plasmids pAP42/pRSF2124 and pAP42/

TABLE 1. Reference Flac Plasmid Transfer Inhibition in C600 Diplasmid Transconjugate Cells

Tested (inhibiting) plasmid	Flac Plasmid IPI	Phage Qβ IRE
pAP42/pRSF2124	$(4.0-8.0)\times10^2$	44.0 – 68.0
pAP42/pUB781	$(1.2-2.0)\times10^3$	0.1 – 1.0
pRSF2124	$(1.6-3.7)\times10^2$	8.2 – 18.0
pUB781	$(1.2-1.7)\times10^2$	26.0 – 32.0
pAP42	1.5 – 3.5	25.0 – 42.0

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TABLE 2. Identification of Cointegrative Plasmid Genetic Regulatin Systems in Tests with F− Like Derepressed Plasmids

Tested (inhibiting) plasmid	F-like plasmid transfer inhibiting capacity (type of plasmid sensitivity to transfer inhibitors)								Type of transfer inhibitor
	pAP53::Tn9(V)		pAP18-1::Tn9(V, W)		pAP22-2::Tn1(U, V)		pAP11-2::Tn5 (OP, U, V)	pAP10-2::Tn9 (OP, Q, U, V)	
	1	2	1	2	1	2	1	2	1	2	
pAP42/pRSF2124	$(1.2-1.4)\times10^2$	+	$(7.7-10.0)\times10$	+	n.t.	n.t.	$(1.5-1.6)\times10^4$	+	$(2.5-33)\times10^3$	+	U + V
pAP42/pUB781	$(2.0-2.2)\times10^2$	+	$(5.0-7.0)\times10$	+ / −	$(5.0-5.2)\times10^4$	+	$(1.2-1.7)\times10^4$	+	$(4.8-5.3)\times10^4$	+	U + V
pRSF2124	$(2.2-5.8)\times10^2$	+	$(8.3-10.0)\times10$	+	n.t.	n.t.	$(0.8-1.0)\times10^2$	+	$(3.0-6.2)\times10^2$	+	V
pUB781	$(2.1-2.5)\times10^2$	+	$(5.0-8.3)\times10$	+ / −	$(6.0-6.4)\times10^2$	+ / −	$(2.1-2.8)\times10^2$	+	$(2.0-2.5)\times10^3$	+	V
pAP42	0.6 − 1.0	−	1.0 − 1.5	−	$(1.8-2.2)\times10$	−	$(9.4-10.0)\times10$	+ / −	$(7.1-7.6)\times10$	+ / −	U

Note. Inhibited functions: 1) drd plasmid IPI; 2) pilus formation function: + (−) capacity (incapacity) to inhibit pilus formation; + / − negligible inhibitory effect; n.t. − not tested.

pUB781, nonconjugative plasmids pRSF2124, pUB781, and pAP42 transfer factor to inhibit transfer of Flac reference plasmid. To this end, diplasmid and monoplasmid transconjugates containing one of the tested plasmids and/or Flac plasmid were selected and tested for sensitivity to Qβ phage and for Flac plasmid conjugation transfer efficacy in hybridization with cells of the C600 Rif recipient strain. The results, summarized in Table 1., demonstrate that all the tested plasmids, nonconjugative ones included, are characterized by the ability to inhibit Flac plasmid transfer functions, the nonconjugative and cointegrative plasmids showing a higher level of reference plasmid transfer inhibition (high IPI values) in comparison with pAP42 conjugative plasmid, whose inhibitory effect is negligible. Nonetheless, all plasmids influence Flac plasmid pilus-determinant function, as is proved by the reduced relative efficacy of pilus-specific phage Qβ inoculation.

Some data on Flac reference plasmid sensitivity to six transfer inhibition types (Fin OP, Q, U, V, W, C) [6] led us to expect that both pAP42 transfer factor and nonconjugative plasmids pRSF2124 and pUB781 and cointegrates pAP42/pRSF2124 and pAP42/pUB781 would obtain determinants of a particular type of transfer inhibitors in their genomes. To test this hypothesis we carried out experiments to identify the possible transfer inhibitor type synthesized under the control of the tested plasmids. For this purpose we selected and studied C6500 Str plasmid transconjugates containing one of these plasmids and/or one of the reference F-like plasmids with a known type of sensitivity to transfer inhibitors. The results of these experiments, presented in Table 2, suggest that a certain type of transfer inhibitor is synthesized in *E.coli* K-12 cells under the genetic control of the tested plasmids. For example, pAP42 factor appears to control type FinU inhibitor synthesis, because the transfer efficacy and particularly the pilus-formation function of reference plasmids pAP22-2::Tn1, pAP11-2::Tn5, and pAP10-2::Tn9 sensitive to this type of inhibitor are inhibited in the presence of this plasmid, in contrast to plasmid pAP53::Tn9 and pAP18-2::Tn9, which are not sensitive to this inhibitor. As for the nonconjugative plasmids pRSF2124 and pUB781, one may suspect the presence of determinants f type FinV inhibitor synthesis in them, as they inhibit transfer functions of all plasmids sensitive to this type of inhibitor (Table 2). As far as cointagrative plasmids pAP42/pRSF2124 and pAP42/pUB781 are concerned, the findings permit us to assume the simultaneous presence in their genomes of genetic

determinants of two different fin systems controlling the synthesis of FinU and FinV transfer inhibitors. This hypothesis is confirmed by the quantitative differences between the levels of inhibition by cointegrative plasmids of the transfer of reference drd plasmids, which are sensitive simultaneously to FinU and FinV inhibitors, and the level of inhibition of the transfer of pAP18-1::Tn9 plasmids, which are sensitive to only one of these inhibitors. FinV. Differences in IPI values of reference plasmids pAP22-2::Tn1, pAP11-2::Tn5, and pAP10-2::Tn9 detected during their inhibition by cointegrative plasmids in comparison with the inhibitory activities of "parent" plasmids pAP42, pRSF2124 and pUB781 also support this hypothesis.

Our data confirm the presence of specific fin systems in the genomes of some nonconjugative plasmids, this permitting us to expect the appearance of such plasmids in the course of evolution of more intricate genetic structures, to which conjugative plasmids belong.

These results allow us to conclude that the formation of conjugative cointegrative plasmids is paralleled by the formation in their genomes of complex fin systems capable of providing synthesis of transfer inhibitors of various functional types; but how many such systems a cointegrative plasmid genome may contain whether there may be "hybrid" fin systems be among them, and how they function are still question to be answered.

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Role of *E.coli* K-12 Chromosomal THR-LEU Segment in Expression of the FIN System of Inhibition of pAP53 F-Like Plasmid Transfer

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Genetic transfer of plasmids is determined by transfer plasmid genes (tra genes) whose functioning is controlled by a plasmid system of genetic regulation (fin system). The ability of plasmids to disseminate in natural bacterial populations depends on fin system activity [1,7]. Meanwhile, certain sites of the bacterial chromosome have an undoubted influence of the expression of tra genes [5,6]. However, the role of chromosomal genes in the expression of a particular fin system in uncnown.

We tried to elucidate the effect of chromosomal genes E cli K-12 cells on the activity of a

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