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Electro-stimulated transformation of *E.coli* cells pretreated by EDTA solution

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The phenomenon of transformation of *E.coli* cells under electric treatment has been studied. The cells of strains MH 1, HB 101 and DH 1 after EDTA treatment in an isotonic medium were transformed with DNA pBR322 by applying a single exponential pulse (E = 10 kV/cm, T = 1.5 ms) to the suspension. The maximum transformation efficiency obtained was 4×10^6 colonies/μg DNA. The maximum transformation frequency was 0.4% at a DNA concentration of 15 μg/ml.

Electrotransfection; Electroporation; *(E.coli)*

1. INTRODUCTION

Advances in biotechnology have aroused considerable interest in the transfer of genetic information to the cell by isolated nucleic acids. In 1982 Neumann and co-workers [1] were the first to describe the phenomenon of transformation of eukaryote cells by high-voltage pulses. In recent years it has been demonstrated that electric treatment can be effectively used for introduction of genetically alien material into animal cells [2,3] as well as protoplasts of plants [4] and yeast [5]. The electrotransformation procedure proved to be applicable to prokaryote cells [6–10]. It seems to be very important since traditional techniques available for some particular bacterium species that provide high yields of transformants are clearly not universal [11,12]. In addition, transformation of prokaryotes is the simplest model for investigating the process of electro-induced translocation of DNA through the cell membrane.

The electrotransformation procedures for *Bacillus cereus* [6], *Streptococcus thermophilus* [7], *Lactobacillus casei* [8] and *Streptomyces livands* [9] as well as the procedure of electrotransformation of intact *Escherichia coli* cells [10] are described already.

In this paper we present a simple method for the electrotransformation of *E. coli* spheroplasts obtained by EDTA treatment. The effect of some factors on the efficiency of this transformation is discussed also.

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2. MATERIALS AND METHODS

2.1. *Chemicals and strains*

We used Tris (Merck, FRG), sucrose, Na_2EDTA (Sigma, USA), $MgSO_4 \cdot 6H_2O$, analytically-pure (Soyuzreaktiv). The DNA of plasmid pBR322 was isolated according to Birnboum and Doly [13], the rest of RNA was removed by gel filtration [14]. In the main part of the study *E. coli* MH 1 [15] was used, while some experiments were performed with the strains HB 101 and DH 1 [12]. For growing *E. coli*, standard media LB and SOB were used. In control experiments, the cells were transformed by a heat shock. Cell competence was induced by treatment with salts of Ca and Mn by the method described in [11].

2.2. *The device and chamber for electric treatment*

By a specially designed generator an exponential discharge

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from a capacitor of 10 μF, pre-charged with a power supply to the needed voltage ($\leqslant$2.4 kV), was performed through a cell suspension.

The amplitude and shape of the pulse as well as the resistance of the chamber were controlled by a storage oscilloscope. The design of the chamber used is shown in fig.1. The distance between the electrodes of stainless steel was 1 mm and maximum volume under treatment was ~130 μl. The coaxial shape of the chamber resulted in some heterogeneity of the field (at the surface of the lower electrode the intensity E was 0.6 of the field intensity at the upper electrode). For simplicity, field intensity was expressed as a maximum value of intensity created within the volume treated.

The cell was sterilized at 170°C for 2 h or autoclaved.

2.3. *Preparation of* E. coli *cells and their electric treatment*

The developed technique of preparation of cells for electric transformation includes several stages. 200 ml of SOB or LB medium in an Erlenmeyer flask was inoculated by a 1 ml, overnight culture of *E. coli* MH 1. After growing at 37°C for 1.5–2 h (A_{550} = 0.4–0.6, the density 5×10^7 cells/ml), 50 ml of the culture was centrifuged at 3000 × g for 5 min at room temperature. The pellet of cells was resuspended at room temperature in 30 ml of solution A containing 10 mM Tris-HCl (pH 8.0), 10 mM EDTA and 10% sucrose. After 1 min of stirring the cells were sedimented by centrifugation at 3000 × g for 5 min at room temperature. The pellet was resuspended in 30 ml of cold (0°C) solution B, containing 10 mM Tris (pH 8.0), 10 mM $MgSO_4$ and 10% sucrose. After 1 min of stirring the cells were centrifuged in the same conditions at 0°C. The pellet was resuspended at 0°C in 1.0 ml of the solution B. The competent cells obtained were stored at 0°C before use. DNA (5 μl) in TE buffer [12] was introduced into a chamber precooled in an ice bath and then 50 μl of the competent cells was added. The solution was stirred by the rotation of the upper electrode and then, after 15 min incubation, was treated with an electric field. The suspension was treated with a single pulse, the upper electrode being the negative pole. The resistance of the chamber at the beginning of the discharge was 190 Ω, the characteristic time of discharge ~1.5 ms. 5 min after electric treatment 450 μl of LB medium containing 10% sucrose was added to 50 μl of the suspension. Changes in the duration of suspension incubation prior to treatment and after it within a time range of 3–25 min did not lead to a considerable change in the results. Subsequently, the culture was plated on LB-agar, containing 13 μg/ml of tetracycline.

