GENETICS

Identification and Characterization of New Systems Regulating Genetic Plasmid Transfer

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Study of the capacity of various repressed plasmids to inhibit derepressed plasmid transfer revealed new systems regulating conjugation plasmid transfer. These systems were denoted Fin A, fin B, fin D, fin E, and fin F.

Key Words: *plasmid; transfer inhibition system; derepressed mutant*

Efficacy of conjugation plasmid transfer and plasmid prevalence in natural populations of bacteria are determined by functioning of plasmid transfer genes (tra-genes), whose expression is regulated by fin systems [3,7,8]. There is evidence [1,4] suggesting the presence of new fin systems in plasmid genomes in addition to the known systems regulating genetic plasmid transfer (fin OP, Q, U, V, W, and C).

This study was devoted to the search for and characterization of such systems in plasmids identified in *Escherichia coli* from natural populations.

MATERIALS AND METHODS

We investigated previously identified repressed (rd) plasmids carrying the drug resistance, colicinogenicity, and hemolytic activity genes, and reference derepressed (drd) plasmids listed in Table 1. *E. coli* K-12 (AP132 Nal, C600 Rif, and C600 Str) were plasmid hosts.

Plasmid transfer was carried out in standard conjugation crossings. The index of drd plasmid transfer inhibition was calculated as the ratio of the frequency of this plasmid transfer from monoplasmid donor cells containing it to the frequency of this plasmid transfer from cells containing drd plasmid and the examined rd plasmid.

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The efficacy of functioning of plasmid-specific "sex" piles was assessed by the sensitivity of bacteria to pile-specific phage MS2. To obtain drd plasmid mutants, populations of plasmid-containing bacteria were routinely treated with N-methyl-N'-nitro-N-nitrosoguanidine mutagen [2]. Plasmid DNA was isolated by a previously described modified method [6]. Restriction of plasmid DNA was carried out routinely using specific EcoR1 endonuclease [5]. The size of restriction DNA fragments was determined by comparing their mobility in agarose gel with the mobility of DNA phage λ fragments.

RESULTS

For detecting plasmids carrying new fin systems, we assessed the capacity of repressed plasmids to inhibit Tra-function of reference drd Flac plasmid. Eight plasmids incapable of such inhibition were detected (Flac plasmid transfer inhibition was 0.3-1.7). Flac plasmid is sensitive to all known rd plasmid transfer inhibitors and, therefore, the detected rd plasmids can carry new fin systems.

To verify this hypothesis, we used the capacity of the rd plasmids to inhibit the functions of tra-genes regulating the production of F-type "sex" piles in ten drd plasmids from our collection. These ten plasmids included drd plasmids previously characterized by the sensitivity to transfer inhibitors [4] and two mutant

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Table 1. Capacity of rd Plasmids to Inhibit the Pile Formation Function in drd Plasmids

drd Plasmid	rd Plasmid										
	pAP 19-1::Tn1 (Col, Ap, finA)	pAP 19-1::Tn9 (Col, Lm, finA)	pAP 20 (Hly, finA)	pAP 20::Tn9 (Hly, Lm, finA)	pAP 22-1::Tn1 (Ap, finB)	pAP 22-1::Tn9 (Lm, finB)	pAP27 (Ap, Lm, Tc, Sm, Su finD)	pAP 68-1 (Tc, finF)	pAP69 (Ap, Lm, Sm, Tc, finE)	pAP83 (Sm, finA)	pAP 85-2 (Sm, finF)
Flac (OP, Q, U, V, W, C)		—		—		—	—	—	—	—	—
pAP11-2::Tn1 (OP, Q, U, V)		—		—		—	—	—	—	—	—
pAP11-2::Tn5 (OP, U, V)		—		—		—	+	—	—	—	—
pAP18-1 (U, V)		—		—		—	—	n.d.	+	—	—
pAP18-1::Tn5 (V)		+		+		+	—	—	+	+	—
pAP19-1::Tn9 (OP, Q, U, V, W)		—		—		+	—	—	—	—	—
pAP20::Tn9 (OP, Q, U, V, W)		—		—		+	—	—	—	—	—
pAP22-2 (OP, V)		—		—		—	—	—	—	—	—
pAP22-2::Tn1 (Q, U, V)		—		—		—	—	—	—	—	—
pAP42::Tn5 (U, V, W)		—		—		+	—	—	+	—	—
pAP53::Tn5 (V)		—		—		—	—	—	+	—	—

Note. Capacity (+) and incapacity (-) of rd plasmid to inhibit pile formation function in drd plasmid; n. d.: not determined.

plasmids (pAP19-1::Tn9 drd and pAP20::Tn9 drd), selected in this study after nitrosoguanidine treatment of bacterial cells containing repressed variants of these plasmids. Testing of these two drd plasmids using reference rd plasmids from N. Willetts' collection revealed their sensitivity to the inhibitors of known fin systems: pAP19-1::Tn9 drd — fin OP, Q, U, V, W; pAP20::Tn9 drd — fin OP, Q, U, V, W.

