THE PLASMID COMPLEX OF *Escherichia coli* B-13

O. A. Glebova and A. P. Pekhov

UDC 579.842.11:579.252.5

KEY WORDS: plasmid; plasmid complex; genetic transfer; genetic transfer factor.

Identification of plasmids in *Escherichia coli* cells isolated from man and animals has shown that many of these strains are carriers of two plasmids or more. In particular, plasmids determining enterotoxigenicity and resistance to ampicillin, streptomycin, chloramphenicol, tetracycline, kanamycin, and sulfonamides have been found in cells of strain *E. coli* B-13 (serogroup 0101) [1, 2]. Continuing the study of this strain, the present writers found that it is also colicinogenic. Cells of *E. coli* B-13 thus contain a complex of plasmids that determine different phenotypes.

The aim of this investigation was to study the structure of the plasmid complex in B-13 cells and also the physical and genetic properties of the R plasmids constituting this complex.

EXPERIMENTAL METHOD

Wild-type strain *E. coli* B-13, whose cells are prototrophic, positive in the phage MS2 titer increase test ($PTIT^+$), enterotoxigenic, colicinogenic, and resistant to ampicillin, streptomycin, chloramphenicol, tetracycline, kanamycin, and sulfonamides, strains *E. coli* C600 thr leu thi rif, *E. coli* VB 1636 trp his lys and *E. coli* Apl15 met thi Nal^r, which are derivatives of *E. coli* K-12, and also strains *Salmonella typhimurium* 18 and *E. coli* B, were used.

The plasmid complex was separated by crossing *E. coli* B-13 cells with *E. coli* C600 and Apl15 recipient cells, followed by selection of transconjugants spearately for resistance to ampicillin (Ap), streptomycin (Sm), chloramphenicol (Cm), tetracycline (Tc), kanamycin (km), and norsulfazole (Su), with determination of the genetic structure and donor ability of the selected transconjugants. Crosses including "three-parent," determination of colicinogenicity, titration of plasmids on types fi^+ and fi^-, and determination of sensitivity of the bacteria to phage MS2 were done by standard methods [2, 3]. Standard methods also were used to study temperature resistance and stability of the plasmids [4, 6].

Plasmid DNAs were isolated by the method of Meagher et al. (1977), with some modifications, from lysates clarified with the detergent Triton X-100, followed by centrifugation in a CsCl density gradient [5]. Restriction analysis was carried out with restriction endonuclease Eco RI, followed by electrophoresis of the restricted DNAs in 0.8% agarose gel. The gels were stained with ethidium bromide. Fragments of DNA of phage λ, obtained with the aid of restriction endonuclease Eco RI, were used as molecular weight standards.

EXPERIMENTAL RESULTS

To study the structure of the plasmid complex in *E. coli* B-13 cells, the first step was to separate the complex into its components, i.e., the plasmids forming the complex. The work therefore began with crossing *E. coli* B-13 cells with *E. coli* C600 cells and selection of transconjugants separately for Ap, Sm, Km, Tc, Cm, and Su markers, followed by determination of their nonselective resistance markers. As a result of several repeated crosses of primary conjugants with suitable recipients, transconjugants of four types with definite sets of resistance markers were obtained: SmCmApSu, SmTcSu, SmKmSu, and Tc. Markers of transconjugants of all these types were transmitted en masse to the recipient cells, without loss of any marker.

Institute of General Genetics, Academy of Sciences of the USSR. Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 4, pp. 453-454, April, 1984. Original article submitted January 12, 1983.

 0007-4888/84/9704-0464$08.50

TABLE 1. Properties of R Plasmids

Transconjugants (donors of R plasmids)	Number of transconjugants of E. coli C600 tested	Nonselective features		
		number of Col^+ transconjugants	number of $PTIT^+$ transconjugants	number of $PTIT^+$ and Col^+ transconjugants
RSmCmApSu	10	0	0	1
RSmTcSu	10	0	0	1
RSmKmSu	10	0	0	1
RTc	10	0	0	5

TABLE 2. Conjugativeness and Mobilizing Activity of R Plasmids of Strain B-13

R plasmid	Frequency of transmission to recipient cells			Frequency of mobilization for transfer of nonconjugative plasmids					
	E. coli K-12, $\cdot 10^4$	E. coli B, $\cdot 10^6$	S. typhimurium, $\cdot 10^6$	pSF1010	pMB9	pBR 322	pACYC184	pMR 5	pSC 101
pAP24-1	2,5	8	1,1	0	0	0	0	0	0
pAP24-2	1,0	1,4	0,46	0	0	0	0	0	0
pAP24-3	1,4	0,06	0,028	0	0	0	0	0	0
pAP24-4	0,6	0,2	0,6	$8{,}4\cdot10^{-5}$	0	0	0	0	0

Assuming that the isolated transconjugants contained one each of the independent conjugative R plasmids, they also were investigated by the phage MS2 titer increase test in order to detect F-likeness and they were also tested for colicinogenicity.

