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EXPERIMENTAL ARTICLES

The Cotransduction of pET System Plasmids by Mutants of T4 and RB43 Bacteriophages

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Abstract—The study of the cotransduction of the plasmid pairs pET-3a–pLysE and pET-3a–pLysS by the mutant phage T4alc7 showed that the antibiotic resistance markers of the plasmids were cotransduced with a high frequency. The analysis of the plasmid DNA of cotransductants and cotransformants showed that the mutant phage T4alc7 can be used for obtaining the monomeric and oligomeric forms of plasmids and for the cotransduction of two-plasmid overproduction systems into *E. coli* strains. The plaque mutants RB43-03 and RB43-13 derived from bacteriophage RB43 were found to be able to cotransduce the antibiotic resistance markers of pET-3a and pLysE plasmids.

Key words: *E. coli*, cotransduction, plasmid, antibiotic resistance, mutant, bacteriophage, T4, RB43, overproduction.

Since the introduction of the expression system based on the RNA polymerase of phage T7 (pET system™, Novagene, Madison, WI, USA) in *E. coli* BL21(DE3) into the practice of genetic engineering, thousands of homologous and heterologous genes have been successfully expressed [1]. However, some problems associated with the use of this system remain to be solved. One of the major problems is related to the expression of genes coding for proteins which are toxic for *E. coli* cell, since the background expression of such genes leads to the selection of bacterial forms defective in the synthesis of desired proteins (for instance, due to mutations that affect the activity of the desired protein or the T7 RNA polymerase itself) [2]. Furthermore, the relatively low stability of pET plasmids with insertions inevitably leads to variable gene expression in attempts aimed at scaling up the production processes.

The coexpression of the T7 phage lysozyme gene with the aid of plasmids pLysE or pLysS was found to considerably decrease the background expression of the pET plasmid gene coding for the desired protein due to the binding and inhibition of the T7 RNA polymerase [1].

In *E. coli* strains differing, for instance, in only one mutation, the levels of protein synthesis upon the use of a pair of pET plasmid with an insertion of the DNA ligase gene and plasmid pLysS may considerably differ [3]. This gives grounds to believe that there are more efficient producers of useful products than are presently known. In view of this, we decided to search for such producers among the *E. coli* strains available in the collection of microbial cultures at the Institute of Biochemistry and Physiology of Microorganisms.

It should be noted that the obtaining of lysogens with the aid of phage λDE3 presents no difficulties, while the transformation of a great number of bacterial strains associated with the production of competent cells and the isolation of plasmid DNA is a serious problem, which is aggravated by the necessity of using toxic reagents. Under these conditions, the cotransduction of plasmid systems similar to pET–pLysE and pET–pLysS may be a simple and useful alternative, which would allow any plasmid to be obtained not only in the monomeric but also in the oligomeric form. The dependence of the level of foreign gene expression on the proportion between monomeric and oligomeric plasmids is of independent interest.

Wilson *et al.* [4] described a mutant of T4 phage, T4alc7 [alc7, am51(g56), NB5060(gdenB-rII), amC87(g42)], which could efficiently bring about generalized transduction [5]. The mutant phage T4alc7 can transduce 1.5 times more extended DNA fragments than phage P1 can. This implies that T4alc7 can transfer DNA fragments carrying two genes located as far away from each other as 3.6 min on the genetic map of *E. coli* [6]. Experiments also showed that plasmid pBR322 can be efficiently transduced by the mutant phage T4alc7 to the $recA^+$ strains of *E. coli*. Phage particles can be stored for a long time without losing their transduction ability. Consequently, they can be used not only for the transfer but also for the conservation of plasmids [7]. The transduction of plasmids by the phage T4alc7 to the $recA^-$ strains of *E. coli* is considerably less frequent than to the rec^+ strains [8].

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Table 1. The cotransduction of plasmids pET-3a and pLysE from the donor strain *E. coli* HMS174 to the recipient strain *E. coli* B834

PFU/ml phagolysate of T4alc7(HMS174(pET-3a)(pLysE))	Initial number of recipient cells	Number of cotransductants	Cotransduction frequency
3.85×10^6	2.2×10^7	0	
3.85×10^5	2.2×10^7	72	1.87×10^{-4}
3.85×10^4	2.2×10^7	6	1.56×10^{-4}
3.85×10^3	2.2×10^7	2	

Note: PFU is plaque-forming unit.

