PILI DETERMINED BY tra GENES OF F-LIKE PLASMIDS

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Since an essential role in plasmid transfer is played by pili (specific surface formations of plasmid-containing donor cells), synthesized under control of plasmids [2, 3, 6, 8, 9], in order to understand the nature of conjugation transfer of plasmids, an explanation of the structural and functional properties of these formations is very important.

The object of this investigation was to study structural and functional features of plasmid-specific pili, determined by tra-genes of F-like plasmids, found in cells of natural strains of E. coli, and also to determine the possible role of transposons in the modification of the properties of the pili.

EXPERIMENTAL METHOD

Altogether 27 F-like plasmids, either repressed (rd) or depressed (drd) with respect to genetic transfer functions, and including various transposon-containing variants of these plasmids, were investigated [1, 3, 5]. A mutant of drd-plasmid pAP39 was obtained in the course of the present investigation. As standards, we used also plasmids Flac, R386, R124, ColB4-K98, R1, and R100, determining synthesis of F-like pili of known type [9]. A complete list of the plasmids studied is given in Tables 1 and 2. Cells of E. coli strain AP132 (Lac, Nal), which is a derivative of E. coli strain K-12, were used as host plasmids.

Electron-microscopic demonstration of F-like pili, adsorbing pili-specific phage f2 on their own lateral surfaces, was carried out by the negative staining method [7]. The sensitivity of bacteria containing drd plasmids to pili-specific phages f1, f2, and Qβ was studied by the agar layers method. The relative seeding efficiency (RSE) of the phage was determined as the ratio, in percent, of the average number of phage zones of lysis formed after seeding

TABLE 1. Relative Seeding Efficiency (RSE) of Pili-Specific Phages f1, f2, and Qβ on E. coli AP132 Cells Containing Derepressed F-Like Plasmids

drd plasmid	RSE of phages, %			Group of F-like pili
	f1	f2	Qβ	
Flac	100	100	100	1
pAP10-2::Tn9	101±6	81±2	91±3	1
pAP11-2	133±2	107±4	112±9	1
pAP11-2::Tn1	119±1	117±2	101±4	1
pAP11-2::Tn5	93±4	116±2	118±2	1
pAP11-2::Tn9	107±5	99±2	125±5	1
pAP18-1	$(63\pm6)\cdot10^{-3}$	116±3	68±2	2
pAP18-1::Tn5	$(53\pm5)\cdot10^{-3}$	106±3	88±2	2
pAP18-1::Tn9	$(36\pm3)\cdot10^{-3}$	96±3	78±1	2
pAP19-1	72±2	102±5	101±2	1
pAP19-1::Tn1	91±2	110±4	77±2	1
pAP19-1::Tn9	103±5	102±3	102±4	1
pAP22-2	88±3	122±7	103±4	1
pAP22-2::Tn1	114±7	147±2	121±11	1
pAP38::Tn9	0	120±3	72±3	2
pAP39::Tn9::Tn5	73±3	98±2	96±2	1
pAP41::Tn9::Tn1721	128±6	118±2	137±5	1
pAP42	69±2	$(61\pm9)\cdot10^{-4}$	63±3	5
pAP42::Tn5	129±3	$(12\pm1)\cdot10^{-2}$	108±4	5
pAP53	$(15\pm1)\cdot10^{-3}$	88±3	61±3	2
pAP53::Tn5	0	92±1	70±2	2
pAP53::Tn9	0	95±6	58±2	2
(plasmid-free control)	0	0	0	—

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TABLE 2. Efficiency of Phage Titer Growth (PTG) of Phages f1, f2, and Qβ in Tests with E. coli AP132 Cells Containing Repressed and Derepressed F-Like Plasmids