Bacteria which accepted the pBR322 plasmid carrying the gene of tetracycline resistance survived on the medium. Calculation of the number of cells that survived makes it possible to evaluate the efficiency of transformation. A 2 h growing period before plating on a selective medium appears to be necessary for the healing of cell membranes after electric treatment [16,17] and for the expression of the genes introduced [12]. It cannot be excluded, however, that amplification of cells during this period may result in an increased number of transformants obtained in the experiments.

The efficiency of transformation was estimated as the number of colonies obtained per 1 μg of DNA introduced. The viability of cells after electric treatment was evaluated by calculating the number of cells that were able to grow in the non-selective medium.

3. RESULTS AND DISCUSSION

For obtaining a reproducible and sufficiently effective electric-induced transformation, a special procedure was developed involving EDTA treatment, which is described above. By analogy with the conventional method of treatment with salts of bivalent cations in the course of transformation by heat shock we shall refer to this procedure as obtaining competent cells.

Fig.2 shows the relationship between the efficiency of transformation and the concentration of EDTA in the solution A. The efficiency of transformation sharply increases with an increase in the EDTA concentration from 0 to 10 mM, then a slow decline in the transformation efficiency begins, which is presumably associated with decline in the cell's viability. Control experiments showed that at the stage of treatment with solution A, the viability of the cells decreases 2-fold and they become sensitive to changes in the medium's tonicity.

The problem of the mechanism of EDTA action on the cells is not yet understood. It is known that EDTA treatment increases permeability of the external membrane of Gram-negative bacteria [18]. Presumably, under such treatment, the external membrane loses its barrier function in relation to the DNA. Special experiments show that the short (5 min) incubation of EDTA-treated cells in the hypotonical solution (10 mM Tris-HCl, pH 8.0) yields a 50-fold decrease of transformation efficiency. It can be concluded that EDTA treatment of cells leads to the appearance of some spheroplasts which are competent for electrotransformation.

It should be emphasized, however, that EDTA treatment without subsequent electric treatment did not result in efficient transformation. Replacement of electric treatment by a heat pulse (0 $\longrightarrow$ 42 $\longrightarrow$ 0°C) [11,12] did not result in transformation either. The relationship between the efficiency of transformation and the intensity of the field applied to the suspension is given in fig.3 on a semi-log scale. The same figure shows a curve of cell viability after treatment with field intensity (also on a semi-log scale). A sharp increase in the efficiency of transformation with an increase of the field intensity applied can be seen in a range of 5–15 kV/cm. A decline in the efficiency of

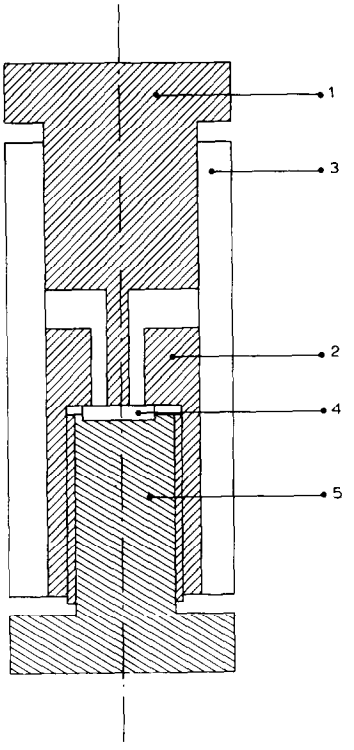

Fig.1. Schematic representation of the chamber for electrical treatment of the cell suspension (section). 1 and 2, upper and lower electrodes of stainless steel; 3, Teflon core; 4, Teflon disc; 5, steel screw.

transformation at higher intensities of the field may be related to a decline in the viability of the cells of *E. coli* after electric treatment (see also [19–21]). It should also be noted that in our experiments we observed a higher viability of cells after electric treatment compared with [19–21]. Presumably, this is explained by the presence in our case of bivalent cations [20] and isoosmoticity of the medium.

The efficiency of transformation is substantially dependent on the suspension's temperature (fig.4). On a semi-log scale this dependence is described as a straight line. The cooling of the suspension from 37°C to 0°C considerably increased the efficiency of transformation. The nature of this effect has not yet been clarified. It is noteworthy that for electric transformation of the eukaryotic cells, a decline of the suspension's temperature in the range 0–37°C, at least in some cases, decreases the transformation frequency [3,22].

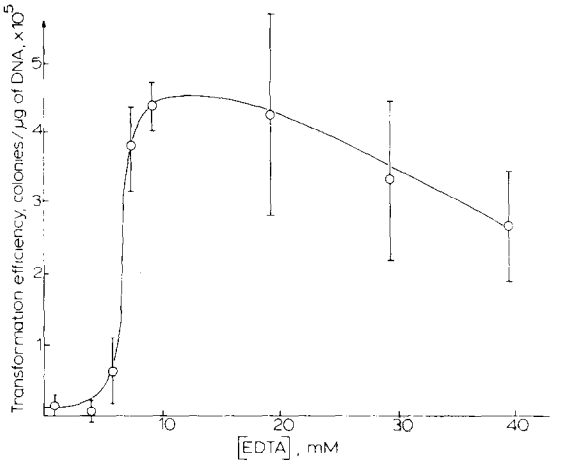

Fig.2. Relationship between the efficiency of electrically induced transformation and the EDTA concentration in solution A (see section 2). The suspension was treated at 0°C with a field intensity of 10 kV/cm, donor DNA concentration in the suspension being 0.01 μg/ml.

