Vol. 82, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30,1978 Pages 477-483

INCREASED TRANSFORMATION FREQUENCY IN E. COLI

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Received March 20, 1978

SUMMARY

The transformation efficiency of Escherichia coli prepared by the calcium-heat shock procedure has been increased sixfold by growth of cells in 0.5 M sucrose and the addition of 1 μg/ml lysozyme along with DNA. A mutant of E. coli, hyt, has been selected which has a tenfold increased efficiency of transformation.

INTRODUCTION

Escherichia coli is the best characterized bacterial species genetically and therefore the logical choice as recipient for transformation with recombinant DNA. Unfortunately the species is very poorly transformable, untreated cells being completely resistant to transformation. E. coli cells rendered competent by treatment with 30 mM $CaCl_2$ at 0°C and then heat pulsed to 42C in the presence of saturating DNA concentrations are transformed at 10^{-5} transformants per cell (1, 2). In this paper we report physiological conditions which increase transformation efficiency to 6×10^{-5} and a mutant strain of E. coli which transforms with an efficiency of 10^{-4}.

MATERIALS AND METHODS

Bacteria and plasmids. A derivative of E. coli C600 r^-m^-

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0006-291X/78/0822-0477$01.00/0

was obtained from Dr. Herbert W. Boyer and used as transformation recipient in these experiments. The strain is hspS thr leu arg. Transforming DNA was prepared from E. coli strains harboring λ C_I857Sam7, pM21 (kanamycin-resistant) or pRSF2124 (ampicillin-resistant). In the text these DNA preparations will be referred to as λ, Km and Ap.

Preparation of transforming DNA. Phage λ DNA was prepared by temperature induction of the lysogen, concentration of the induced cells by centrifugation, chloroform lysis, purification of intact λ by differential centrifugation and phenol extraction. Plasmid DNAs were expanded overnight in the presence 100 μg/ml chloramphenicol, released by gentle lysis in Sarkosyl-lysozyme, separated from chromosomal DNA by low speed centrifugation and finally purified in CsCl-EthBr.

Transformation. Recipient cells were prepared according to the procedure of Cohen et al (2) as modified from Mandel and Higa (1). Lambda DNA transfectants were assayed in soft agar seeded with E. coli Ymel (supF$^+$). Km or Ap transformants were plated on agar containing the appropriate antibiotic following dilution into prewarmed L broth and incubation at 37C for 2 h. Efficiency of transformation was calculated as transformants per viable cell.

Mutagenesis. N-methyl-N'-nitro-N-nitrosoguanidine was employed according to the procedure of Adelberg et al (3).

RESULTS

Physiological conditions which enhance transformation

TABLE 1. Enhancement of transformation efficiency by 0.5 M sucrose.

Experiment	Infectious centers per ng λ DNA	
	- sucrose	+ sucrose
1	47*	70
2	34	73
3	41	102

*Each value was determined from three plates which contained 100-400 plaques.

frequency. Many perturbations of the Cohen et al (2) procedure were tested for improved transformation efficiency in strain C600 r^-m^-, including variations in $CaCl_2$ concentration and temperature and duration of the heat pulse. In our hands 50 mM $CaCl_2$ worked consistently better than 30 mM, but the differences were minor. The procedure as stated (2) has been used in the experiments described below.

When cells were grown in L broth containing 0.5 M sucrose, a 2-3 fold enhancement of transformation frequency was consistently seen (Table 1). This enhancement was only seen if 0.5 M sucrose was also incorporated in the 10 mM NaCl wash and first 30 mM $CaCl_2$ treatment as described by Cohen et al (2).

Various concentrations of lysozyme (Sigma, 3X crystallized from eggwhite) were added to the incubation mixture of calcium-treated cells and DNA during the 60 min treatment at 0°C. A twofold increase in transformation efficiency was seen with 1 μg/ml lysozyme (Table 2). No

TABLE 2. Enhancement of transformation efficiency by 1 μg/ml lysozyme.

Concentration of lysozyme (μg/ml)	Infectious centers per ng λ DNA *
0.0	42.5
0.1	40.0
1.0	77.5
10	32.0

*Values are the averages of two experiments. See also footnote to Table 1.

enhancement was seen if lysozyme was added solely during the heat pulse. The effect was less profound if lysozyme was added prior to DNA.