Table 2. The cotransduction of plasmids pET-3a and pLysS from the donor strain *E. coli* HMS174 to the recipient strain *E. coli* B834

PFU/ml phagolysate of T4alc7(HMS174(pET-3a)(pLysE))	Initial number of recipient cells	Number of cotransductants	Cotransduction frequency
2.2×10^7	2.2×10^7	2	
2.2×10^6	2.2×10^7	162	7.36×10^{-5}
2.2×10^5	2.2×10^7	12	5.45×10^{-5}
2.2×10^4	2.2×10^7	3	

The ability of the mutant phage T4alc7 to cotransduce plasmids has already been reported by us [9], without the details and molecular mechanisms of this phenomenon. This paper describes for the first time the results of the experimental study of the cotransduction of the plasmid pairs pET-3a–pLysE and pET-3a–pLysS with the aid of the mutant phage T4alc7 [4].

It is known that the nucleotide sequences of the homologous genes of T-even phages differ by no more than 5%. At the same time, some morphologically similar bacteriophages (such as RB42, RB43, and RB49) have considerably differing genomic sequences. Under strict hybridization conditions, only about 10% of the DNA of these phages (called pseudo-T-even phages) hybridize with the DNA of T4 phage [10].

Earlier, we studied the transduction of plasmids by pseudo-T-even phages [11, 12]. It was of interest to investigate the possibility of existence of such mutant phages that could transfer the markers of two-plasmid systems. This paper describes the results of our attempts to cotransduce the antibiotic resistance determinants of the plasmid pair pET-3a–pLysE with the aid of the plaque mutants of bacteriophage RB43.

MATERIALS AND METHODS

Experiments were carried out with the *E. coli* strains B, B_A, B_E, B_{40} supI, B_{40} supIII, B834, BL21(F^- ompT $r_B^- m_B^-$), HMS174(F^- recA$^-$ $r_{K12}^- m_{K12}^+$ Rif^R) [1, 12], K12(λ^+) r6$^-$ r2,4$^-$ sup0, C600 supE tonA, C600(pR386), 803met$^-$ hsd$_S$K$^-$ supE, QR47 recA$^+$ supE, QR48 recA$^-$ supE, ED8538 sup^0 lacZam, W3101 recA$^-$ sup^0, ED 8689 sup^0 trpR hsdR_K^-, 5K(hsdR_K^- derivative of C600), 5KRI (5K with plasmid RI of Yoshimori), and W3350, as well as the bacteriophages T4alc7 and RB43 [4, 11, 12].

The bacteria and phages were enumerated using L agar (Difco) plates. Pseudo-T-even phages were obtained using *E. coli* strains B_E and B834. To be used as donors for plasmid transduction, pseudo-T-even phages were grown in *E. coli* strains containing the respective plasmids [5]. Phage suspensions were diluted with M9 buffer and mixed with recipient cells grown in 10 ml of M9 medium containing 0.05 ml of 40% glucose, 0.1 ml of 25% casamino acids, 0.01 ml of 10% $CaCl_2$, 0.01 ml of 1 M $MgCl_2$, and 0.01 ml of a 1% solution of vitamin B_1. A 200-μl aliquot of the recipient cell suspension was mixed with an equal volume of the appropriate phage suspension dilution. After incubation at room temperature for 30 min, aliquots of this mixture were plated onto agar media containing the necessary antibiotics. Transduction frequency was calculated as described by Young and Edlin [6]. The mutants of phage RB43 were obtained as described earlier [11].

Plasmid DNA was isolated by the method of Summerton *et al.* [13]. According to those authors, different forms of the DNA of plasmid pBR322 electrophoresed in 0.7% agarose gel has mobilities of 0.68, 0.48, and 0.32 (relative to the circular plasmid DNA). In this case, chromosomal DNA has a relative mobility of 0.16. DNA was digested with restriction endonucleases according to the manufacturer's instructions (Fermentas, Lithuania).

Electrophoresis in 0.8% agarose gel was performed in a BioRad vertical system.

Bacterial growth was monitored by measuring the optical density of culture suspensions at 540 nm using a KFK-2MP photoelectric colorimeter (Russia). The other methods used in this work were described in our recent paper [12].

RESULTS AND DISCUSSION

The cotransduction of the plasmid pairs pET-3a–pLysE and pET-3a–pLysS by the mutant phage T4alc7. The mutant bacteriophage T4alc7 contains dC-DNA, which, unlike the glucosylated hydroxymethylcytosine-containing DNA of the wild-type phage T4, is sensitive to the known restriction endonucleases. Before starting transduction experiments, we made sure that the T4 phage mutations were not lost during the phage storage. For this, we isolated the phage DNA, digested it with restriction enzymes, and analyzed the restriction digest by electrophoresis. A specific restriction fragment pattern of the digest served as an indication that the mutations were not lost and that the phage could be used in transduction experiments [4].

