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COMPATIBILITY OF F-LIKE PLASMID FB1drd WITH STANDARD F-GROUP PLASMIDS IN STRAINS OF *Escherichia coli* K12

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UDC 576.851.48.097.2:575

Compatibility of derepressed F-like plasmid FB1drd, integrated into the chromosome of *Escherichia coli* K12 cells with standard plasmids of compatibility groups FI-FVI was studied. The results show that such plasmids can coexist in a stable state in the same cell with plasmid FB1drd. This suggests that it belongs to a new compatibility group (FVII).

KEY WORDS: bacterial plasmids; compatibility; plasmid transconjugants.

One of the chief criteria for determination of the degree of phylogenetic kinship between different bacterial plasmids is the compatibility test, based on the inability of two closely related plasmids to coexist in a stable state in the same cell [3, 4]. According to this criterion, all currently known F-like plasmids can be divided into six compatibility groups (FI-FVI) [3-5].

The object of this investigation was to study compatibility of the F-like plasmid FB1drd, previously identified by the writers in cells of *Escherichia coli* serogroup O6 [2].

EXPERIMENTAL METHOD

Cells of *E. coli* strain AP106, containing standard plasmids belonging to groups FI-FVI were used as donors of the genetic material. The original strains carrying plasmids of groups FI-FV were obtained from Dr. Dennison (England); the strain with plasmid Hly-P212 (FVI) was obtained from Dr. Monti-Bragadin (Italy). Strain *E. coli* AP117 trp, thi, lac, nal (derived from strain 200PSF$^-$), bred by the present writers, and its derivatives AP118 and AP119 were used as recipients. Strain AP118 contains integrated plasmid FB1drd and is a lac$^+$-recombinant obtained by conjugating *E. coli* AP3Hfr [2] with AP117 cells. Strain AP119 was bred by the writers as a lactose-negative mutant from an AP118 population treated with nitrosoguanidine.

The bacteria were conjugated by the following standard method. 18-Hour broth cultures were diluted 1:10 in fresh nutrient broth (NB) and grown for 3 h at 37°C, after which they were mixed in the proportion of 1:4 by volume in 50-ml flasks. The conjugation mixtures were incubated for 2 h at 37°C and then seeded on media for selecting clones of recipient cells receiving the corresponding plasmid marker from the donor (plasmid transconjugants).

To study compatibility of the plasmids, the isolated transconjugants (at least three from each conjugation) were grown for 18 h in NB not containing any of the selective agents (at 37°C), and then seeded on dishes with nutrient agar (NA). The resulting clones (at least 50 from each sample) were replicated on dishes with selective agar in order to determine preservation of the corresponding plasmid markers.

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 0007-4888/78/8506-0786$07.50

TABLE 1. Compatibility of Plasmid FB1drd with Standard Plamids of F-Group

Donor containing standard plasmid	Compatibility group	Recipient	Transferred plasmid marker	Frequency of transfer (per donor's cell)	Analysis of resulting plasmid transconjugants	
					total number studied	number retaining plasmid marker
AP106	FI	AP117	Lac	1,0—1,2	1076	1042
F′ lac+		AP119 Hfr	Lac	$3,0\times10^{-3}$—$1,0\times10^{-2}$	568	568
AP106	FI	AP117	Tc	$5,0\times10^{-2}$	150	150
R368		AP118 Hfr	Tc	$0,8$—$1,5\times10^{-2}$	150	150
AP106	FII	AP117	Lm	0,9—2,0	150	150
R1-19 drd		AP118 Hfr	Lm	1,0	150	150
AP106	F III	AP117	Lm	$1,9\times10^{-2}$	150	150
ColB-R_3 AP106		AP119 Hfr	Lm	$1,6\times10^{-2}$	150	150
ColBK-98	F III	AP118 Hfr	ColB	0,38	150	150
AP106	F IV	AP117	Tc	$2,4\times10^{-2}$	150	150
R 124		AP118 Hfr	Tc	$1,2\times10^{-2}$	150	150
		AP119 Hfr	Tc	$1,8\times10^{-2}$	150	150
AP106	F V	AP117	Lac	$1,0\times10^{-5}$	917	917
Folac		AP119 Hfr	Lac	$3,0\times10^{-6}$	965	965
AP106	F VI	AP117	Hly	5×10^{-3}	1464	1461
Hly-P212		AP119 Hfr	Hly	11×10^{-3}	888	886

The sensitivity of the bacteria to antibiotics was tested by seeding them on NA containing the corresponding preparation. To determine hemolytic activity, 4% blood agar containing human erythrocytes was used. The colisinogenicity of the transconjugants was studied by the stab method [1].

EXPERIMENTAL RESULTS

Since recipient strains AP118 and AP119 contained plasmid FB1drd in the integrated state, i.e., were strains of the hfr type, the character of compatibility was judged from the degree of preservation of the marker of the superinfecting standard (autonomous) plasmid in cells of the strains indicated above (plasmid transconjugants). The analogous crosses in which strain AP117 not containing any plamids was used as the recipient served as the control.

It will be clear from Table 1 that different standard plasmids differed in the frequency of their transmission into the recipient cells. The marked decrease in the frequency of transmission of the F′lac$^+$ plasmid into cells of strain AP119Hfr compared with AP117 is evidence of the marked surface exclusion of that plasmid by the "resident" plasmid FB1drd. In the case of the other standard plasmids, this exclusion was less marked or absent.

The results (Table 1) are also evidence that plasmids belonging to all six groups of F-like plasmids so far known can coexist in a stable state in cells containing integrated plasmid FB1drd, i.e., they are compatible with it.

As these results show, the test plasmid FB1drd evidently belongs to none of the above-mentioned compatibility groups. On this basis it can be postulated that it may belong to a new compatibility group. In accordance with the recommendations [6], this compatibility group can be designated group FVII.

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