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SURFACE EXCLUSION SYSTEMS OF F-LIKE PLASMIDS OF *E. coli* AND THEIR GENETIC CONTROL

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Surface exclusion of plasmid F and of F-like plasmids is controlled by traS and traT genes [5, 7, 9, 11]. The problem of the distribution of the different types of surface exclusion systems (Sfx systems) determined by these genes and of their location in the genome of different plasmids remains unsolved.

The aim of the investigation was to identify Sfx systems determined by genes of F-like plasmids in cells of natural strains of *E. coli,* to study their specificity, and to determine the possible localization of these genes in genomes of individual plasmids.

EXPERIMENTAL METHOD

We studied 17 F-like drd-plasmids, including their transposon-containing variants [1-4]. As standard strains we used plasmids Flac, R124, R1, and R100, determining known types of Sfx systems [12]. We also used plasmids pAP53::Tn5 and pAP53::Tn9, shown to belong to the SfxII group (Tables 1 and 2). As host cells for the plasmids we used cells of strains of *E. coli* AP115 (Lac, Met, Nal) and C600 (Lac, Thr, Leu, Rif). Competent cells of *E. coli* HB101 were used in the transformation experiments.

Surface exclusion was studied in standard (direct and reverse) conjugation crosses of bacteria containing plasmids. The surface exclusion index (SEI) was determined as the ratio of the number of plasmid transconjugants found by the use of a plasmid-free recipient strain to their number obtained for an isogenic strain containing the plasmid. Plasmid DNA was isolated by the method in [10], the purified cell lysates being centrifuged in a CsCl density gradient. Restriction of plasmid DNA by endonucleases EcoRI and SalI, elution of the restriction fragments from the gel, and subsequent molecular cloning of these fragments in the composition of vector plasmid pBR325 were carried out by the usual methods [8]. The restriction fragments were fractionated in 0.65% agarose gel by horizontal slab electrophoresis. The dimensions of the restriction fragments of DNA were determined by comparing their mobility in agarose gel with that of DNA fragments from phage λ [6].

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TABLE 1. Surface Exclusion Index of F-Like Plasmids in Direct Crosses of *E. Coli* C600 × AP115

Plasmid of recipient stain (resident)	Plasmid of donor strain (incoming)				
	Flac (Sfx I)	R124 (Sfx II)	pAP53::Tn5 or pAP53::Tn9, [sfx ii]	RI (Sfx III)	R100 (Sfx IV)
pAP10-2::Tn9	$(1,0—2,1)\cdot10^2$	6,0—10,7		2,8—5,7	2,4—4,1
pAP11-2	1,8—1,9	0,8—1,5		9,5—10,4	1,8—2,0
pAP11-2::Tn5	6,1—9,2	4,8—5,5		3,1—3,8	3,2—3,9
pAP18-1	$(0,9—8,5)\cdot10^2$		$(6,3—8,3)\cdot10^3$	9,3—10,7	$(2,1—6,0)\cdot10^1$
pAP18-1::Tn5	2,3—3,4		$(2,0—9,1)\cdot10^2$	3,0—8,0	$(1,7—3,7)\cdot10^1$
pAP18-1::Tn9	1,4—3,0		$(0,9—1,9)\cdot10^2$	2,4—8,3	$(2,0—5,4)\cdot10^1$
pAP19-1::Tn1	$(0,1—3,2)\cdot10^4$	$(0,1—1,6)\cdot10^4$		$(1,0—3,6)\cdot10^2$	$(0,5—2,2)\cdot10^4$
pAP19-1::Tn9	$(1,0—1,4)\cdot10^1$	$(1,1—2,4)\cdot10^1$		1,6—4,3	$(1,2—1,6)\cdot10^1$
pAP22-2	0,5—5,0	1,8—10,9		2,7—4,6	2,1—6,0
pAP22-2::Tn1	1,0—2,1	$(1,2—2,3)\cdot10^1$		$(0,6—1,9)\cdot10^1$	2,3—10,0
pAP38::Tn9	1,1—1,5	$(2,0—2,9)\cdot10^1$		1,8—3,2	3,4—8,2
pAP39::Tn9::Tn5	1,2—2,8	$(1,4—2,6)\cdot10^1$		1,6—2,4	0,8—1,6
pAP41::Tn9::Tn1721	$(4,3—5,5)\cdot10^1$		1,8—2,0	0,9—1,1	1,7—2,7
pAP42::Tn5	2,3—3,0	1,3—1,8		1,1—2,1	1,8—2,8
pAP53	7,7—10,3	$(0,2—2,4)\cdot10^2$		2,8—3,4	1,1—1,8
pAP53::Tn5	5,9—6,2	$(0,9—2,9)\cdot10^2$		$(1,2—6,6)\cdot10^2$	1,0—1,1
pAP53::Tn9	5,9—6,2	$(0,8—5,9)\cdot10^2$		1,0—3,0	0,9—1,2