Plasmid	Plasmid type	PTG index			Relative efficiency of PVG			Group of F-like pili
		f1	f2	Qβ	f1	f2	Qβ	
Flac	drd	$(1,2—3,8)\cdot10^6$	$(1,5—3,6)\cdot10^7$	$(3,5—6,5)\cdot10^7$	100	100	100	1
R386	drd	$(2,7—8,2)\cdot10^5$	$(0,4—1,1)\cdot10^7$	$(0,4—1,3)\cdot10^7$	26±3	26±3	20±4	1
R124	rd	$(1,8—3,2)\cdot10^4$	$(1,8—3,0)\cdot10^5$	$(3,5—7,1)\cdot10^5$	$(14\pm1)\cdot10^{-1}$	$(11\pm2)\cdot10^{-1}$	$(10\pm1)\cdot10^{-1}$	1
ColB4-K98	rd	$(3,3—8,3)\cdot10^2$	$(3,9—7,2)\cdot10^5$	$(0,8—1,3)\cdot10^6$	$(29\pm4)\cdot10^{-3}$	$(25\pm3)\cdot10^{-1}$	$(20\pm3)\cdot10^{-1}$	2
RI	rd	0,6—1,2	$(2,1—3,3)\cdot10^3$	0,8—1,4	$(38\pm4)\cdot10^{-6}$	$(16\pm3)\cdot10^{-3}$	$(19\pm2)\cdot10^{-7}$	3
R100	rd	0,7—1,1	0,6—0,9	0,9—1,4	$(42\pm6)\cdot10^{-6}$	$(38\pm2)\cdot10^{-7}$	$(22\pm3)\cdot10^{-7}$	4
pAP10-2	rd	$(0,5—1,2)\cdot10^4$	$(2,3—5,7)\cdot10^4$	$(1,2—1,4)\cdot10^5$	$(52\pm6)\cdot10^{-2}$	$(16\pm2)\cdot10^{-2}$	$(31\pm6)\cdot10^{-2}$	1
pAP10-2::Tn9	rd	$(0,4—1,0)\cdot10^3$	$(0,4—1,2)\cdot10^4$	$(1,4—2,4)\cdot10^4$	$(40\pm2)\cdot10^{-3}$	$(28\pm2)\cdot10^{-3}$	$(48\pm14)\cdot10^{-3}$	1
pAP10-2::Tn9	drd	$(4,3—7,0)\cdot10^5$	$(4,5—8,6)\cdot10^6$	$(0,7—1,5)\cdot10^7$	36±3	28±2	24±3	1
pAP18-1	rd	$(0,6—1,8)\cdot10^3$	$(0,4—1,0)\cdot10^5$	$(0,8—1,3)\cdot10^5$	$(49\pm4)\cdot10^{-3}$	$(28\pm2)\cdot10^{-2}$	$(18\pm4)\cdot10^{-2}$	2
pAP18-1	drd	$(0,7—3,2)\cdot10^4$	$(0,4—1,1)\cdot10^7$	$(2,2—2,8)\cdot10^7$	$(87\pm21)\cdot10^{-2}$	33±4	42±4	2
pAP38::Tn9	rd	0,6—1,8	$(1,1—1,6)\cdot10^5$	$(4,3—5,5)\cdot10^5$	$(43\pm6)\cdot10^{-6}$	$(56\pm8)\cdot10^{-2}$	$(10\pm1)\cdot10^{-1}$	2
pAP38::Tn9	drd	0,7—2,0	$(3,0—4,4)\cdot10^6$	$(2,8—6,3)\cdot10^6$	$(49\pm17)\cdot10^{-6}$	14±2	9±2	2
pAP39::Tn9	rd	5—13	$(3,8—9,7)\cdot10^4$	$(1,4—3,3)\cdot10^5$	$(52\pm3)\cdot10^{-5}$	$(26\pm3)\cdot10^{-2}$	$(63\pm15)\cdot10^{-2}$	2
pAP39::Tn9::Tn5	drd	$(3,8—6,8)\cdot10^5$	$(0,3—1,0)\cdot10^7$	$(0,9—1,7)\cdot10^7$	33±2	26±3	31±2	1
pAP41::Tn9	rd	$(1,0—2,9)\cdot10^4$	$(1,2—1,8)\cdot10^5$	$(3,9—8,1)\cdot10^5$	$(96\pm14)\cdot10^{-2}$	$(66\pm8)\cdot10^{-2}$	$(15\pm3)\cdot10^{-1}$	1
pAP41::Tn9::Tn1721	drd	$(3,7—5,3)\cdot10^5$	$(0,6—1,6)\cdot10^7$	$(0,8—1,5)\cdot10^7$	30±7	38±3	26±2	1
(plasmid-free control)		0,5—0,8	0,6—0,8	0,7—0,8	$(28\pm4)\cdot10^{-6}$	$(29\pm4)\cdot10^{-7}$	$(15\pm2)\cdot10^{-7}$	—