To improve the efficiency of pBR322-mediated transduction, we attempted to find a more efficient recipient strain than the common recipient strain *E. coli* B834. The presence of deletion NB5060 within the rII region in the phage T4alc7 was thought to make more promising the use of the lysogenic strain *E. coli* K12(λ^+) r6$^-$ r2,4$^-$ sup0, since the presence of prophage lambda in bacterial cells is known to block the development of phage T4 with the deleted rII region. On the other hand, the strain *E. coli* C600(pR386) also seemed to be a promising recipient, since cells with plasmid pR386 block the development of the alc mutants of phage T4 [14]. Experiments showed, however, that both of these strains are less efficient recipients than *E. coli* B834.

Cotransduction experiments were carried out as follows. M9 medium (10 ml) with necessary supplements was inoculated with 0.5 ml of an overnight culture of *E. coli* grown in the same medium and incubated for 6 h. Aliquots of this 6-h-old culture were tested for the number of bacterial cells and mixed with appropriate serial dilutions of the suspension of phage T4alc7 grown in the *E. coli* strains containing the plasmid pairs pET-3a–pLysE and pET-3a–pLysS. After 30 min of incubation at room temperature, the aliquots (200 μl) of this suspension were plated onto agar media with chloramphenicol (30 μg/ml), or ampicillin (25 μg/ml), or both.

In the experiments with the plasmid pair pET-3a–pLysE, among the 102 colonies grown on the agar plates with ampicillin, 10 were found to be also able to grow on the agar plates with chloramphenicol. And vice versa, of the 90 colonies grown on the agar plates with chloramphenicol, 14 were also able to grow on the agar plates with ampicillin.

In the experiments with the plasmid pair pET-3a–pLysS, among the 204 colonies grown on the agar plates with ampicillin, 15 were found to be also able to grow on the agar plates with chloramphenicol. And vice versa, of the 102 colonies grown on the agar plates with chloramphenicol, 10 were also able to grow on the agar plates with ampicillin.

The results of the analysis of the cotransductants, which are able to grow on the agar plates containing both antibiotics, are presented in Tables 1 and 2.

Two of the cotransductants obtained at a multiplicity of infection not greater than 0.001 (under this condition, no more than one phage particle infects one recipient *E. coli* B834 cell) were chosen for further analysis. These cotransductants contained the plasmid pairs pET-3a–pLysE and pET-3a–pLysS. The cotransformants obtained by cotransformation with the same plasmid pairs were used as the control. The samples of plasmid DNA isolated from the cotransformants, cotransductants, and transformants (they contained only one of the plasmids used) were analyzed with the aid of *Eco*RI and *Hin*dIII restriction endonucleases.

In the experiments performed by Takahashi and Saito with the pBR322–*E. coli* B834 transductants [7], more than 50% of the grown clones were found to contain plasmid monomers, whereas the other clones contained plasmid dimers and trimers. After 20 transfers of the latter clones in the presence of the suitable antibiotic, more than 50% of the clones still contained plasmid dimers and trimers. At the same time, all of the transformants subjected to analysis contained only plasmid monomers.

The results of our experiments are in agreement with the results of Takahashi and Saito. Namely, cotransformants and one of the two cotransductants taken for analysis (with the plasmid pair pET-3a–pLysE) contained plasmid monomers. In the case of the other cotransductant (with the plasmid pair pET-3a–pLysS), at least one of the plasmids retained its oligomeric form in the course of a few serial passages (Figs. 1, 2). The digestion of all DNA preparations with individual *Eco*RI and *Hin*dIII restriction endonucleases gave rise to only linear monomers (Fig. 1). The combined digestion of all DNA samples by the two enzymes gave rise to three fragments (Fig. 2). Actually, the number of DNA fragments produced was likely four, but the fourth fragment was too small (30 bp) to be visible on the electropherogram [1].

These data suggest that the phenomenon of packaging different truncated DNA molecules in one capsid, which was described for phage P1, is also typical for the mutant phage T4alc7.