TABLE 2. Surface Exclusion Index of F-Like Plasmids in Back Crosses of *E. coli* AP115 × C600

Plasmid of donor strain (incoming)	Plasmid of recipient strain (resident)			
	Flac (Sf×I)	R124 (Sf×II)	pAP153::Tn5 or pAP53::Tn9 (Sf × 11)	R100 (Sf×IV)
pAP10-2::Tn9	$(0,9—1,8)\cdot10^2$	1,4—1,7		
pAP11-2::Tn5	$(2,9—4,3)\cdot10^1$	$(1,5—8,8)\cdot10^2$		$(1,3—1,4)\cdot10^1$
pAP18-1*	1,3—5,0		$(1,2—6,6)\cdot10^3$	
pAP18-1::Tn5	$(0,8—1,6)\cdot10^1$		$(0,6—1,3)\cdot10^2$	$(2,0—6,5)\cdot10^1$
pAP18-1::Tn9	$(1,4—1,7)\cdot10^1$		$(2,3—3,4)\cdot10^2$	
pAP19-1::Tn1	$(0,4—1,0)\cdot10^2$	$(1,3—2,7)\cdot10^2$		$(3,9—6,1)\cdot10^2$
pAP19-1::Tn9	$(1,4—1,7)\cdot10^1$	$(2,4—3,0)\cdot10^2$		
pAR22-2::Tn1	$(2,1—3,1)\cdot10^1$	4,0—9,4		$(1,2—1,7)\cdot10^1$
pAP38::Tn9	2,5—4,5	$(1,0—4,2)\cdot10^2$		
pAP39::Tn9::Tn5	3,7—9,8	$(0,8—3,0)\cdot10^2$		
pAP41::Tn9::Tn1721	$(4,6—6,2)\cdot10^1$	1,1—2,3		
pAP42::Tn5	4,5—6,1	2,8—4,4		2,1—3,8
pAP::Tn5	4,0—5,7	$(3,0—9,5)\cdot10^2$		6,5—11,3
pAP53::Tn9	7,0—9,6	$(1,2—5,4)\cdot10^2$		

*Asterisk indicates that surface exclusion of this plasmid by plasmid R1 (SEI = 1.0-3.0) also was studied.

EXPERIMENTAL RESULTS

The results of the experiments to determine surface exclusion are given in Tables 1 and 2. Considering that the Sfx genetic system has a sufficiently complex phenotypic manifestation, which can be attributed to the simultaneous action of the plasmid-containing product (protein) of the traS gene, giving a stable and marked surface exclusion effect, and the nonspecific action of the product of the traT gene, giving a low level of surface exclusion [7], we regarded surface exclusion between the two test plasmids as specific only if it appeared in response to their transmission in two directions (each plasmid serves alternately as incoming or resident), and was adequately represented quantitatively (SEI of the order of 20 or more). It can accordingly be concluded from analysis of the results in Tables 1 and 2 that most of the F-like drd-plasmids studied belonged to one or other specific Sfx group. As will be clear from Tables 1 and 2, plasmids pAP10-2::Tn9 and pAP41::Tn9::Tn1721 can be classed in the SfxII group, described by other workers, and plasmids pAP38::Tn9, pAP39::Tn9::Tn5, pAP53::Tn5, and pAP53::Tn9 can be classed in group SfxII. At the same time the results show that certain plasmids belong to different Sfx groups, i.e., they are atypical. For instance, the surface exclusion system of plasmid pAP19-1::Tn1 is characterized by belonging to no fewer than three different Sfx groups simultaneously (I, II, and IV), whereas the system of plasmid pAP18-1::Tn5 belongs to two different groups (II, IV). In the case of plasmid pAP42::Tn5, and also plasmids pAP11-2 and pAP22-2 (and their transposon-containing analogs), no surface exclusion could be found with any of the standard plasmids of the four known Sfx groups. This suggests that they may belong to a new (not previously discovered) Sfx group (or groups).