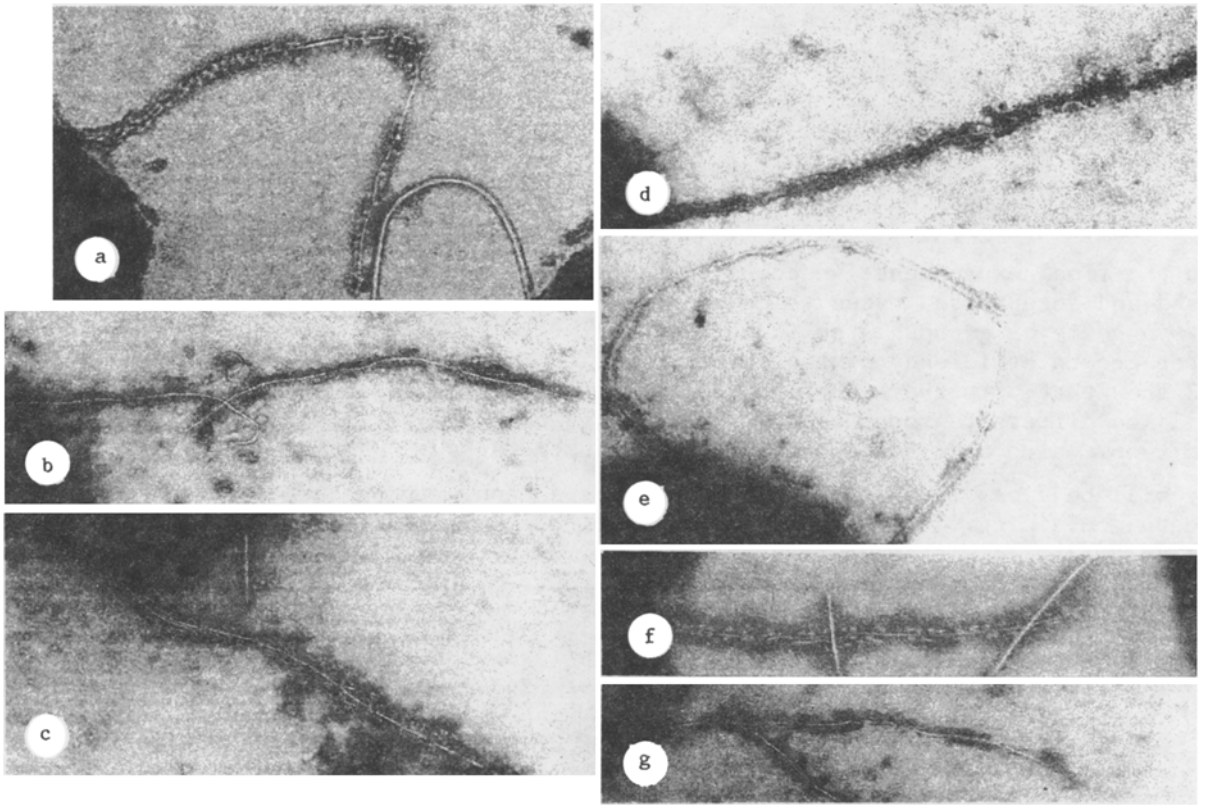

Fig. 1. Cells of E. coli strain AP132 containing depressed F-like plasmids. Magnification 15,000. a) Flac; b) pAP11-2::Tn1; c) pAP18-1; d) pAP38::Tn9; e) pAP39::Tn9::Tn5; f) pAP42; g) pAP53::Tn9.