Experiments showed that the growth of the cotransductant with plasmids pET-3a and pLysS (as was already mentioned, this cotransductant contained the multimeric form of one of the plasmids) was slow at the

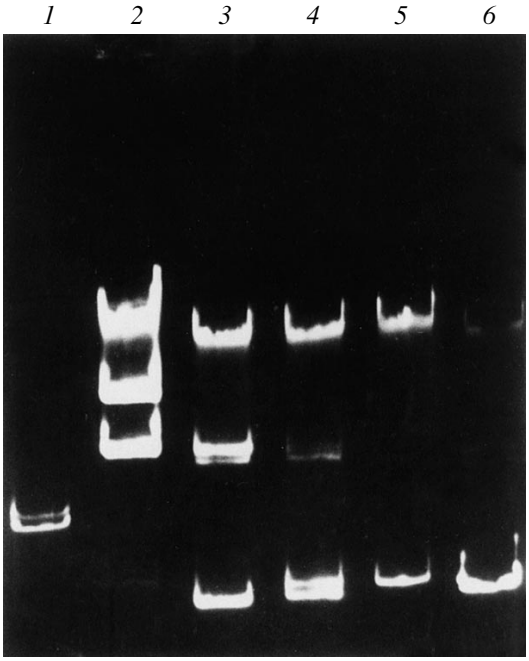

Fig. 1. The electrophoresis of plasmid DNA isolated from cotransformants and cotransductants. Lanes: (*1*) the *Hin*dIII digest of plasmid DNA isolated from a cotransductant containing the plasmid pair pET-3a–pLysE (plasmid DNA from all other cotransformants and cotransductants with the plasmid pairs pET-3a–pLysE and pET-3a–pLysS had identical *Hin*dIII and *Eco*RI restriction fragment patterns); (*2*) plasmid DNA from a cotransductant with the plasmid pair pET-3a–pLysS; (*3*) plasmid DNA from a cotransductant with the plasmid pair pET-3a–pLysE; (*4*) plasmid DNA from a cotransformant with the plasmid pair pET-3a–pLysE (plasmid DNA from a cotransformant with the plasmid pair pET-3a–pLysS had the identical electrophoretic pattern); (*5*) DNA of plasmid pLysE (DNA of plasmid pLysS had the identical electrophoretic pattern); (*6*) DNA of plasmid pET-3a.

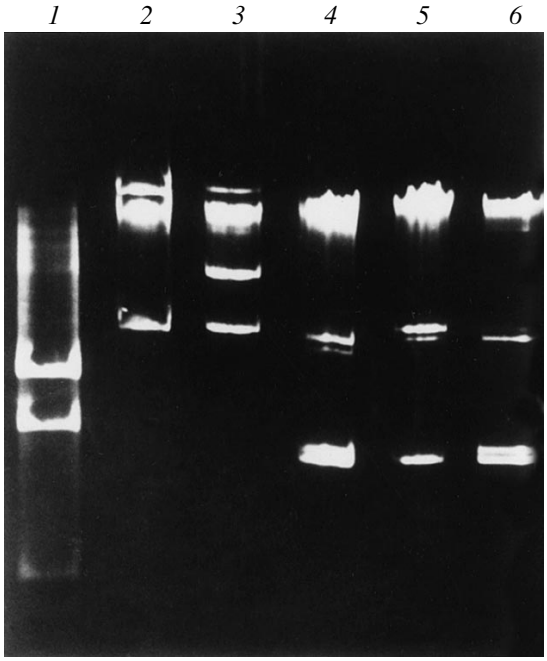

Fig. 2. The electrophoresis of plasmid DNA isolated from cotransformants and cotransductants and purified using a nitrocellulose filter. Lanes: (*1*) the *Eco*RI–*Hin*dIII digest of plasmid DNA isolated from a cotransductant containing the plasmid pair pET-3a–pLysE (plasmid DNA from all other cotransformants and cotransductants with the plasmid pairs pET-3a–pLysE and pET-3a–pLysS had identical *Hin*dIII–*Eco*RI restriction fragment patterns); (*2*) plasmid DNA isolated from a cotransductant with the plasmid pair pET-3a–pLysS after four passages on a solid nutrient medium; (*3*) plasmid DNA isolated from a cotransductant with the plasmid pair pET-3a–pLysS after five passages on a solid nutrient medium; (*4*) plasmid DNA from a cotransformant with the plasmid pair pET-3a–pLysE; (*5*) plasmid DNA from a cotransductant with the plasmid pair pET-3a–pLysE; (*6*) plasmid DNA from a cotransformant with the plasmid pair pET-3a–pLysE.

early stages of incubation. This finding is in agreement with the observations of other researchers [7, 15] and may serve as the basis for the simple differentiation of clones containing monomeric and multimeric plasmids.

