INDUCTION OF *Escherichia coli* K-12 MUTANTS WITH HIGHLY EFFICIENT PLASMID TRANSFORMATION

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The method of transformation of *Escherichia coli* cells treated with Ca^{++} ions with isolated plasmid DNA molecules is widely used in genetic engineering research. However, the low efficiency of the method, due to the fact that only about 1% of viable cells become transformable by saturating concentrations of plasmid DNA [4], reduces its usefulness.

The aim of the present investigation was to obtain *E. coli* K-12 mutants with highly efficient plasmid transformation.

EXPERIMENTAL METHOD

The following strains of *E. coli* K-12 were used: Hfr KL 16 as the recipient, Ga9/pMB9 and C-600/pBR322 as carriers of plasmids. To isolate DNA, donor strains were cultured in L broth at 37°C with aeration up to a concentration of 5×10^8 cells/ml, after which chloramphenicol was added to the medium in a final concentration of 170 μg/ml for amplification of the plasmid DNA and incubation continued under the same conditions for 16-18 h. The next stage of isolation of plasmid DNA, namely obtaining "clarified" lysates, was carried out as in [1]. Cesium bromide was added to the lysate up to a density of 1.57 g/cm^3. After centrifugation at 25,000 rpm (for 1 h) the protein fraction was removed. Subsequent centrifugation at 25,000 rpm (for 1 h) the protein fraction was removed. Subsequent centrifugation was carried out at 45,000 rpm for 36 h.

Of the two bands clearly distinguishable in UV light the lower band, which was supercoiled plasmid DNA, was selected. Ethidium bromide was removed by extraction 4 times with 2 volumes of isopropyl alcohol. The resulting preparation was dialyzed against 0.02 M Tris buffer, pH 7.6, in the cold for 24 h. The DNA concentration was 30-40 μg/ml.

Bacteria were transformed by plasmid DNA by the method in [2]. Recipient cells were grown in synthetic medium of the following composition: 0.17 g KH_2PO_4, 3 g $(NH_4)_2SO_4$, 3.7 g KCl, 1 g $MgSO_4$, 6 g Tris, 10 g casaminic acid, 0.2% glucose, 1% vitamin B_1, 1000 ml water, pH 7.6. The number of recipient cells was estimated from optical density and by seeding the cells on Hottinger's agar.

EXPERIMENTAL RESULTS

To obtain mutants with high efficiency of plasmid transformation, recipient strain *E. coli* K-12 Hfr KL 16 was treated with the mutagen N-methyl-N'-nitro-N-nitrosoguanidine (NG). Separate Hfr KL 16 clones after treatment with NG were tested for transformability by isolated pMB9 plasmid DNA, using tetracycline resistance as the criterion.

To screen the mutants a new rapid method of plasmid transformation was developed, as follows. Plasmid DNA was applied in a dose of 0.3-0.6 μg to the surface of agarized medium containing 0.05M $CaCl_2$ and 50 μg/ml tetracycline. The minimal inhibiting dose of tetracycline, incidentally, was exceeded because partial binding of the antibiotic takes place in midea with Ca^{++} ions [3]. Next the separate clones of the recipient strain obtained after

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TABLE 1. Comparison of Efficiency of Plasmid Transformation by pMB9 DNA of Mutants and Original Strain Mfr KL 16

Recipient	Number of transformants/μg DNA	Excess of transformation relative to original strain (number of times)
KL16	$3 \cdot 10^4$	—
Mutant 2	$7{,}8 \cdot 10^5$	26
Mutant 3	$2{,}4 \cdot 10^5$	8
Mutant 4	$2{,}1 \cdot 10^5$	7
Mutant 6	$2{,}5 \cdot 10^5$	8,3
Mutant 7	$3 \cdot 10^5$	10
Mutant 8	$1{,}4 \cdot 10^5$	4,6
Mutant 10	$1{,}6 \cdot 10^5$	5,3
Mutant 12	$8 \cdot 10^4$	2,6
Mutant 14	$1{,}1 \cdot 10^5$	3,6
Mutant 15	$6{,}1 \cdot 10^4$	2
Mutant 16	$2{,}5 \cdot 10^5$	8,3
Mutant 17	$8{,}7 \cdot 10^4$	2,9

TABLE 2. Comparison of Efficiency of Plasmid Transformation by pMB9 DNA of Mutants and Initial Strain Mfr KL 16

Recipient	Number of transformants/μg DNA	Excess of transformation relative to original strain (number of times
Mutant 3	$2 \cdot 10^5$	7,7
Mutant 4	$1{,}6 \cdot 10^5$	6
Mutant 6	$1{,}9 \cdot 10^5$	7,3
Mutant 7	$2{,}2 \cdot 10^5$	8,4
KL16	$2{,}6 \cdot 10^4$	

treatment with mutagen were resuspended in physiological saline and the suspensions were plated out in sectors on medium with pMB9 DNA. The plates thus seeded were kept for 1 h at 4°C and then transferred to an incubator at 37°C. In parallel experiments suspensions of these same clones were plated out in sectors on medium without DNA. After incubation for 48 h at 37°C the sectors with the largest number of tetracycline-resistant transformants were selected. The dose of plasmid DNA applied to the surface of the agarized medium was chosen so that it did not cause transformation of the original strain. The final efficiency of plasmid transformation was estimated by the standard method.

Altogether 12 mutants with high efficiency of plasmid transformation were selected. The results of one of four experiments are given in Table 1.

As Table 1 shows, the mutants differed in their level of plasmid transformation, and this is evidently a sign that their genetic lesions differed in character. The level of plasmid transformation of the mutants was between 2 and 26 times higher than that of the original strain.

Some mutants were tested for efficiency of plasmid transformation by DNA of plasmid pBR322 by using tetracycline resistance as the criterion. The results of one of four experiments are shown in Table 2.

The level of plasmid transformation of the tested mutants by pBR322 DNA was the same number of times higher than that of the original strain as when pMB9 DNA was used.

The method of obtaining mutants with high efficiency of plasmid transformation described above can be used to study the mechanism of this process.

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