The relationship between the number of transformants and the concentration of plasmid DNA is shown in fig.5 on a log scale. This relationship represents a straight line within a range of 0.003–15 μg DNA in the treated suspension. The slope of the line corresponds to the efficiency of transformation, 6.6×10^5 colonies/μg DNA. The highest efficiency attained in our experiments was

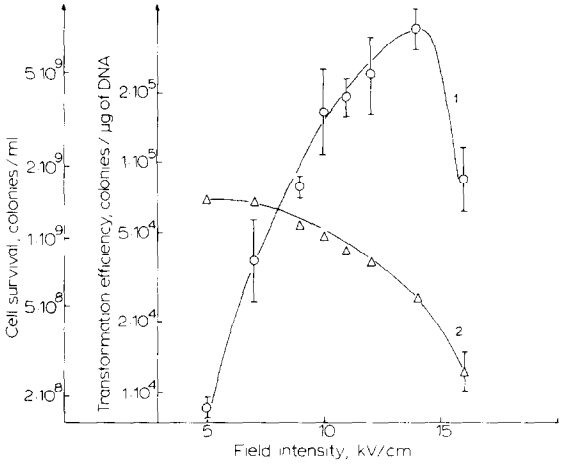

Fig.3. Transformation efficiency (—○—) and the survival of cells (—△—) as a function of field intensity. Concentration of donor DNA in the suspension was 0.01 μg/ml.

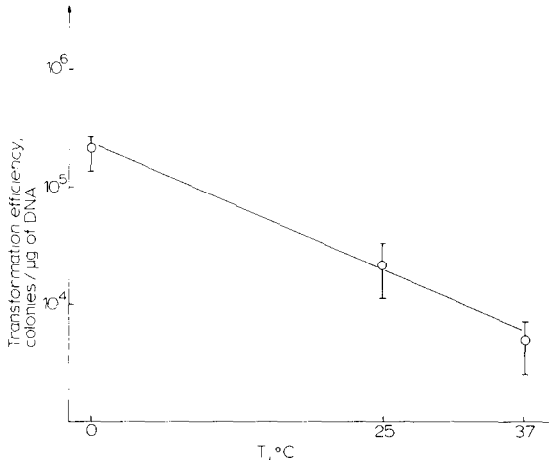

Fig.4. Relationship between the efficiency of electrically induced transformation and suspension temperature in the chamber. DNA concentration, 0.01 µg/ml; field intensity applied, 10 kV/cm.

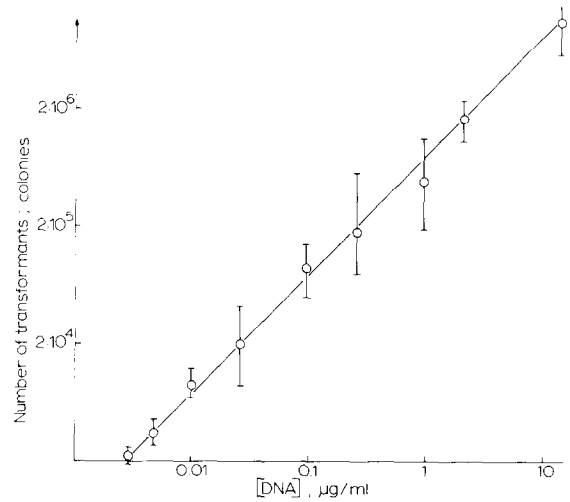

Fig.5. Relationship between the number of transformants and the concentration of donor DNA. Field intensity applied, 10 kV/cm.

4×10^6 colonies/µg DNA in the treated suspension. The frequency of transformation, i.e. the portion of transformed cells among all viable cells attained 0.4% at a concentration of 15 µg DNA/ml.

There are grounds to believe that both the efficiency and the frequency of transformations could be increased in the course of further optimization of the proposed procedure. However, the efficiency $\sim 10^6$ colonies/µg DNA attained in our study for the MH 1 strain approximates that provided by the use of traditional methods of the transformation of the *E. coli* cells.

Special experiments performed with other *E. coli* strains (HB 101 and DH 1) revealed that their electrically induced transformation is similar in efficiency to that of strain MH 1.

It is important to note that the main regularities described here on the electrotransformation of *E. coli* spheroplasts obtained by EDTA treatment are similar to results published in [10] on the electrotransformation of intact bacterial cells.

The mechanism of electric transformation remains to be understood. There are reasons to assume that a substantial role in this phenomenon is played by the membrane electroporation proceeding in the same range of parameters as for electric treatment [17,23]. In which case the universality of electroporation as a phenomenon determined by the properties of membrane lipid matrix [24] provides grounds to believe that the electric transformation method proposed is applicable for a wide range of prokaryote cells.

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