The cotransduction of plasmids pET-3a and pLysE by mutants of phage RB43. In 1957, Fraser reported that phage T3 can form so-called lasting complexes with infected cells [16]. The development of the phage in these complexes stopped at the stage intermediate between the beginning of infection and maturation. Some mutants of phage T3 produced lasting complexes with more than 50% of infected cells, i.e., much more frequently than did the wild-type phage. These mutants were characterized by small size plaques. The lasting complexes could elongate, divide, and produce uninfected phage-susceptible segregants until the phase of lysis was achieved. Fraser concluded that infected cells in the lasting complexes occur in the state of pseudolysogeny, which ends by the lysis of these cells.

Our recent works dealt with the transduction of plasmids by pseudo-T-even phages and the analysis of the relationship between transduction and pseudolysogeny [11, 12]. We wished to reveal mutants of pseudo-T-even phages which could be able to produce lasting complexes similar to those described by Fraser. With this in mind, we analyzed the mutants of bacteriophage RB43 for ones that could produce small plaques and would be different from the wild-type phage RB43 in the characteristics of transduction. Using the same approach as was used in obtaining the amber mutants RB43-21 and RB43-33 [11, 12], we succeeded in the isolation of mutant RB43-03, which produced such transductants that differed in the type of colonies from the transductants produced by the wild-type mutant RB43 (Fig. 3a) [11, 12]. Later, we isolated another such mutant of RB43, the plaque mutant RB43-13.

Experiments on the cotransduction of the antibiotic resistance markers of the pET-3a and pLysE plasmids by the wild-type phage RB43 led to a failure, while

