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ROLE OF *Escherichia coli* HOST CELLS IN GENETIC CONTROL OF PLASMID TRANSFER

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The genomes of different plasmids contain the genetic fin-systems OP, Q, U, V, W, and C, which control the synthesis of inhibitors of transfer of F and other F-like plasmids [5, 8]. The use of derepressed plasmids with known type of sensitivity toward transfer inhibitors, suppressing the action of tra-genes, has led to the identification of the genetic fin system in a number of repressed plasmids [1]. However, the regulating activity of these systems in all known experiments has been established by the use only of cells of a serologically untyped laboratory strain *E. coli* K12 and its derivatives. Yet under natural conditions plasmids are inhabitations of cells of *E. coli* strains which differ significantly from the K12 strain, thus raising the question of the role of host cells in the genetic control of plasmid transfer.

The aim of the investigation was to study genetic control of plasmid transfer in serologically typed *E. coli* cells.

EXPERIMENTAL METHOD

Strains *E. coli* C600 Str and AP132 Nal, derivatives of *E. coli* K12 forming rough colonies (the R form) were used. Genetically labeled strains of serologically types *E. coli* forming smooth colonies (S) were bred in the course of the present investigation. The S and R forms of the bacteria were differentiated in tests with boiling of the corresponding cultures. O antigens were detected in the linear agglutination test in tubes, using heated cultures of bacteria and standard OB-coli diagnostic sera.

R plasmids pAP3 (Lm), pAP17-2 (Tc), pAP18-1 (Tc), pAP30-2 (Ap, Lm, Tc), and Hly-plasmid pAP17-1::Tn9, controlling synthesis of known types of inhibitors [1], were used as plasmids repressed for transfer functions. The list of derepressed plasmids with known types of sensitivity to inhibitors used is given in Table 1. Plasmids were eliminated from bacteria with the aid of ethidium bromide by the standard method [7]. Conjugation transfer of plasmids was carried out in standard crosses of plasmid donor bacteria with suitable recipient cells [3].

The ability of *E. coli* cells to form specific F-pili was judged by their sensitivity to F-pili-specific phage MS2, determined by the agar layers method [3]. The relative seeding efficiency of the phage was determined as the ratio between the mean number of infectious phage centers, formed after seeding biplasmid conjugants (cells containing a derepressed and a repressed plasmid at the same time) to the number of such centers formed in the case of monoplasmid bacteria (containing one derepressed plasmid only), in percent. The transfer inhibition index of the derepressed plasmid was determined as the ratio of the frequency of conjugation transmission of this plasmid from cells of the monoplasmid strain to the corresponding parameter for the biplasmid strain.

EXPERIMENTAL RESULTS

The investigation began with the obtaining of genetically labeled recipient (plasmid-free) strains of serologically types *E. coli*. For this purpose, after treatment of the plasmid-containing cells of *E. coli* of strains AP15 (serogroup O106), AP58 (O147), and AP70 (O128), isolated previously [2, 4], with ethidium bromide, plasmid-free variants of these

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TABLE 1. Inhibition of Functions of tra-Genes of Derepressed L-Like Plasmids by Test (repressed) Plasmids in Cells of Serologically Typed and Untyped *E. coli* Strains

Test plasmids and most probable type of transfer inhibitor	Derepressed plasmids (type of sensitivity to transfer inhibitor)	Strain of host bacteria	Results of study of biplasmid transconjugants	
			relative seeding efficiency of phage, %	index of inhibition of transfer of derepressed plasmid
pAP3 (Fin OP)	pAP11-2:: (OP, U, V)	AP15-3	23	50
		AP58-3	63	240
		AP70-3	27	800
		AP132 (K12)	13	2 500
pAP17-1::Tn9 (Fin U)	pAP22-2::Tn1 (U, V)	AP15-3	13	500
		AP58-3	36	400
		AP70-3	0,6	120
		AP132 (K12)	0,3	2 400
pAP17-2 (Fin U)	pAP22-2::Tn1 (U, V)	AP15-3	0,6	0,5
		AP58-3	14	30
		AP70-3	0,6	50
		AP132 (K12)	0,3	20 000
	pAP42::Tn5 (U, V, W)	AP15-3	0,5	2,5
		AP58-3	0,5	20
		AP70-3	0,4	20
		AP132 (K12)	0,3	150
pAP18-1 (Fin V)	pAP53::Tn5 (V)	AP15-3	0,5	500
		AP58-3	0,8	110
		AP70-3	0,5	1 200
		AP132 (K12)	0,5	4 000
pAP30-2 (Fin OP)	pAP11-2::Tn5 (OP, U, V)	AP15-3	2	30
		AP58-3	12	30
		AP70-3	1	40
		AP132 (K12)	0,2	1 000