on cells containing the test plasmid to the number of these zones on cells containing the standard plasmid Flac. The efficiency of titer growth reactions of these phages (PTGR) was studied by the standard scheme [4], using a ratio of phage to bacteria in the mixture of the order of 1:100, and using E. coli AP132 Flac as the indicator strain. The phage titer growth index (PTGI) was determined as the ratio of the number of phage zones of lysis found by titration of the phage-bacterial mixture after incubation for 18 h at 37°C to the number of zones found after seeding samples of this same mixture, taken before the beginning of incubation. The relative efficiency of PTG was calculated as the ratio, in percent, of the value of PTGI for bacteria containing the test plasmid to the value of PTGI for cells with the Flac plasmid.

EXPERIMENTAL RESULTS

Pili determined by F-like plasmids of the derepressed type can be identified by adsorption of pili-specific phages on them [7, 8]. The study of pili determined by the plasmids

used in this investigation was therefore begun with an electron-microscopic investigation of adsorption of pili-specific phage f2 on cells of the AP132 strain, containing one or other plasmid. As the experiment showed, cells of strain AP132 containing plasmids pAP11-2::Tn1, pAP18-1, pAP38::Tn9, pAP39::Tn9::Tn5, pAP42, and pAP53::Tn9 possess F-like sex pili, which are illustrated in Fig. 1b-g. According to the widely used classification [6], all the pili discovered belonged to the flexible type. Since the identified pili are morphologically indistinguishable from pili determined by the standard Flac plasmid (Fig. 1a), on the basis of the morphological investigation it is impossible to differentiate features of F-like pili determined by individual plasmids.

To discover functional differences between plasmid-specific pili, in the next experiments the sensitivity of the corresponding bacteria was compared to three different pili-specific phages (filamentous DNA-containing phage f1 and isometric RNA-containing phages f2 and Qb). Data on RSE of these phages on cells of strain AP132, containing the test drd plasmids, are given in Table 1.

To estimate levels of sensitivity of bacteria to individual phages, the F-like pili which we studied can be divided into three different functional groups (Table 1). Pili of two of these groups (1 and 2) are probably identical to pili of the first two groups (1 and 2) of the four groups of F-like pili described by other investigators after a study of plasmids identified in other regions of the world [8, 9]. Conversely, pili determined by genes of plasmid pAP42 and its transposon-containing variant (pAP42::Tn5), which maintain the normal level of sensitivity to phage f1 an Qβ, but a low level of sensitivity to phage f2, can be combined on the basis of these properties into a new (not previously described) functional grop of F-like pili (group 5, see Table 1). Thus pili determined by F-like plasmids can be divided functionally into at least five groups.

In the final experiments we compared bacteria of strain AP132 containing plasmids of repressed and derepressed types which we had identified, and also standard plasmids determining synthesis of groups established previously (1, 2, 3, and 4) of F-like pili [9] on the titer growth efficiency of the three specified phages in PTGR tests. The data given in Table 2 show that with the aid of the tests used it is possible to detect and classify F-like pili on different groups of cells containing both drd-plasmids and pladmids of repressed type, which are found most frequently in bacteria of natural populations

It will be clear from Table 2 that there is a change in PTG levels in bacteria possessing transposon-containing plasmids (pAP10-2::Tn9 rd, pAP10-2::Tn9 drd) compared with bacteria possessing a plasmid without a transposon (pAP10-2 rd) or a change in the group to which the pili belong (comparison of data for plasmids pAP39::Tn9 rd and pAP39:: Tn9::Tn5 drd).

It can thus be concluded that bacterial pili determined by F-like plasmids are characterized by functional diversity. One of the causes leading to a change in the functional properties of pili may perhaps be incorporation of transposons into the plasmid genomes.

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