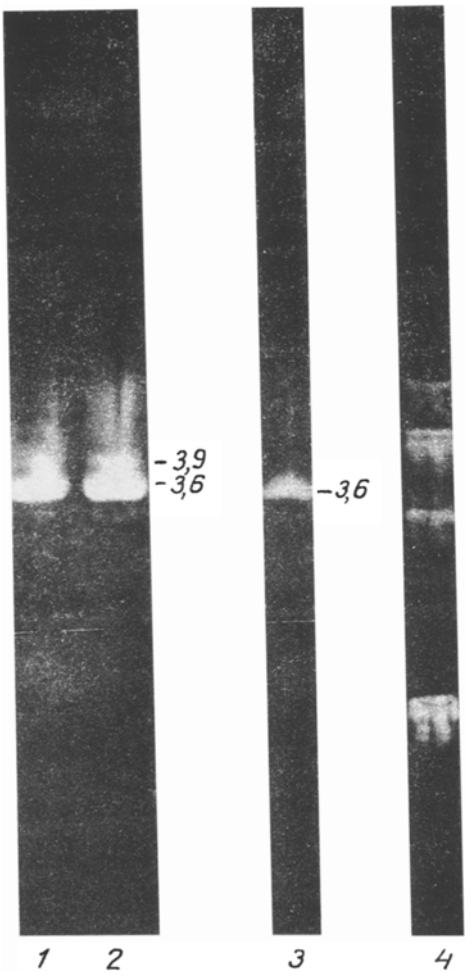

Fig. 1. Surface exclusion systems of F-like plasmids of *E. coli* and their genetic control. 1) DNA of pAP105, SalI; 2) DNA of pAP106, SalI; 3) DNA of pBR325, SalI; 4) DNA of phage λ, EcoRI.

To test this hypothesis experiments were undertaken to study the behavior of these plasmids relative to each other. Plasmids pAP11-2::Tn5 and pAP22-2::Tn1 were shown not to exhibit two-way surface exclusion with respect to each other (SEI = 2.0-2.3 and 2.2-2.7), plasmid pAP11-2 cannot exclude plasmid pAP22::Tn1 (SEI = 1.2-1.3), and plasmid pAP22-2 cannot exclude plasmid pAP11-2::Tn5 (SEI = 2.1-7.2). Meanwhile plasmid pAP11-2 excluded plasmid pAP11-2::Tn5 (SEI = 103-265), and plasmid pAP22-2 excluded plasmid pAP22-2::Tn1 (SEI = 218-242). In addition, no surface exclusion could be detected between plasmids pAP11-2 and pAP42::Tn5 (SEI = 4.8-9.6), pAP22-2 (pAP22-2::Tn1) and pAP42::Tn5 (SEI = 1.0-9.8). These results thus show that plasmids pAP42::Tn5, pAP11-2 (pAP11-2::Tn5), and pAP22-2 (pAP22-2::Tn1) do in fact belong to new Sfx groups, which can be designated SfxV, SfxVI, and SfxVII respectively.

Considering the atypical character of the surface exclusion of plasmid pAP18-1 we next carried out molecular cloning of its EcoRI- and SalI-fragments in the composition of the vector plasmid pBR325 in order to determine the possible localization of the genetic determinants of the Sfx phenotype. During the study of the ability of constructed recombinant plasmids to exclude plasmids pAP53::Tn5 and pAP53::Tn9, belonging to the SfxII group, this ability was found in two plasmids, selected during cloning of the SalI-fragments f5 of plasmid pAP18-1, and designated as pAP105 and pAP106 (SEI = 15-30 and 17-24 respectively). However, these plasmids were unable to exclude plasmid R100 — SfxIV (SEI = 1-2 and 2-3 respectively). In control experiments with vector plasmid pBR325 surface exclusion was absent in all cases (SEI = 0.5-4.0).

For the molecular characterization of these recombinant plasmids, we isolated their DNA and undertook its restriction analysis with the aid of endonuclease SalI.

The results of electrophoresis to study DNA of recombinant plasmids pAP105 and pAP106 are given in Fig. 1. They show that these plasmids each contain a SalI-fragment f5 (3.9 megadaltons) of plasmid pAP18-1. Consequently, the genetic locus determining the SfxII character is located in the SalI-fragment f5 of plasmid pAP18-1, the restriction map of which was drawn by the writers previously [4].

It can be concluded from a generalization of these results that different surface exclusion systems exist in the F-like plasmids identified in bacteria of various natural populations, and that some of them behave atypically as regards their membership of the Sfx group. The results of molecular cloning of plasmid pAP18-1 suggest that this atypical nature is linked with the presence of different genes determining the Sfx phenotype in the genomes of these plasmids.

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