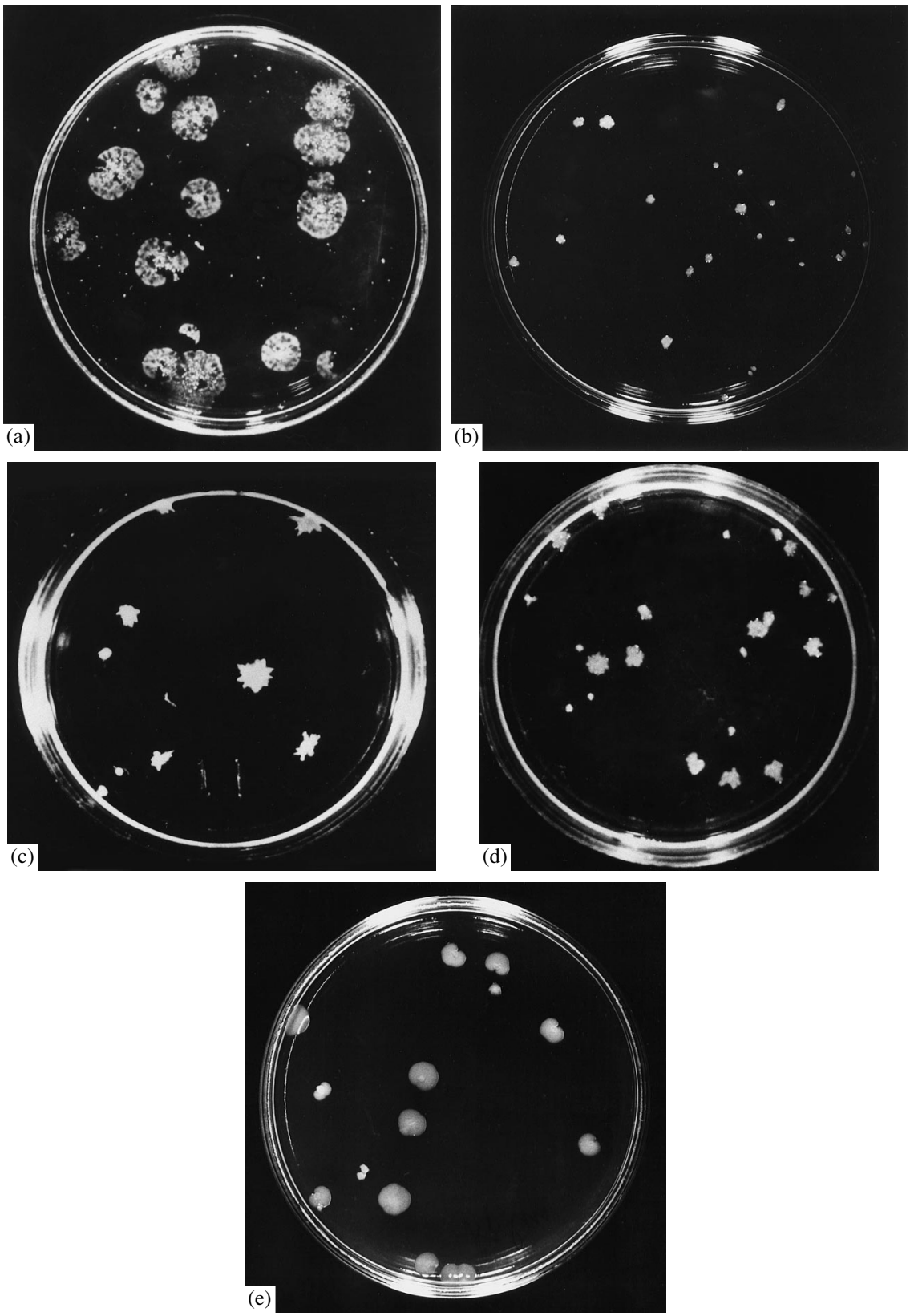

Fig. 3. Colonies of (a) *E. coli* B_E transductants produced with the aid of the mutant phage RB43-03 grown in *E. coli* B834(pBR322), (b) *E. coli* 803 cotransductants produced with the aid of the mutant phage RB43-03 grown in *E. coli* HMS174(pET-3a)(pLysE), (c) *E. coli* B_E cotransductants produced with the aid of the mutant phage RB43-03 grown in *E. coli* HMS174(pET-3a)(pLysE), (d) *E. coli* B_E cotransductant produced with the aid of the mutant phage RB43-13 grown in *E. coli* HMS174(pET-3a)(pLysE), and (e) *E. coli* B_E cotransductants produced with the aid of the mutant phage T4alc7 grown in *E. coli* HMS174(pET3a)(pLysE).

contransduction with the RB43 mutants was successful. In the latter experiments, strain HMS174(pET-3a)(pLysE) was used as the donor, the mutants RB43-03 and RB43-13 were used as transducing phages, the mutant phage T4alc7 was used as the control, and the *E. coli* strain B_E was used as the recipient. In separate experiments, the antibiotic resistance determinants were cotransduced by the mutant phage RB43-03 from the donor strain HMS174(pET-3a)(pLysE) to the recipient strain *E. coli* 803 (Fig. 3b).

The cotransduction frequencies of the marker Amp^R of plasmid pET-3a and the marker Cam^R of plasmid pLysE by the phages RB43-03, RB43-13, and T4alc7 to the recipient strain *E. coli* B_E were 1.8×10^{-7}, 4×10^{-8}, and 1.5×10^{-7}, respectively. The cotransduction frequency of the same markers by the phage RB43-03 to the recipient strain *E. coli* 803 was 5.8×10^{-7}.

Figure 3 shows the photographs of the cotransductants obtained in these experiments. As can easily be seen from these photographs, the colonial morphology of the cotransductants obtained with the aid of the phages RB43-03 and RB43-13 (Figs. 3c, 3d) considerably differs from that of the cotransductants of the T4alc7 phage (Fig. 3e). As was already mentioned, the latter cotransductants contain only plasmid DNA and do not contain the DNA (or its fragments) of the T4alc7 phage.

In turn, the colonies of the cotransductants of the phages RB43-03 (Fig. 3c) and RB43-13 (Fig. 3d) differed slightly in morphology.

To understand the molecular mechanisms of cotransduction mediated by the mutant bacteriophages RB43-03 and RB43-13, it is necessary to localize mutations in these phages, determine their nature, and study the structural organization of plasmid DNA and changes occurring in DNA during the growth of the cotransductants. Under certain conditions, cotransduction can likely generate new hybrid molecules similar to those described for phage P1 [17]. The possibility cannot be excluded that investigations along this line will lead to the development of a new variant of genetic engineering in vivo.

To conclude, the transformation and cotransformation techniques require the obtaining of competent cells, the isolation and purification of plasmids, and, consequently, the use of toxic reagents. At the same time, the cotransduction of two-plasmid pET expression systems by the mutant bacteriophage T4alc7 may be a useful and simple alternative.

The cotransduction of the antibiotic resistance determinants of two-plasmid pET systems can be also brought about by the plaque mutants of bacteriophage RB43 (but not by the wild-type bacteriophage). The study of the structural organization of plasmid DNA in the cotransductants obtained with the aid of these mutants is in progress in our laboratory.

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