The effects of sucrose and lysozyme upon the enhancement of transformation efficiency are synergistic as shown in Table 3. The additive effect of these two treatments is to increase *E. coli* transformation efficiency sixfold.

E. coli mutation which enhances transformation efficiency. Strain C600 r^-m^- was mutagenized with nitrosoguanidine. Survival was 45 percent and the frequency of rifampicin-resistant mutants increased from 10^{-6} to 10^{-3}. Following mutagenesis the culture was diluted 20-fold into 20 separate flasks containing L broth at 37C. Bacteria from each flask were treated independently by the calcium-heat shock procedure and transformed with 1 μg/ml Km plasmid DNA. The efficiency of transformation to kanamycin resistance was nearly 10^{-5} in all 20 cultures. Each day for four weeks ten colonies were inoculated into L broth containing

TABLE 3. Enhancement of transformation efficiency by sucrose and lysozyme.

Modification of Cohen et al (2) procedure	Infectious centers per ng λ DNA*
None	38.5
0.5 M sucrose in broth, 10 mM NaCl and 1st 30 mM $CaCl_2$	87.0
1 μg/ml lysozyme added with DNA	75.5
0.5 M sucrose and 1 μg/ml lysozyme	216.0

*Values are the averages of two experiments. See also footnote to Table 1.

25 μg/ml kanamycin and the resulting cells treated independently by the calcium-heat shock procedure. This time each culture was transformed with 1 μg/ml Ap plasmid DNA, however, and the bacteria then plated for ampicillin resistance. All cultures but one showed 20-60 ampicillin-resistant colonies per plate. The exception, designated hyt, showed 300 ampicillin-resistant colonies, indicative of an increased transformation efficiency by an order of magnitude.

The hyt mutant was cured of its plasmids by overnight growth in L broth containing 0.5 μg/ml ethidium bromide and 10 μg/ml rifampicin. A kanamycin-sensitive, ampicillin-sensitive isolate was chosen for further study. This isolate shows a tenfold greater transformation efficiency than the parental C600 r^-m^- strain when transformed with λ, Km or Ap DNA preparations (Table 4).

TABLE 4. Enhanced transformation in the *hyt* mutant of *E. coli* C600.

Recipient strain	Transformants per ng DNA*		
	λ DNA	Km DNA	Ap DNA
C600 *r⁻m⁻*	35.5	22.0	17.5
C600 *r⁻m⁻ hyt*	297.0	213.5	119.0

*Values are the averages of two experiments. Each value was determined from three plates containing 100-400 plaques or colonies.

DISCUSSION

Curtiss (4) has described several mutations which enhance transformation frequency in *E. coli* K12. These include *dap* (deficient in diaminopimelic acid synthesis), *galE* (UDP-galactose-4-epimerase) and *end* (endonuclease I-deficient), each of which may increase transformability by three- to tenfold. Cumulatively these mutations may result in a 50-fold increase in transformation frequency (4). *E. coli* C600 *r⁻m⁻ hyt*, the hypertransformable mutant described in this paper is *dap*⁺, *gal*⁺, and *end*⁺ (Molholt, unpublished). The biochemical nature of the *hyt* defect is unknown, but it seems likely that there are at least four types of mutations which can enhance transformation frequency in *E. coli*. It is suggested that further mutations be sought in an EK-2 strain, *e.g.*, *chi1776*, by a selective procedure similar to that outlined in this paper.

Hypotonic expansion of *E. coli* grown in 0.5 M sucrose and lysozyme digestion of the murein layer have

been shown to release endonuclease I (5, 6). Perhaps the release of this surface-bound enzyme forms the basis of the sixfold increased transformation frequency after growth in 0.5 M sucrose and upon addition of 1 μg/ml lysozyme reported herein. The delicate nature of competence in E. coli is unclear, however, too much damage to the murein layer decreases transformability (4).

ACKNOWLEDGMENTS

We thank Ms. Věra Doušová for technical assistance and the U.S. National Academy of Sciences for support of B.M. during this study. Some of the experiments reported herein were performed in the Institute for Molecular Genetics, University of Heidelberg.

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