Thus, all ten drd plasmids are characterized by specific sensitivity to inhibitors of known fin systems and differ from the "classical" Flac plasmid.

Table 1 demonstrates functional heterogeneity of transfer inhibitors produced in bacterial cells under regulation by the studied rd plasmid fin-genes. The differences in the capacity of individual rd plasmids to inhibit Tra-functions of a derepressed plasmid made it possible to assign fin systems of 8 examined plasmids into at least 5 groups, arbitrarily denoted A, B, D. E. and F.

Data presented in Table 1 allow us to refer to fin A group plasmids pAP19-1::Tn1, pAP19-1::Tn9, pAP20, pAP20::Tn9, and pAP83; fin B — pAP22-1::Tn1 and pAP22-1::Tn9, fin D — pAP27; fin E — pAP69; and fin F — pAP68-1 and pAP85-2.

Induced drd mutants of plasmids pAP19-1::Tn9 drd, pAP20::Tn9 drd, and initial rd variants of these plasmids were compared in special experiments. Unlike the initial repressed plasmids, their drd analogs were characterized by capacity to render plasmid-containing host cells a derepressed pattern of functioning of plasmid-specific ("sex") piles, ensuring contacts between conjugated bacteria.

Table 2. Number and Size of Restriction EcoR1 Fragments of Examined Plasmids (MD)

Fragment No.	Plasmid			
	pAP19-1::Tn9 rd	pAP19-1::Tn9 drd	pAP20::Tn9 rd	pAP20::Tn9 drd
1	15.8	15.8	18.5	15.8
2	12.5	12.5	13.7	12.5
3	4.9	8.8	6.7	6.7
4	3.5	4.9	3.8	3.8
5	2.9	3.5	3.6	3.6
6	2.2	2.2	3.3	3.3
7	2.1	2.1	2.7	2.7
Total molecular weight, MD	43.9	49.8	52.3	48.4

For comparative study of molecular structure of the above-mentioned drd mutants and initial rd plasmids, plasmid DNA was isolated from the cells containing these plasmids, which was subjected to restriction analysis. The sizes of restriction fragments of plasmid DNA detected by slab electrophoresis in 0.65% agarose gel are shown in Table 2.

Appearance of plasmid pAP19-1::Tn9 drd mutant can be explained by rearrangement of its genome structure caused by deletion of EcoR1 fragment f5 of the initial plasmid with molecular weight 2.9 MD and appearance of a new EcoR1-fragment f3 (8.8 MD) in drd mutant genome. No genome restructuring of such kind seemed to occur in plasmid pAP20::Tn9 drd.

Our results demonstrate a great variety and functional heterogeneity of genetic transfer regulating systems in genomes of different plasmids from natural bacterial populations. Probably, the pattern of fin system activity determines the efficacy of plasmids and their genes in natural populations of bacteria. Besides reference plasmids from N. Willetts' collection, drd plasmids with a definite sensitivity to inhibitors of different fin systems, selected by us, can be used for practical identification of such systems.

REFERENCES

1. L. V. Maksimenko and A. P. Pekhov, *Byull. Eksp. Biol. Med.*, **114**, 196-198 (1992).
2. D. Miller, *Experiments in Molecular Genetics* [in Russian], Moscow (1976).
3. A. P. Pekhov, *Fundamentals of Plasmidology* [in Russian], Moscow (1996).
4. A. P. Pekhov, V. P. Shchipkov, and K. S. Krivskaya, *Byull. Eksp. Biol. Med.*, **120**, No. 7, 6-12 (1995).
5. T. Maniatis, E. F. Fritsch, and I. Sambrook, *Molecular Cloning: A Laboratory Manual*, New York (1982).
6. R. B. Meagher, R. C. Tait, M. Betlach, and H. B. Boyer, *Cell*, **10**, 521-536 (1977).
7. N. S. Willetts and R. Skurray, *Annu. Rev. Genet.*, **14**, 41-76 (1980).
8. N. S. Willetts and R. Skurray, in: *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*, Washington (1987), Vol. 2, pp. 1110-1133.