TABLE 2. Efficiency of Conjugation Transfer of Test Plasmids into Recipient Cells of Serologically Typed and Untyped Strains of *E. coli*

Donor	Plasmid	Frequency of plasmid transfer into cells of recipient strains, %			
		AP15-3 (O106)	AP58-3 (O147)	AP70-3(O128)	AP132 (K12)
AP15-2 (O106)	pAP3	0,005	0,0003	0,005	1,1
	pAP17-1::Tn9	0,005	0,0005	1,0	1,0
	pAP17-2	0,0008	0,0002	0,03	0,03
	pAP18-1	0,00004	0,00003	0,01	0,0003
	pAP30-2	0,3	0,8	2,3	3
AP58-2 (O147)	pAP3	0,0005	0,00005	0,005	0,06
	pAP17-1::Tn9	0,05	0,00006	5,0	1,0
	pAP17-2	0,0005	0,08	0,02	0,003
	pAP18-1	0,0006	0,0003	0,001	0,01
	pAP30-2	0,01	0,03	1,1	2,5
AP70-2 (O128)	pAP3	0,01	0,00001	0,5	0,3
	pAP17-1::Tn9	0,1	0,00006	0,8	1
	pAP17-2	0,01	0,00004	0,2	0,08
	pAP18-1	0,001	0,0002	1	0,00006
	pAP30-2	1,1	0,002	2	4

bacteria were selected and were designated AP15-1, AP58-1, and AP-70-1, respectively. On subsequent seeding of cultures of these bacteria on dishes with nutrient agar + antibiotic, mutants resistant to streptomycin (AP15-2, AP58-2, AP70-2) or to nalidixic acid (AP15-3, AP58-3, AP70-3) were selected. Additional investigations of bacteria of mutant strains in the culture boiling test and in linear agglutination tests showed that they preserve the S colony form and belong to serogroups O106, O147, and O128, respectively.

Conjugation crosses of serologically untyped *E. coli* AP132 cells containing one of the repressed plasmids pAP3, pAP17-1::Tn9, pAP17-2, pAP18.1, or pAP30-2, with serologically types AP15-2, AP58-2, and AP70-2 and untyped C600 recipient cells, yielded transconjugants which were used in subsequent experiments as plasmid donors in crosses with recipient cells of various serologically types and untyped strains of *E. coli*. The results of these crosses are given in Table 2.

As Table 2 shows the efficiency of conjugation transfer of the repressed plasmids tested is determined by the particular features both of the plasmids themselves and of the recipient

strains of *E. coli*. In most cases cells of untyped strain AP132 and typed strain AP70-3 (serogroup 0128) were found to be the most efficient recipients.

To study the characteristics of function of the fin systems of genetic control of transfer functions (functions of tra-genes) of the test plasmids in serologically typed *E. coli* cells, biplasmid transconjugants of these bacteria containing one of the test (repressed) plasmids and a standard derepressed plasmid, sensitive to the particular transfer inhibitor, were obtained. Similar biplasmid transconjugants of cells of a serologically untyped strain *E. coli* AP132 were obtained for comparison. The transconjugants selected were tested for sensitivity to F-pili-specific phage MS2 and for efficiency of plasmid transfer in crosses in which the recipients were *E. coli* C600 Str cells. The results of these experiments are given in Table 1.

It will be clear from Table 1 that in most cases the genetic fin systems of the repressed plasmids (of the fin OP, fin U, and fin V types) function in cells of both serologically typed and untyped strains of *E. coli*. However, plasmids contained in cells of the serologically untyped strain were characterized by much higher transfer inhibition indices than plasmids contained in cells of serologically typed bacteria. Similar platterns also were observed with respect to the level of inhibition of synthesis of F-specific pili, as may be judged by the relative seeding efficiency of phage MS2 (Table 1).

Analysis of the results suggests that the lower degree of inhibition of tra-gene function of serologically typed compared with serologically untyped *E. coli* cells may be collected either with a lower level of inhibitor synthesis or with lower activity of the inhibitor in these cells. Whatever the case, the results are evidence that the genome of the host cells exerts a considerably influence on the degree of expression of the fin systems for genetic control of plasmid transfer, which, in turn, is probably one of the factors determining the range of distribution of the different plasmids and their complexes in natural communities of bacteria.

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