Some transconjugants of *E. coli*, which inherited R plasmids, were shown to possess Col^+ and $PTIT^+$ features simultaneously (Table 1). Since the $PTIT^+$ characteristic in this complex is determined by conjugative plasmid pAP10-2 Ent [1], this means that these transconjugants can be classed, depending on their plasmid content, in 8 types: 1) RSmCmApSu; 2) RSmCmApSu Ent Col; 3) RSmTcSu; 4) RSmTcSu Ent Col; 5) RSmKmSu; 6) RSmKmSu Ent Col; 7) RTc; 8) RTc Ent Col.

It is also clear from Table 1 that the Col plasmid was not found in any transconjugant cell cultures containing only one of the R plasmids. Col plasmid was present only in those transconjugants which contained Ent plasmid. In other words, Col plasmid is nonconjugative and is mobilized for transfer by Ent plasmid. It can thus be concluded from these results that the plasmid complex found in *E. coli* B-13 cells consists of 6 different plasmids: conjugative R plasmids pAP24-1 (SmCmApSu), pAP24-2 (SmTcSu), pAP24-3 (SmKmSu) and pAP24-4(Tc), nonconjugative Col plasmid pAP24-5, and conjugative F-like plasmid pAP10-2 Ent.

In the next experiments conjugative activity of R plasmids was tested by transmission into *E. coli* K-12, *E. coli* B, and *S. typhimurium* cells, and the ability of these plasmids to mobilize nonconjugative plasmids pSF 1010, pMB 9, pBR 322, pACYC 184, pMR 5, and pSC 101 for transfer in "three-parent" crosses, in which the donors were *E. coli* AP115 cells with one of the R plasmids chosen for study, the intermediate recipients were *E. coli* UB1636 and C600 cells, with one of the nonconjugative plasmids, and the final recipients were *E. coli* C600 cells, for transfer also were tested. The R plasmids tested were found to be capable of transmission in bacteria belonging to different species (Table 2). Ability to mobilize nonconjugative plasmids for transfer was possessed only by R plasmid pAP24-4, which mobilizes nonconjugative plasmid pSF1010 for transfer (Table 2).

To determine the character of mobilization of nonconjugative plasmid pSF1010, 100 transconjugants were selected from *E. coli* AP115 (pAP24-4) • *E. coli* UB1636(pSF 1010) • *E. coli* C600 crosses, and each was tested for the presence of markers of the mobilizing and mobilized plasmids. Some transconjugants were found to carry only markers of the mobilized plasmid whereas the rest carried markers of both mobilizing and mobilized plasmids. This suggested that the genetic transfer factor in bacterial cells of strain AP115 with plasmid pAP24-4, used as donors and, consequently, in *E. coli* B-13 cells, may be in a state free from determinants of resistance to Tc.

In experiments to study the sensitivity of R plasmids to temperatures of 28 and 37°C, and also as a result of their titration on types fi^+ and fi^-, they were found to be temperature resistant and fi^-, except plasmid pAP24-1, which is fi^+.

The study of elimination (spontaneous and induced) of R plasmids showed that they are maintained in a stable condition in the cells and are eliminated only by treatment of the cells with ethidium bromide, dodecylsulfate, and acridine orange.

The study of plasmid DNAs isolated from *E. coli* C600 cells, containing one or other R plasmid showed that the molecular weight of plasmid pAP24-1 is 53.6 megadaltons, of pAP24-2 — 40.9 megadaltons, pAP24-3 — 73.8 megadaltons, and pAP24-4 — 51.3 megadaltons.

It can be concluded from these results as a whole that the plasmid complex found in *E. coli* B-13 cells consists of six different plasmids: F-like conjugative plasmid Ent(fi^+), nonconjugative plasmid Col, and conjugative R plasmids pAP24-1 (fi^+), pAP24-3 (fi^-), pAP24-3 (fi^-), pAP24-4 (fi^-). It is postulated that in the case of R plasmid pAP24-4 the genetic transfer factor may exist in the free state (without determinants of drug resistance).

LITERATURE CITED

1. N. I. Buyanova, V. P. Shchipkov, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 2, 210 (1981).
2. A. P. Pekhov, V. P. Shchipkov, T. Arai, et al., Zh. Mikrobiol., No. 9, 45 (1979).
3. V. P. Shchipkov, N. A. Drobysheva, N. I. Shchipkova, et al., Zh. Mikrobiol., No. 9, 134 (1977).
4. J. Davies, Methods Enzymol., 43, 41 (1975).
5. R. B. Meagher, R. C. Tait, M. Betluch, and H. W. Boyer, Cell, 10, 521 (1977).
6. H. W. Smith and Z. Parsell, J. Gen. Microbiol., 109, 37 (1978).